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Thèse

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par **Liliane MAJED**

Sous la direction de Mme Sylvie DOUSSET

**Teneurs en résidus de pesticides dans les raisins et les
feuilles de vigne au Liban et conséquences**

4 Avril 2024

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Résumé

La vigne est étroitement liée à l'histoire du Liban, où elle est cultivée tant pour ses fruits que pour ses feuilles. L'exportation des produits de la viticulture (raisins ou feuilles de vigne) est entravée par la présence de résidus de produits phytosanitaires, entraînant parfois le rejet de ces denrées par les douanes des pays importateurs. Cette situation est plus préoccupante pour les feuilles de vigne, produit de consommation régionale qui n'ont pas été étudiées suffisamment par la communauté scientifique pour établir de Limites Maximales de Résidus (LMR) propres à cette denrée alimentaire. Ainsi, cette thèse évalue l'état des teneurs des résidus de pesticides dans les produits viticoles au Liban et met en lumière les facteurs influençant leurs teneurs dans les feuilles, tels que le type de formulation et les processus de conservation (à sec et en saumure) et de lavage (eau de robinet, solution de vinaigre à 10% ou eau bouillante).

Les données de surveillance des pesticides obtenues par les campagnes d'échantillonnages réalisées par le ministère de l'agriculture (2012-2014) comportant 1594 échantillons de raisins de table ont été analysées. Le suivi de dissipation de l'imidaclopride appliqué sous forme de deux formulations (SL et WDG) a été effectué pendant 18 jours dans les raisins et les feuilles de vigne. L'effet de la conservation et du lavage sur l'élimination des résidus de pesticides a été évalué pour trois molécules, azoxystrobine, fenazaquin et indoxacarbe. Les résultats de surveillance ont montré que 73 % des raisins de table libanais contenaient des résidus de 48 molécules dont 23 % dépassant les LMR du Codex avec une détection fréquente de cocktails de pesticides (60% des échantillons). La formulation SL a présenté un dépôt initial significativement plus élevé que la formulation WDG sur les raisins et les feuilles de vigne, et l'étude de la cinétique de dissipation a révélé des demi-vies de 0,5 jours pour les deux formulations dans les deux matrices. Les résidus d'imidaclopride se sont révélés être 20 à 70 fois plus élevés dans les feuilles de vigne que dans les raisins. La conservation à sec a montré une réduction moyenne de 60 % des résidus pour les trois pesticides testés comparée à 48% pour la conservation en saumure. Le lavage à l'eau bouillante a montré une élimination moyenne de 30 % pour les trois molécules contre 26 et 18 % pour l'eau de robinet et la solution vinaigrée, respectivement. La consommation des raisins et des feuilles de vigne ne présentait aucun risque pour la santé des consommateurs mais contribue à l'exposition aux résidus de pesticides.

L'ensemble des résultats obtenus souligne la nécessité d'une gestion plus stricte des pratiques phytosanitaires dans la viticulture libanaise pour garantir la conformité aux normes internationales et la protection de la santé humaine et de l'environnement. Le choix de formulations granulées plutôt que liquides est préférable, en raison de l'accumulation significative plus élevée de résidus dans les feuilles par rapport aux raisins. Finalement, étant donné que la conservation et le lavage des feuilles de vigne ne garantissent pas l'élimination totale des résidus, il serait recommandé d'établir un programme de traitements phytosanitaires spécifique pour la production de feuilles de vigne.

Mots-clés : *Formulation, Conservation, Lavage, QuEChERS, LCMSMS, GCMSMS, LMR, Azoxystrobine, Fenazaquin, Imidaclopride, Indoxacarbe*

Abstract

The vine is closely linked to the history of Lebanon, where it is cultivated for both its fruits and its leaves. The export of viticulture products (grapes or vine leaves) is hindered by the presence of pesticide residues, sometimes resulting in the rejection of these commodities by the customs of importing countries. This situation is more concerning for vine leaves, a due to its regional pattern, which have not been sufficiently studied by the scientific community to establish Maximum Residue Limits (MRLs) specific to this commodity. Thus, this thesis evaluates the state of pesticide residue levels in viticultural products in Lebanon and highlights factors influencing their levels in leaves, such as the type of formulation and conservation processes (dry and in brine) and washing (tap water, 10% vinegar solution, or boiling water).

Pesticide surveillance data obtained from sampling campaigns conducted by the Ministry of Agriculture (2012-2014), comprising 1594 samples of table grapes, were analyzed. The dissipation study of imidacloprid applied in two formulations (SL and WDG) was carried out for 18 days in grapes and vine leaves. The effect of conservation and washing on pesticide residue elimination was evaluated for three molecules: azoxystrobin, fenazaquin, and indoxacarb. Surveillance results showed that 73% of Lebanese table grapes contained residues of 48 molecules, with 23% exceeding the Codex MRLs, frequently detecting pesticide cocktails (60%). The SL formulation exhibited significantly higher initial deposits than the WDG formulation on grapes and vine leaves, and dissipation kinetics study revealed half-lives of 0.5 days for both formulations in both matrices. Imidacloprid residues were found to be 20 to 70 times higher in vine leaves than in grapes. Dry conservation showed an average reduction of 60% in residues for the three tested pesticides compared to 48% for brine conservation. Washing with boiling water showed an average elimination of 30% for the three molecules, compared to 26% and 18% for tap water and vinegar solution, respectively. Consumption of grapes and vine leaves posed no health risk to consumers but contributes to exposure to pesticide residues

All results underline the need for stricter management of phytosanitary practices in Lebanese viticulture to ensure compliance with international standards and the protection of human health and the environment. Choosing granulated formulations over liquid ones is preferable due to significantly higher residue accumulation in leaves compared to grapes.

Finally, since the conservation and washing of vine leaves do not guarantee total residue elimination, it would be recommended to establish a specific phytosanitary treatment program for vine leaf production.

Keywords: *Formulation, Conservation, Washing, QuEChERS, LCMSMS, GCMSMS, MRL, Azoxystrobin, Fenazaquin, Imidacloprid, Indoxacarb*

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Liste des abréviations

| | |
|------------------|---|
| ACN | Acetonitrile |
| ADI / DJA | Acceptable Daily Intake / Dose Journalière Admissible |
| aHQ | Acute Hazard Quotient /Quotient de Risque Aigue |
| ANOVA | Analysis of variance |
| ARfD | Acute Reference Dose /Dose de référence aiguë |
| BCF | Facteur de Bioconcentration |
| BPA | Bonnes Pratiques Agricoles |
| CAC | Commission du Codex Alimentarius |
| CBI | Center for the Promotion of Imports from developing countries - The Netherlands |
| CCIAT | Chamber of Commerce, Industry and Agriculture of Tripoli |
| CE | Commission Européenne |
| CFR | US Code of Federal Regulations |
| cHQ | Chronic Hazard Quotient /Quotient de Risque Chronique |
| CID | Compound Identifier |
| DAR | Durée Avant Récolte |
| DDT | Dichlorodiphényltrichloroéthane |
| DL50 | Dose Léthale 50 |
| d-SPE | Dispersive Solid-Phase Extraction |
| DT50 | Demie-vie / Half-life |
| EDI - DJE | Estimated Daily Intake - Dose Journalière Estimée |
| EDTA | Ethylenediaminetetraacetic acid |

| | |
|------------------|--|
| EFSA | Autorité Européenne de Sécurité des Aliments |
| EPA | U.S. Environmental Protection Agency |
| ESI | Electrospray Ionisation |
| ESTI | Estimated Short Term Intake |
| FAO | Food and Agriculture Organization |
| FEL | Frank Effect Level |
| FFDCA | Federal Food and Cosmetic Act |
| FRAC | Fungicide Resistance Action Committee |
| FT | Facteur de Transformation |
| GAP | Good Agricultural Practices |
| GCB | Graphitized Carbon Black |
| GC-MS-MS | Chromatographie Gaseuse - Spectrométrie de Masse en tandem |
| ha | Hectare |
| HI | Risk Index - Indice de Risque |
| HQ | Hazard Quotient - Quotient de Risque |
| IRAC | Insecticide Resistance Action Committee |
| ISTAT | Italian National Institute of Statistics |
| JMPR | Joint FAO/WHO Meeting on Pesticide Residues |
| K | Dissipation Rate - Constante de Dissipation |
| Kd | Coefficient d'adsorption du sol |
| Koc | Coefficient de partage Carbone Organique/Eau |
| Kow | Coefficient de partage Octanol / Eau |
| LC-MS-MS | Chromatographie Liquide - Spectrométrie de Masse en tandem |
| Libnor | Institut Libanais de Normalisation |
| LMR - MRL | Limites Maximales en Résidus – Maximum Residue Limit |
| LOAEL | Lowest Observed Adverse Effect Level |
| LOD | Limit Of Detection - Limite de Détection |
| LOEL | Lowest Observed Effect Level |
| LOQ | Limite Of Quantification - Limite de Quantification |
| MoA | Ministry of Agriculture |
| MRM | Multiple Reaction Monitoring |
| NOAEL | No Observed Adverse Effect Level |
| NOEL | No Observed Effect Level |
| OECD | Organisation de Coopération et de Développement Economiques |
| OMS | Organisation Mondiale de la Santé |
| OP | Organophosphate Pesticides |
| PF | Processing Factor |
| PHI | Pre-Harvest Interval |
| pKa | Constante de dissociation acide |
| PPDB | Pesticide Properties Database |
| PSA | Primary Secondary Amine |
| QuEChERS | Quick, Easy, Cheap, Efficient, Rugged and Safe |
| RASFF | Réseau d'Alerte Rapide Européen pour l'Alimentation Humaine et Animale |
| RM | Recovery Mean |
| RSD | Relative Standard Deviation |
| SANCO | Directorate-General for Health and Consumers - European Commission |
| SANTE | Directorate-General for Health and Food Safety - European Commission |

| | |
|--------------|--|
| SD | Standard Deviation |
| SweEt | Swedish ethyl acetate extraction method |
| UE | Union Européenne |
| USAID | United States Agency for International Development |
| WHO | World Health Organisation |

Les Formulations

| | |
|------------|--------------------------------|
| CS | Concentré Soluble |
| EC | Concentré émulsionnable |
| EW | Emulsion aqueuse |
| F | Fluide |
| GL | Gel |
| ME | Formulation Microcapsules |
| S | Solution |
| SC | Concentré pulvérisable |
| SL | Concentré soluble |
| SP | Poudre soluble |
| WDG | Granulé dispersible dans l'eau |
| WG | Granulée Mouillables |
| WP | Poudre mouillable |

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Introduction générale

Les produits de la vigne au Liban

De nos jours, la culture de la vigne est largement répandue dans le monde entier, avec une superficie estimée à 7,6 millions d'hectares (Creasy et Creasy 2018). Cette culture liée à l'histoire du Liban y trouve des conditions pédoclimatiques propices avec une grande diversité de terroirs (ROBY 2003) . Appartenant principalement à l'espèce *Vitis vinifera* (Mohasseb, Sassine, et el Sebaaly 2019), la vigne s'étend au Liban sur 10 609 hectares, dont 70 % sont destinés à la production de raisins de table (Tabaja, Siblini, et Chalak 2020). Actuellement, la production annuelle de raisin est de 120 000 tonnes, dont seulement 20 000 tonnes sont exportées, principalement vers les pays du Moyen-Orient, et un faible pourcentage (3,5 %) vers les pays européens (principalement l'Allemagne et le Royaume-Uni) (USAID/LEBANON 2021). Avec le raisin de table, les principaux produits de la viticulture libanaise sont le vin, l'arak et les feuilles de vignes (Roby 2003).

Les feuilles de vigne farcies sont l'un des plats classiques du mezzé libanais et sont consommées dans de nombreux pays méditerranéens, dont Chypre, la Turquie, la Grèce, l'Espagne, l'Italie et le Maroc (Rizzuti et al. 2013). Selon Oruç, Cangı, et Ergün (2020), les familles (de 4 personnes en moyenne) de la région de Tokat en Turquie consomment en moyenne 13,9 kg de feuilles de vigne par an par famille (un équivalent de 5 feuilles de vigne par jour par personne). Selon qu'elles soient préparées de manière végétarienne ou non végétarienne, les feuilles de vigne peuvent être farcies avec de la viande, du riz, du persil, des tomates, des oignons et des épices, le tout rehaussé d'huile d'olive et de jus de citron. Ces feuilles sont devenues un ingrédient important de la cuisine internationale, entraînant une augmentation de la production de feuilles de vigne (Rizzuti et al. 2013; Aktop, Şanlıbaba, et Güçer 2023). En effet, les feuilles de vigne possèdent un potentiel commercial substantiel et prometteur. Selon les données du commerce mondial, le Liban exporte principalement ses feuilles de vigne vers le Ghana, l'Ouganda et la Colombie (Volza 2023). Il en exporte également pour plus de 2 millions de dollars américains vers les pays du Golfe (Hayar, Zeitoun, et Maestroni 2021b). Parmi les principaux exportateurs mondiaux, les États-Unis sont en tête suivis du Vietnam et de la Chine (Volza 2023).

Les consommateurs préfèrent les feuilles de vigne minces, glabres et peu lobées ; ainsi la Turquie cultive des variétés spécifiques de vigne destinées à l'effeuillage gastronomique (exemple du Narince, du Sultani et du Yapıncak). De plus, les feuilles de vigne obtenues à

partir de variétés telles que le Muscat Hamburg, le Hesap Ali (Müşküle), le Kober 5 BB, le Karaerik et le Kabuğu Yufka sont également utilisées à des fins comestibles (Aktop, Şanlıbaba, et Güçer 2023). Parmi les autres pays cultivant de telles variétés, on peut citer la Grèce et la Bulgarie (Maia et al. 2021). En Grèce, des expérimentations sont en cours pour cultiver les feuilles de vigne en serre, visant à obtenir une production tout au long de l'année plutôt que de se limiter à la période de récolte de deux mois (Chatzigeorgiou et al. 2022). Effectivement, étant donné la courte période de récolte des feuilles généralement de mai à juin afin de rester consommables, de nombreuses méthodes de conservation sont traditionnellement utilisées pour préserver les feuilles de vigne : conservation à sec, saumurage et blanchiment/congélation (Cantwell et al. 2022). Au Liban, la pratique de conservation des feuilles de vigne est considérée comme faisant partie de la préparation de la "Mouneh" dans les foyers, qui consiste à conserver des produits locaux d'une saison à l'autre. Pour la conservation à sec, les feuilles de vigne fraîches sont directement stockées dans un bocal sans ajouter de sel ni d'agents de conservation (Başoğlu et al. 1996), tandis qu'avec le saumurage, différents niveaux de sel, d'acide citrique et d'acide acétique peuvent être ajoutés (Cantwell et al. 2022). Bien que le marché mondial des feuilles de vigne soit vaste (EastFruit 2019), le commerce international de ces feuilles est entravé par le manque de normes internationales spécifiques à cette denrée, notamment celles concernant les limites en résidus de produits phytosanitaires dans les denrées alimentaires (Hayar, Zeitoun, et Maestroni 2021b).

Les teneurs en résidus de pesticides dans les produits de la vigne

La production de raisin est confrontée à des défis importants en raison de fortes pressions biotiques. Des maladies fongiques courantes telles que le mildiou (*Plasmopara viticola*), l'oïdium (*Uncinula necator*) et la pourriture grise (*Botrytis cinerea*), ainsi que les attaques de ravageurs tels que les acariens du raisin (*Eriophyes vitis*), la tordeuse de la grappe (*Lobesia Botrana*), les cochenilles (*Planococcus vitis*, *Pseudococcus sp*) et les thrips du raisin (*Frankliniella occidentalis*, *Drepanotrips reuterii*) sont fréquentes au Liban (Hobeika et El Khouri 2011; Gabur et al. 2021). Parmi les nombreux modèles de gestion des ravageurs, l'utilisation de pesticides est l'une des pratiques les plus courantes. Selon les douanes libanaises, le Liban importe annuellement 6641 tonnes de pesticides (Abou Zeid et al. 2020) avec l'une des utilisations de pesticides par hectare de terres cultivable les plus élevées au monde (6.5

kg/ha)(FAOSTAT 2021) par rapport à la moyenne mondiale de 1,8 kg/ha en 2020 (S. R. Patil et Patil 2022). En viticulture, les pesticides sont largement utilisés pour garantir une production de haute qualité et des quantités économiquement viables (Gabel 2019). En effet, les vignes sont sujettes à des traitements fréquents, en particulier les fongicides, qui peuvent atteindre jusqu'à 18 traitements par an (Gabel 2019). En Italie, en 2020, les vignes ont reçues 24,89 kilogrammes de pesticide en moyenne par hectare (ISTAT 2023). En Espagne, les quantités utilisées sur la vigne sont supérieures, avec une utilisation estimée de 34,2 kg/ha (Alonso González, Parga-Dans, et Pérez Luzardo 2021; Alonso González et al. 2022). Au Liban, il n'existe pas de données officielles sur l'utilisation des pesticides en viticulture ou dans d'autres cultures (Akkouch et Halwani 2023).

Selon l'autorité européenne de sécurité des aliments (EFSA), en 2021, 86 % des échantillons de raisins produits en Europe contenaient un résidu de pesticide et 68 % contenaient plusieurs résidus (Alonso González et al. 2022). Dans une étude récente au Liban, les échantillons de raisins et de feuilles de vigne présentaient les teneurs les plus élevées en résidus de pesticides parmi les 49 produits alimentaires d'origine végétale étudiés (Khazaal et al. 2022). L'utilisation des pesticides suscite une double préoccupation en raison de leur impact sur les écosystèmes et de leur persistance dans les aliments sous forme de résidus (Gabur et al. 2021). Pour surmonter ces défis, les autorités telles que le *Codex Alimentarius* et la Commission Européenne ont établi un certain nombre de normes, émis des réglementations et mis en œuvre divers systèmes de certification/agrément pour s'assurer d'une utilisation sûre et durable des pesticides. La conformité à ces règles et directives relève des "bonnes pratiques agricoles" (BPA) (Abou Zeid et al. 2020). Ces BPA reposent sur l'application des connaissances disponibles sur l'utilisation des ressources naturelles de manière durable pour obtenir des aliments et des produits sains et sûrs, tout en réalisant la viabilité économique et la stabilité sociale (Kharel, Dahal, et Raut 2022). Les BPAs doivent permettre le respect de la dose recommandée spécifiée sur l'étiquette des flacons de pesticides et les délais d'attente avant récolte (DAR) afin que les teneurs en résidus de pesticides dans les denrées alimentaires puissent être inférieures aux limites maximales en résidus (LMRs) (Li 2022). La non-conformité aux normes phytosanitaires constitue l'un des principaux défis auxquels sont confrontées les exportations de raisins libanais (Koubeissy 2014). L'auteur a vivement recommandé le respect des BPAs par les agriculteurs et a souligné l'importance d'utiliser les pesticides autorisés par le ministère de l'agriculture et de la santé

du Liban. Récemment, la présence de résidus de pesticides non autorisés sur le raisin (chlorpyrifos et monocrotophos) et des teneurs en résidus de lambda-cyhalothrine et cyperméthrine dépassant les LMRs a été pointé du doigt par la Commission européenne dans les raisins de table importés du Liban (RASFF 2023).

Les feuilles de vigne : une culture orpheline

Les "cultures orphelines" correspondent à des plantes qui, du fait des habitudes de consommation régionale, n'ont pas bénéficié de soutien politique, ni des avancées agricoles (Tadele 2019; Talabi et al. 2022). C'est par exemple le néflier, la corète potagère et les feuilles de vigne (sous-produit de la viticulture) alors que la valorisation des sous-produits agricoles est importante afin d'améliorer la durabilité alimentaire mondiale et de relever le défi de l'insécurité alimentaire (Lau, Sabran, et Shafie 2021). Ainsi, la valorisation des feuilles de vigne se heurte à l'absence de normes spécifiques, en particulier de LMRs, ce qui constitue un obstacle à son exportation. Les LMRs sont absentes du Codex Alimentarius et la Commission Européenne spécifie une LMR qui correspond à la limite inférieure de détermination analytique pour 98 % des matières actives et est retenue par principe de précaution sans aucune évaluation d'un point de vue toxicologique (FAO-WHO 2023; European Commission 2023).

Il existe très peu d'études sur les teneurs en résidus de pesticides et les facteurs influençant ces résidus dans les raisins et les feuilles de vigne au Liban (Hayar, Zeitoun, et Maestroni 2021; Khazaal et al. 2022). Par ailleurs, la présence de résidus de pesticides non autorisés et/ou dépassant les LMRs ont été signalés dans les raisins et les feuilles de vigne provenant du Liban par les douanes des pays importateurs (RASFF 2023). Il est donc indispensable d'évaluer les teneurs en résidus de pesticides dans les produits de la vigne au Liban et de proposer des moyens de réduire et/ou d'éliminer ces résidus.

Les travaux de cette thèse s'inscrivent dans le cadre d'une initiative nationale plus vaste visant à établir des LMRs dans les feuilles de vigne et autres cultures orphelines au Liban. Les résultats obtenus permettront à l'Institut libanais de normalisation (Libnor) d'établir des LMRs nationales. À son tour, cet institut collaborera avec d'autres pays engagés dans des recherches similaires pour demander officiellement aux organisations internationales telles que le Codex Alimentarius de proposer des LMRs pour les feuilles de vigne.

Objectifs de la thèse et questions de recherches

Les objectifs de cette thèse sont donc :

- i. De synthétiser les recherches menées sur les feuilles de vigne au Liban et à l'échelle internationale ;
- ii. D'interpréter les teneurs en résidus de pesticides dans les raisins de table produits au Liban entre 2012 et 2014 et d'évaluer leur impacts sur la santé humaine et environnementale ;
- iii. D'explorer les différences potentielles d'accumulation et de dissipation des pesticides dans les feuilles et les raisins ;
- iv. D'investiguer les principaux facteurs influençant la dissipation de résidus de pesticides dans les feuilles de vigne, notamment la formulation des produits commerciaux des pesticides ;
- v. D'évaluer l'efficacité des méthodes de transformation des feuilles de vigne industrielles et traditionnelles à réduire les teneurs en résidus de pesticides, en se concentrant sur les pratiques de lavage et de conservation, et en évaluant les risques potentiels pour la santé humaine.

En réponse à ces objectifs, la thèse cherche à répondre aux questions de recherche suivantes :

- 1.** Quel est l'état des pratiques phytosanitaires dans la viticulture libanaise ?
- 2.** La nature de la matrice (feuilles et raisins) influence-t-elle les teneurs et la dissipation des résidus de pesticides ?
- 3.** La formulation des pesticides affecte-t-elle leurs dépôts initiaux et leurs cinétiques de dissipation dans les feuilles de vigne et les raisins ?
- 4.** Les méthodes de lavage sont-elles efficaces pour éliminer les résidus de pesticides dans les feuilles de vigne ?
- 5.** Les méthodes de conservation des feuilles de vigne influencent-elles leurs teneurs en résidus de pesticides ?

Ainsi, dans cette thèse, une synthèse bibliographique (chapitre 1) a permis d'évaluer l'état des connaissances sur les teneurs en résidus de pesticides dans les feuilles de vigne au Liban et à l'échelle internationale, ainsi que leurs conséquences sur la santé humaine. Le **chapitre 2** vise à répondre à la question de recherche n°1 au moyen d'une étude rétrospective

utilisant des données obtenues auprès du ministère de l'agriculture sur les teneurs en résidus de pesticides dans le raisin de table de janvier 2012 à décembre 2014. Les données ont été analysées quantitativement et qualitativement au moyen de méthodes statistiques et d'évaluation des risques, en tenant compte des propriétés et des modes d'action des fongicides et des insecticides. Le **Chapitre 3** aborde les questions de recherche n°2 et 3 ; il concerne l'étude de la vitesse de dégradation de l'imidaclopride sous forme de deux formulations (liquide soluble ou SL et granulés dispersables dans l'eau ou WDG) dans les matrices feuilles de vigne et raisin. Des intervalles pré-récoltes pour les deux formulations et les deux matrices ont été proposés. Le **Chapitre 4** aborde les questions de recherche n° 4 et 5 en examinant l'impact de deux techniques de conservation industrielles courantes (saumurage et à sec) et de trois méthodes de lavage couramment utilisées (lavage à l'eau du robinet, à l'eau bouillante, et avec une solution de vinaigre à 10%) sur les teneurs en résidus d'azoxystrobine, de fenazaquin et d'indoxacarbe dans les feuilles de vigne. Enfin, une **conclusion générale** synthétise l'ensemble des résultats obtenus et permet de proposer des perspectives à ces travaux.

CHAPITRE I
ÉTAT DE L'ART

1. Définition des pesticides

Conformément à la définition énoncée par l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO), les pesticides englobent un ensemble de substances comprenant les insecticides, les huiles minérales, les herbicides, les fongicides, les bactéricides, les régulateurs de croissance des plantes et les rodenticides, ainsi que d'autres combinaisons de composés. Ces substances méticuleusement formulées sont conçues pour répulser, éliminer ou contrôler efficacement divers parasites s'étendant aux organismes agissant en tant que vecteurs de maladies. Elles englobent les formulations appliquées pour la protection des cultures après la récolte, pendant les processus de stockage et de transport (FAO 2021).

2. Classification des pesticides

Les pesticides peuvent être regroupés de manière systématique en catégories distinctes en tenant compte de divers critères tels que leur cible, leur origine, leur groupe chimique, leur forme commerciale, leur mode d'action, leurs caractéristiques physico-chimiques (persistance) et leur toxicité. On se limite à décrire les quatre dernières critères qui constituent ultérieurement la base de l'interprétation des résultats (Hassaan et El Nemr 2020; Ansari et al. 2021; FAO 2021; Pathak et al. 2022).

2.1. Selon leur forme commerciale (formulation) :

Les formulations de pesticides sont composées d'un mélange de substances actives et de divers composants "inertes" (University of Kentucky 2016). Ces derniers jouent un rôle essentiel dans la modification des propriétés physico-chimiques des substances actives, facilitent leur épandage et leur manipulation par les agriculteurs et améliorent leur stabilité lors du stockage. Ces "inertes" peuvent donner lieu à diverses formulations de pesticides, telles que des liquides, des granulés, des poudres mouillables, des émulsions, et des suspensions, etc. (Ansari et al. 2021). Cependant, certains composants inertes spécifiques peuvent présenter des risques potentiels pour la santé humaine (Nagy et al. 2020).

2.2. Selon leur mode d'action :

Les modes d'action des pesticides peuvent être largement regroupés en deux principales catégories : les pesticides de **contact** et les pesticides **systemiques**. Les pesticides de contact agissent par contact physique direct, entraînant la mortalité des ravageurs. En revanche, les

pesticides systémiques sont absorbés par les plantes et acheminés par la sève élaborée et/ou brute pour atteindre les diverses parties non traitées (Das 2013). Les bases de données du FRAC et du l'IRAC répertorient les pesticides en 34 et 14 groupes respectivement selon leurs modes d'actions (FRAC 2023; IRAC 2023). Elles indiquent que la plupart des pesticides ciblent les systèmes nerveux et musculaires des insectes et qu'ils peuvent également affecter la croissance en interférant directement avec la formation ou le dépôt de la cuticule ou avec la biosynthèse des lipides des ravageurs. D'autres pesticides affectent la respiration mitochondriale par l'inhibition du transport d'électrons et de la phosphorylation oxydative.

2.3. Selon leurs caractéristiques physico-chimiques :

Les propriétés physico-chimiques des pesticides revêtent une importance significative car elles dictent leur persistance et leur toxicité. Ces propriétés englobent le poids et la structure moléculaire, la pression de vapeur, la solubilité, le coefficient de partition octanol/eau (K_{ow} ou $\log K_{ow}$), le coefficient d'adsorption au sol (K_{oc}/K_d), la constante de Henry et la constante de dissociation acide (pK_a) (Zacharia et al. 2011). Par ailleurs, les pesticides peuvent être catégorisés comme persistants, semi-persistants ou non persistants en fonction de leur taux de dégradation dans l'environnement, exprimé sous forme de demi-vie (DT_{50}), une mesure intrinsèquement liée à leurs caractéristiques physico-chimiques et au compartiment environnemental. Il convient de mentionner que les pesticides qui sont fortement liés au sol ont généralement des valeurs élevées de K_{ow} , ce qui entraîne des valeurs élevées de K_{oc} , et ces deux propriétés se traduisent par une forte adsorption sur la matière organique du sol. Ainsi, les pesticides classés comme hydrophobes, persistants et bioaccumulables devraient s'accumuler et persister dans les sols (Tudi et al. 2021). En effet, le $\log K_{ow}$, également appelé $\log P$, présente une corrélation inverse avec la solubilité dans l'eau et une corrélation directe avec le poids moléculaire de la substance. Les valeurs du $\log K_{ow}$ s'étendent généralement de 3 (très hydrophile) à +10 (extrêmement hydrophobe) (Cumming et Rucker 2017; Stefl 2017). Il est à noter que les pesticides subissent deux types principaux de dégradation : la dégradation physico-chimique dans l'eau ou l'atmosphère (par oxydation, réduction, hydrolyse et photolyse) et la dégradation biologique au sein des organismes vivants (impliquant oxydation, réduction, hydrolyse et conjugaison) (Zacharia et al. 2011; Ansari et al. 2021).

2.4. Selon leur toxicité :

En ce qui concerne leur toxicité, l'Organisation mondiale de la santé (OMS) utilise la dose létale DL_{50} par voie orale et cutanée (DL_{50} - la dose à laquelle une substance est mortelle pour 50 % des animaux testés) chez les rats pour classer les pesticides (OMS 2019; Pathak et al. 2022). Il est important de noter que cette classification aborde principalement la toxicité aiguë des pesticides plutôt que la toxicité chronique qui constitue la majeure préoccupation au sein de la communauté scientifique (Pathak et al. 2022). Dans ce but, les organisations internationales ont formulé une série de paramètres et de normes toxicologiques pour évaluer la toxicité potentielle des pesticides, garantissant la sécurité des personnes exposées à ces substances et des produits alimentaires traités avec des pesticides. Ces paramètres sont élucidés dans l'étude menée par Fahed et Hayar (2021) et englobent les définitions suivantes telles que détaillées dans le glossaire de l'EFSA et de l' EPA (2023) :

Limite maximale de résidus (LMR): les organisations mondiales (FAO, Commission Européenne, EPA..) établissent la quantité maximale admissible de résidus de pesticides pouvant être détectables dans ou sur les denrées alimentaires. Ce seuil, appelé Limite Maximale de Résidus (LMR), est quantifié en milligrammes par kilogramme.

Dose Journalière Admissible (DJA): la Dose Journalière Admissible désigne la quantité maximale d'une substance à laquelle un individu peut être exposé quotidiennement sur une période prolongée (durée de vie) sans subir d'effets indésirables. Cette mesure est généralement exprimée en milligrammes de la substance par kilogramme de poids corporel (humain) par jour.

Dose de Référence Aiguë (ARfD): la Dose de Référence Aiguë est une estimation de la quantité maximum d'une substance qui peut être ingérée en une fois ou sur une période courte (généralement dans les 24 heures ou moins), sans entraîner un risque dangereux pour la santé. L'ARfD peut être déduite du NOAEL, LOAEL. Les composés ayant une ARfD sont généralement plus toxiques que les composés sans ARfD (Zarn et O'Brien 2018).

Niveau sans effet observé (NOEL) : niveau d'exposition auquel il n'y a pas d'augmentations statistiquement ou biologiquement significatives de la fréquence ou de la gravité d'aucun effet entre la population exposée et son groupe témoin.

Niveau le plus bas d'effet observé (LOEL or LEL) : dans une étude, la dose ou le niveau d'exposition le plus bas auquel un effet statistiquement ou biologiquement significatif est

observé dans la population exposée par rapport à un groupe témoin non exposé approprié.

Niveau sans effet adverse observé (NOAEL) : niveau d'exposition le plus élevé auquel il n'y a pas d'augmentations biologiquement significatives de la fréquence ou de la gravité des effets indésirables entre la population exposée et son groupe témoin approprié ; certains effets peuvent être observés à ce niveau, mais ils ne sont pas considérés comme indésirables ou comme précurseurs d'effets indésirables.

Niveau le plus bas d'effet adverse observé (LOAEL) : niveau d'exposition le plus bas auquel des augmentations biologiquement significatives de la fréquence ou de la gravité des effets indésirables sont observées entre la population exposée et son groupe témoin approprié.

Niveau d'effet de Frank (FEL) : Un niveau d'exposition ou de dose qui produit des effets indésirables irréversibles, avec une augmentation statistiquement ou biologiquement significative de la fréquence ou de la gravité entre les individus exposés et ceux qui ne le sont pas.

En essence, NOEL, LOEL, NOAEL, LOAEL et FEL sont des paramètres distincts illustrés dans la Figure I - 1.

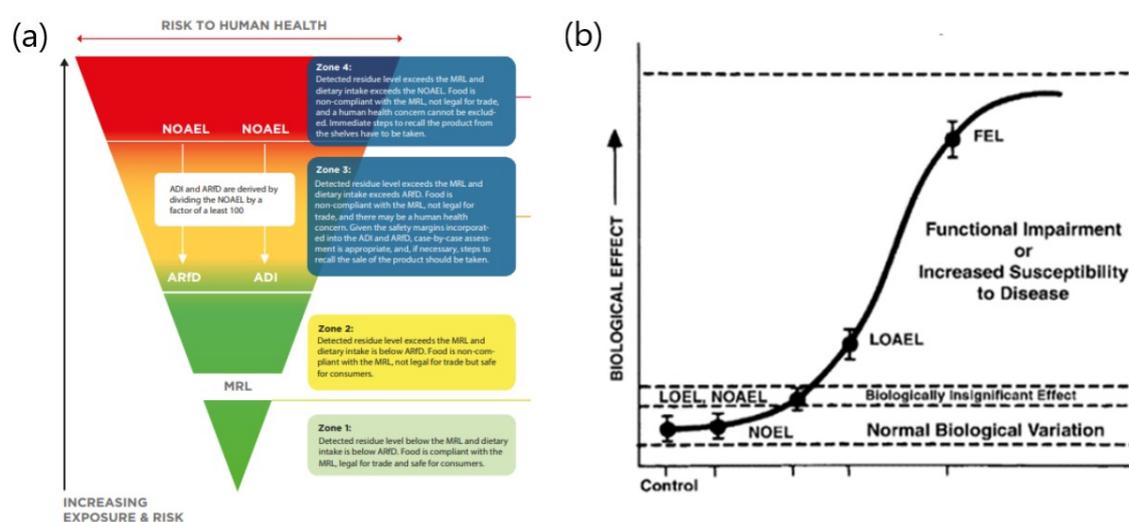


Figure I-1: a) Représentation du risque potentiel accru pour la santé humaine lorsque les niveaux de résidus de pesticides dépassent les limites établies par les organisations internationales (EFSA, Codex). L'illustration décrit également les actions recommandées en fonction des niveaux de résidus de pesticides dans les aliments (CropLife Europe 2022) b) Démonstration de l'impact biologique accru en fonction du degré d'exposition et mettant en évidence que les altérations fonctionnelles ou la vulnérabilité accrue aux maladies commencent au-delà du LOAEL (Ecobichon 1997).

Le facteur de bioconcentration (BCF): le facteur de bioconcentration (BCF) est une mesure de la tendance d'un composé à s'accumuler dans les tissus des poissons ou d'autres

organismes. L'accumulation biologique est souvent corrélée à la lipophilicité. Pour les produits chimiques organiques, un $\log K_{ow} \geq 3$ indique un potentiel d'accumulation biologique. La stabilité d'un composé est un autre indicateur d'accumulation. Le composé est considéré comme stable lorsqu'une perte de moins de 90 % de la substance originale sur 24 heures via l'hydrolyse est observée (EFSA 2013).

Dose journalière estimée (DJE) : la dose journalière estimée (EDI, Estimated Daily Intake), concerne les quantités totales de pesticides mesurées en milligrammes par kilogramme de poids corporel par jour (mg/Kg pc/j) et est utilisé pour calculer le risque chronique pour la santé des consommateurs. Ce calcul nécessite deux facteurs essentiels : (1) la consommation quotidienne d'aliments pouvant potentiellement contenir la substance active (F_i), (2) la concentration de cette substance active dans la denrée alimentaire (MR_i) et (3) le poids corporel moyen (B_w) (Gu et al. 2021). Elle est calculée grâce à la formule suivante : $EDI = \frac{F_i \times MR_i}{B_w}$

L'apport estimé à court terme : l'Apport Estimé à Court Terme (ESTI, Estimated Short Term Intake) est utilisé pour approximer le niveau d'exposition des consommateurs en se basant sur l'apport maximal signalé en une seule journée et est utilisé pour prédire le risque aigu pour la santé des consommateurs. L'ESTI est quantifié en milligrammes par kilogramme de poids corporel par jour (mg/Kg pc/j) et nécessite deux éléments clés : (1) la quantité maximale quotidienne d'une denrée consommée pouvant inclure la substance active (LP), (2) la teneur maximale des résidus de cette substance active dans cette denrée et (3) le poids corporel moyen (B_w) (Gu et al. 2021). Elle est calculée grâce à la formule suivante : $ESTI = \frac{LP \times HR}{B_w}$

Les Quotients de Risque Chronique et Aigu et l'Indice de Risque : Le Quotient de Risque (HQ), Chronique (cHQ) ou Aigu (aHQ), reflète respectivement le risque pour la santé du consommateur à long terme et à court terme. Il est sans unité. Lorsque le cHQ est < 1 , cela signifie que la consommation à vie d'un produit contenant le niveau mesuré de résidus de pesticides ne pourrait pas poser de risques pour la santé des consommateurs. Pour le aHQ, cela signifie que la consommation à court terme (24h ou moins) d'un produit contenant le niveau mesuré de résidus de pesticides ne pourrait pas poser de risques pour la santé des consommateurs. Enfin, l'Indice de Risque (HI) est la somme de tous les cHQ ou aHQ pour les résidus trouvés dans un produit (Goumenou et Tsatsakis 2019; Gu et al. 2021; Jensen et al. 2022). Les quotients et indice de risque sont calculés par les formules suivantes :

$$cHQ = \frac{EDI}{ADI}; \quad aHQ = \frac{ESTI}{ARfD}; \quad HI = \Sigma HQ$$

Où : cHQ est le quotient de risque chronique ; EDI est la dose journalière estimée ; ADI est dose journalière admissible ; aHQ est le quotient de risque aigu ; ESTI est l'apport estimé à court terme ; ARfD est la dose de référence aiguë ; HI est l'indice de risque et HQ est le quotient de risque.

3. Utilisation des pesticides : Avantages et Défis

L'industrie mondiale des pesticides est devenue l'un des secteurs les plus rentables à l'échelle mondiale. Le volume commercial des pesticides a atteint 5,9 millions de tonnes, équivalant à une valeur de 37,6 milliards de dollars américains en 2018. Les exportations de pesticides sont passées de 1 992 898 millions de tonnes en 2015 à 2 454 480 millions de tonnes en 2019. L'Asie émergeant récemment comme le principal exportateur mondial de pesticides (Aktar, Sengupta, et Chowdhury 2009; Tudi et al. 2021; Tucker, Dumitriu, et Teodosiu 2022).

L'utilisation extensive de pesticides en agriculture pour lutter contre les ravageurs pré- et post-récolte demeure efficace même si une petite proportion des pesticides appliqués sont directement impliqués dans leur action prévue (Pimentel et Burgess 2012). Cependant, une grande majorité des composants actifs sont plutôt laissés sous forme de résidus dans l'environnement (Hwang, Seok, et Kim 2019). Cela est particulièrement vrai pour les résidus provenant de la dérive de pulvérisation ou ceux qui persistent dans les aliments après la récolte. Comme le soulignent (Popp, Pető, et Nagy 2013), la dérive de pulvérisation constitue une préoccupation majeure en ce qui concerne le déplacement non intentionnel des pesticides vers des organismes non ciblés, entraînant des pertes hors cible qui peuvent varier de 50 % à 70 % du pesticide initialement appliqué.

3.1. Voies de transport des pesticides dans l'environnement

Au-delà de leur impact sur le sol et les cultures, les pesticides ont tendance à étendre leur portée à travers l'air, le sol et l'eau, laissant leurs résidus comme des polluants significatifs dans les écosystèmes (Sameut Bouhaik 2009).

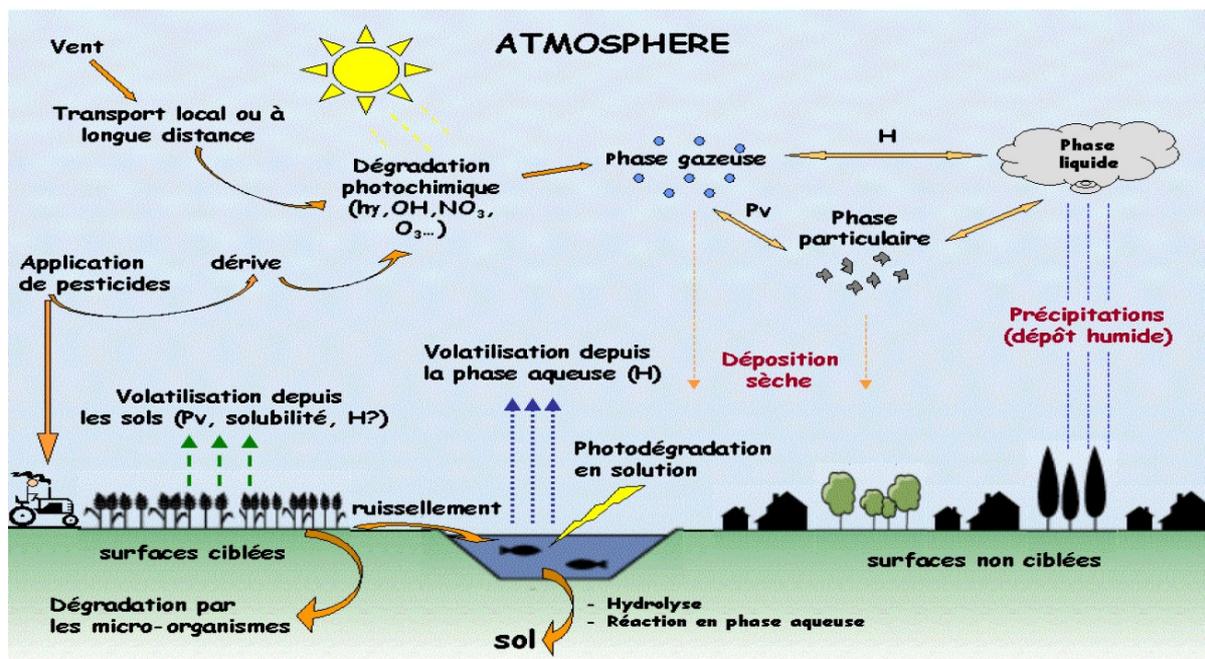


Figure I- 2: Voies de transport des pesticides et destinée dans l'environnement (Sameut Bouhaik 2009)

La figure I-2 illustre le devenir des pesticides d'après Sameut Bouhaik 2009. Socorro et al. (2016) ont exploré huit pesticides : le difénoconazole, le tétraconazole, le fipronil, l'oxadiazon, le deltaméthrine, le cyprodinil, la perméthrine et le pendiméthaline, lorsqu'ils sont enrichis dans la phase particulaire atmosphérique. Leurs conclusions montrent la remarquable capacité à être transportés sur de vastes distances, s'étendant sur des milliers de kilomètres à partir de leurs sites d'application initiaux.

Cette dissémination généralisée ne s'arrête pas à l'air ; elle s'étend aux plans d'eau par l'intermédiaire des précipitations atmosphériques affectant potentiellement la vie aquatique (Jürgens et al. 2016). L'écosystème aquatique sert de réservoir pour les pesticides dissous, comme le montre une étude dans l'écosystème de la rivière South Litani au Liban (Youssef et al. 2015). Cette étude a mis en évidence la détection de certains pesticides interdits tels que l'aldrine, l'heptachlore époxyde et le dichlorodiphényltrichloroéthane (DDT), dans les eaux de surface et les eaux souterraines à des teneurs bien inférieures aux limites autorisées. En effet, la contamination du sol est une préoccupation importante car les pesticides appliqués directement sur le sol pour la protection des cultures peuvent infiltrer le sol par l'écoulement de l'eau, entraînant des interactions telles que l'adsorption, la désorption et la dégradation (Katagi et Tanaka 2016). Sánchez-Bayo, Goka, et Hayasaka (2016) ont souligné la contamination de l'environnement aquatique par les néonicotinoïdes. La concentration

moyenne de tous les néonicotinoïdes dans l'eau dépassait les directives européennes dans 27 % des cas, et 66 à 79 % des cas les directives canadiennes et américaines, respectivement, tandis que les concentrations maximales pouvaient dépasser de 35 % les directives européennes. Selon les auteurs, ces résultats sont préoccupants car ils indiquent que les résidus dans le sol, où la plupart de ces insecticides sont appliqués, s'accumulent au fil des ans (Sánchez-Bayo, Goka, et Hayasaka 2016).

3.2. Aperçu des effets toxicologiques et écotoxicologiques des pesticides

Les résidus de pesticides peuvent subir des processus de bioaccumulation et de biomagnification, qui correspond à l'accumulation de résidus le long de la chaîne alimentaire, où les organismes plus petits sont consommés par des plus grands, ce qui aboutit à l'amplification de la toxicité cumulative, comme montré par Sánchez-Bayo, Goka, et Hayasaka (2016). Par conséquent, la présence de pesticides dans la chaîne alimentaire, l'absorption des pesticides par les organismes, la durée de l'exposition, la dynamique de la toxicité, la dispersion, le métabolisme et l'élimination contribuent collectivement à l'impact sur différentes espèces. Ce phénomène engendre ainsi des conséquences préjudiciables à la fois pour les organismes non ciblés et bénéfiques (Pathak et al. 2022). Sánchez-Bayo, Goka, et Hayasaka (2016) ont mené une exploration approfondie de l'impact des néonicotinoïdes sur les organismes aquatiques, révélant leurs effets neurologiques irréversibles. De plus, l'étude a mis en évidence les conséquences sous-létales des néonicotinoïdes, notamment l'inhibition de l'alimentation, le mouvement altéré, la fécondité réduite, la diminution de la taille corporelle chez les éphémères et les poissons, ainsi que la suppression immunitaire chez les poissons.

De manière similaire, les mélanges ou cocktails composés de divers ingrédients actifs de pesticides et de coformulants peuvent présenter des effets toxiques additifs sur la santé humaine et environnementale, comme souligné dans le rapport de l'OMS de 2018 (WHO 2018). Les pesticides aux fonctions analogues ont la capacité d'exacerber leurs effets néfastes, aboutissant à un préjudice intensifié par le biais de mécanismes tels que l'addition de dose/concentration, l'addition de réponse ou des effets biologiques cumulatifs. Ces interactions peuvent également englober l'augmentation synergique et les effets antagonistes (WHO 2018).

Du côté toxicologique, les résidus de pesticides peut perturber la croissance, le métabolisme, la physiologie et les mécanismes de défense chez l'homme entraînant l'accumulation de toxines dans les organes corporels sur de longues périodes, donnant ainsi lieu à des affections chroniques telles que la neurotoxicité, le cancer, la nécrose, l'asthme, les troubles de la reproduction, les affections cardiaques, le diabète, les dommages génétiques, les malformations congénitales et la perturbation endocrinienne (Aktar, Sengupta, et Chowdhury 2009; Pathak et al. 2022). De plus, de nombreuses études ont documenté des cas d'exposition des nourrissons aux résidus de pesticides par le biais du lait maternel, qui sert de bioindicateur environnemental, accumulant des pesticides organochlorés et pouvant potentiellement perturber le développement néonatal (Limon-Miro et al. 2017; Salama 2017; Mehta et al. 2020; Mekonen et al. 2021; Qi et al. 2022).

3.3. Importance des normes et des réglementations et organismes impliqués

L'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO), a mis sur-place les Bonnes Pratiques Agricoles (BPA) pour garantir une utilisation des pesticide sûrs tout en protégeant la santé humaine et environnementale. Le respect des BPAs assure aussi la production d'aliments sains tout en préservant simultanément la viabilité économique et la stabilité sociale. En effet, au cœur des BPAs vient l'utilisation de la dose recommandée par hectare, telle qu'indiquée sur les étiquettes des produits et le respect des DARs qui contribuent à la diminution des résidus de pesticides aux seuils autorisés (LMRs) et même plus bas, et par la suite ne présentant ainsi aucun risque pour la santé humaine ou l'environnement (Li 2022). Les normes internationales sont établies par la Commission du Codex Alimentarius (CAC), fournissent un code de pratique (CAC/RCP 1-1969, Rev. 4-20031) par l'Autorité européenne de sécurité des aliments (EFSA) et l'Agence de protection de l'environnement (EPA). Ainsi, l'EFSA recommande des réglementations relatives aux résidus de pesticides dans les aliments européens en vertu de divers règlements, notamment le Règlement (CE) n° 178/2002, le Règlement (CE) n° 396/2005 et le Règlement (CE) n° 1107/2009. Parallèlement, l'EPA aux États-Unis réglemente les LMR, appelées "tolérances", en vertu du Federal Food and Cosmetic Act (FFDCA) et du Code of Federal Regulations (CFR) Title 4, Part 180. Pour garantir le respect des LMR, les autorités locales et internationales mènent régulièrement des campagnes de surveillance des résidus pour identifier les risques potentiels pour la sécurité alimentaire et environnementale (Shokoohi et al. 2019). Les données de surveillance aident les

autorités à prévenir ou à atténuer ces risques et peuvent également mettre en lumière d'éventuelles lacunes dans le cadre réglementaire et aider à prendre des décisions fondées sur des données probantes concernant les actions de gestion des risques, telles que l'établissement, la modification ou l'harmonisation des LMR (EFSA 2022). Ces campagnes sont menées en utilisant des stratégies d'échantillonnage basées sur le risque ou peuvent être effectuées dans le cadre d'une surveillance ciblée avec des objectifs d'échantillonnage limités (Ambrus et al. 2023).

3.4. Les résidus de pesticides dans les aliments

Les résultats des efforts de surveillance mondiaux et locaux, ainsi que des études examinant la dissipation des résidus dans divers produits alimentaires sont présentés ci-dessous. Cela englobe une perspective internationale et met l'accent sur le contexte libanais. La partie suivante aborde également la limitation de la recherche spécifiquement axée sur les feuilles de vigne.

En 2022, Jensen et al. ont mené une étude d'évaluation des risques alimentaires cumulatifs des pesticides au Danemark. Sur la période 2012–2017, leur étude a utilisé des données du Programme de surveillance des pesticides danois, impliquant 13 492 échantillons de 200 groupes de produits. Des résidus ont été détectés dans 116 groupes, révélant la contribution relative des pesticides à l'indice de risque. Pour les enfants de 4 à 6 ans, cinq substances ont dépassé un HQ de 0,01, dont l'aldrine/dieldrine, le chlorpyrifos, le dichlorvos, les dithiocarbamates et le pirimiphos-méthyl. Les adultes avaient également trois substances dépassant 0,01 : l'aldrine/dieldrine, le chlorpyrifos et les dithiocarbamates (Jensen et al. 2022).

En 2023, Zhang et al. ont examiné 208 pesticides dans les menthes comestibles chinoises. Parmi les 16 résidus détectés, huit étaient des fongicides, six étaient des insecticides et deux étaient des herbicides. Le difénoconazole était le plus courant, détecté dans 25 % des échantillons de menthe dont 53% contenaient au moins un résidu de pesticide. L'évaluation des risques à l'aide des quotients de risque chroniques (cHQ) et des quotients de risques aigus (aHQ) a donné des valeurs inférieures à 1, indiquant des niveaux de risque sanitaire acceptables pour les adultes et les enfants (Zhang et al. 2023) . De même, Yang et al. (2017) ont rapporté des résultats comparables pour la myrte (*Myrica rubra*) en Chine, trouvant des résidus de pesticides détectables dans 63 % des échantillons mais concluant à l'absence de

risques pour la santé. Schusterova et al. (2021) ont trouvé un cocktail de résidus de pesticides (>12) et leurs métabolites (>20) dans 22 échantillons testés de raisins et de vins sur le marché tchèque, mettant en évidence plus de résidus de fongicides que d'insecticides. En Arabie saoudite, Ramadan et al. (2020) ont évalué les résidus de pesticides dans les légumes de la région d'Asir, avec 68,7 % des échantillons contenant des résidus détectables, dépassant ou non les limites maximales de résidus de l'Union européenne. Notamment, les piments et les concombres dépassaient fréquemment les LMRs. Nougadère et al. (2020) ont évalué l'exposition chronique des nourrissons et des jeunes enfants français aux résidus de pesticides. Leur résultats ont montré que 67 % des échantillons contenaient des résidus avec 78 différentes molécules distinctes détectées. Ces investigations soulignent l'importance mondiale de la surveillance et de l'évaluation des résidus de pesticides dans les produits alimentaires pour garantir la sécurité de santé des consommateurs.

Études sur les résidus de pesticides au Liban

L'étude de Kfoury, Hilan, et El-Amil (2002) menée sur les fraises et couvrant différentes régions du Liban tout au long de la saison de récolte, a révélé des substances non autorisées telles que le fénamiphos et le propamocarbe ainsi qu'un autre pesticide non approuvé pour les fraises, le fenarimol, dépassant les LMRs. En 2016, Nasreddine et al. ont mené une évaluation de l'exposition alimentaire aux pesticides chez les adultes libanais, en considérant 47 résidus de pesticides. Ils ont constaté que les niveaux d'exposition à la plupart des pesticides étaient cohérents avec les résultats mondiaux. L'étude de Bou-Mitri et al. (2022) a examiné les teneurs de glyphosate dans différents types de pain et de farine disponibles dans la région du Mont-Liban. Le glyphosate, un herbicide largement utilisé, a été retrouvé dans 80 à 100 % des divers échantillons. Bien que les concentrations de glyphosate dans tous les échantillons soient inférieures aux LMRs établies, l'étude a conclu que les consommateurs libanais sont exposés à des niveaux de glyphosate par la consommation de pain dans des limites acceptables. Smadi, Jammoul, et El Darra (2019) ont évalué des échantillons de lait maternel de mères réfugiées syriennes dans les camps du Nord du Liban. Parmi 120 échantillons, seulement 5 % contenaient des résidus de pesticides. Les pesticides les plus détectés étaient le lufénuron, le méthamidophos et le chlorpyrifos, avec une concentration maximale de 13 µg/L pour le méthamidophos dans un échantillon. Le niveau de contamination relativement faible était attribué à l'exposition maternelle aux fruits et

légumes. El Hawari et al. (2019) ont évalué les résidus de pesticides dans les pommes libanaises de 2012 à 2016. Parmi 212 échantillons, 23 % ne contenaient aucun résidu détectable, tandis que 77 % présentaient des résidus, dont 61 % dépassaient les LMRs. Les pesticides fréquemment détectés comprenaient le chlorpyrifos, le méthidathion, le cyperméthrine, le lambda-cyhalothrine, le myclobutanil et le diazinon. Les calculs ont indiqué que le Quotient de Risques (HQ) pour une exposition à long terme variait de 0,1 % à 8 % de la dose journalière admissible (DJA), suggérant un risque minimal pour la santé humaine. L'étude menée par Abou Zeid et al. (2021) a fourni des informations sur la dynamique des résidus de pesticides dans les nèfles. Sur 128 échantillons, 51 présentaient des irrégularités dans les résidus détectés, avec des pesticides non autorisés, comme le cyperméthrine et le diméthoate étant les plus courants. L'étude a également déterminé les cinétiques de dissipation du penconazole dans les pommes et les nèfles, proposant des durées avant récolte (DAR) sûres et des LMRs. L'enquête de Khazaal et al. (2022) a exploré les résidus de pesticides dans divers aliments d'origine végétale collectés à Beyrouth. Sur 478 échantillons représentant 49 produits alimentaires, 32,2 % présentaient des résidus détectables. Plus de la moitié des échantillons positifs dépassaient les LMRs de l'Union européenne. Bien que les valeurs des quotients de risques indiquent généralement des niveaux d'exposition sûrs, le chlorpyrifos dans les concombres a présenté une exception avec une valeur de HQ de 1,79. Ces résultats soulignent l'importance d'une surveillance continue en matière de résidus dans les denrées de l'industrie libanaise.

Études sur les résidus de pesticides dans les feuilles de vigne

Dans la littérature, il existe peu d'études portant sur les feuilles de vigne, principalement dans les pays ayant des intérêts culinaires pour cette denrée alimentaire comme le Liban, la Turquie, l'Égypte, la Palestine et la Syrie. Dans ce contexte, nous présentons un aperçu des études disponibles malgré les difficultés linguistiques étant donné que plusieurs études ont été publiées en turque (Ertürk 2009; Cangi et al. 2014; Gülcü et Demirci 2014; Yanar, Cangi, et Ozata 2015; Şensoy, Ersayar, et Doğan 2017; Turkoz Bakirci 2018; Kutlu Kuşaksiz et Çimer 2019). Nous soulignons une augmentation notable des activités de recherche depuis l'année 2018, sur les résidus de pesticides dans les feuilles de vigne par les communautés scientifiques de ces pays afin d'élucider leur devenir dans cette matrice et d'aboutir à des résultats concrets pouvant contribuer à proposer une liste des LMRs et promouvoir le commerce

international de cette denrée.

Teneurs en résidus des feuilles de vigne et des raisins : effet des pratiques agricole

En 2004, Sama'neh, a étudié l'impact de nombre de six pulvérisations et des délais après pulvérisation (de 1 à 14 jours) sur les teneurs dans les raisins et feuilles de vigne en résidus de chlorpyrifos et de penconazole avec un taux d'application de 0,96 et 50 mg/L respectivement. L'auteure a montré que les teneurs en résidus des deux pesticides ont augmenté avec le nombre de pulvérisations. Dans les feuilles de vigne, les teneurs en résidus des deux molécules ont passé de 2,74 à 9,93 µg/kg et de 15,01 à 51,56 µg/kg pour le penconazole et le chlorpyrifos respectivement. Dans les pellicules des raisins de 2,46 à 12,04 et de 53,27 à 157,03 µg/kg pour les deux pesticides. Tandis que dans les pulpes les teneurs en résidus ont passé de 0,27 à 0,44 et de 0,39 à 1,19 µg/kg chlorpyrifos. Shokr, Nasr, et Mahmoud (2015) ont montré que les dépôts initiaux de fénarimol et de flusilazole ont été respectivement 12 et 5 fois plus élevés sur et dans les feuilles de vigne que dans les raisins. Ces auteurs ont trouvé que la dissipation des deux molécules a été 5 et 9 fois plus rapide dans les raisins que dans les feuilles de vigne, respectivement, pour les deux molécules testées. Salama (2009) a obtenu des résultats similaires avec l'utilisation de l'azoxystrobine, du lambda-cyhalothrine, du fénoxéthanol et du cyprodinil et la dégradation des molécules actives a été plus marquée dans les raisins que dans les feuilles de vigne. D'une manière comparable, Arora et al. (2009) ont trouvé que les feuilles de vigne montrent des dépôts initiaux beaucoup plus élevés que les raisins. En effet après quatre applications de Confidor 200SL aux doses de 400 et 800 ml/ha, les dépôts initiaux d'imidaclopride sur les feuilles de vigne ont été observés à 10,01 et 16,97 mg kg⁻¹ aux doses unique et double respectivement, tandis que dans les raisins une concentration de 0,05 mg/kg et 0,06 mg/kg pour les doses unique et double respectivement. Des résultats semblables ont été obtenus par Jyot et al. (2010) où le doublement du taux d'application de la trifloxystrobine et de la tébuconazole entraîne une augmentation des dépôts initiaux ainsi que des demi-vies des molécules dans les feuilles de vigne. Les travaux de Mohapatra et al. (2010) ont également mis en évidence une augmentation des durées de demi-vies de même ordre de grandeur lorsque le fipronil a été appliqué à double dose. Les résultats de l'étude de Maestroni et al. (2018) ont indiqué que les taux de dissipation pour toutes les doses appliquées (chlorpyrifos, 2,4 et 0,67 kg/ha ; chlorpyrifos-méthyl, 4 et 11,2 kg/ha ; diazinon, 3 et 0,84 kg/ha ; diméthoate, 2 et 0,56 kg/ha), entraînant des demi-vies allant

de 2,9 à 3,9 jours dans les feuilles de vigne. Pour respecter les LMRs actuelles et minimiser les risques pour les consommateurs, la dose d'application recommandée nécessitait des Durées Avant Récoltes (DAR) de 8,9 à 37,1 jours avant la consommation. Récemment, Balkan et Kara (2023) ont montré que la dissipation des résidus de cyperméthrine, boscalide, deltaméthrine, kresoxim-méthyl, lambda-cyhalothrine, metalaxyl-M, métrophénone et triadiménol dans les feuilles de vigne a abouti à des demi-vies comprises entre 1,85 et 7,22 jours. Ils ont également conclu que le processus de dégradation varie selon que les applications de pesticides ont été faites de manière individuelle ou en mélange et cela est influencé par la nature des molécules. Par conséquent, ils recommandent de ne pas utiliser de mélanges de pesticides dans le champ sans avoir des informations détaillées sur leurs ingrédients.

Etat de connaissances sur les méthodes analytiques pour l'analyse des résidus de pesticides dans les feuilles de vigne

Patil et al. (2018) ont optimisé et validé une méthode d'extraction basée sur la technique QuEChERS (Anastassiades et al. 2003) pour un mélange de 363 pesticides, en utilisant la chromatographie en phase gazeuse et liquide couplée à la spectrométrie de masse en tandem (GC-MS/MS et LC-MS/MS) avec des limites de quantification (LOQ) inférieures aux LMRs de l'UE. De manière similaire, Maestroni et al. (2018) ont validé une méthode SweEt/QuEChERS modifiée et miniaturisée pour l'analyse de 59 résidus de pesticides dans les feuilles de vigne par GC/MSMS. De même, Hayar, Zeitoun, et Maestroni (2021) ont validé une méthode QuEChERS sur les feuilles de vigne pour l'analyse de 33 pesticides, couvrant 17 groupes chimiques distincts couramment utilisés dans la production de raisin. Récemment, Zorlu Ünlü et al. (2023) ont validé une méthode d'analyse des résidus dans les feuilles de vigne pour 243 pesticides par LC-MS-MS.

Surveillance des résidus de pesticides dans les feuilles de vigne au cours de différentes campagnes d'échantillonnages

Turkoz Bakirci (2018) ont fait l'analyse quantitative de 327 résidus de pesticides présents dans 666 feuilles de vigne marinées collectées dans la région égéenne de la Turquie entre 2014 et 2016. Les résultats de l'étude ont révélé que, sur les échantillons analysés, 173 contenaient des résidus de pesticides détectables dont 124 échantillons dépassaient les LMR turques. L'azoxystrobine, en particulier, a été le résidu le plus courant dépassant les LMR, tandis que le métalaxyl a été fréquemment détecté dans les feuilles de vigne marinées. En 2018, Din,

Azab, et Shalaby (2018) ont évalué les niveaux de résidus de pesticides dans un total de 96 échantillons de raisins et de feuilles collectés en 2012 et 2013 dans diverses régions égyptiennes. Les résultats ont révélé que tous les résidus de pesticides détectés dans les échantillons de raisins ou de feuilles dépassaient les LMR. Dans les feuilles, l'oxamyl a été le plus fréquemment détecté suivi par le chlorpyrifos (Din, Azab, et Shalaby 2018). Hayar, Zeitoun, et Maestroni (2021), ont examiné les résidus de pesticides dans 17 marques de feuilles de vigne disponibles sur le marché libanais, appartenant à trois méthodes de conservation différentes. Les feuilles de vigne séchées ont présenté une contamination plus élevée en résidus de pesticides par rapport aux feuilles conservées dans de la saumure ou sous forme de feuilles de vigne farcies. Parmi les divers composés phytosanitaires couramment utilisés dans la production de raisin, les fongicides systémiques ont été les plus fréquemment détectés. En 2022, Hamzawy, a réalisé une évaluation de 400 pesticides présents dans les feuilles de vigne (*Vitis vinifera L.*) disponibles sur le marché égyptien. Les résultats de cette analyse ont révélé que, sur les échantillons testés, 78 contenaient des traces de 36 résidus de pesticides distincts provenant de différents groupes chimiques, dépassant les LMRs établies par l'UE. Parmi les pesticides détectés, les fongicides étaient les plus couramment présents (59 %).

4. Facteurs influençant les résidus de pesticides

Dans l'ensemble de ce que nous avons exposé, le suivi de résidus de pesticides et leurs dépôts initiaux ont été réalisés sur les deux matrices baies de raisins et feuilles de vigne pour évaluer les effets des paramètres physico-chimiques des molécules et de la physiologie des plantes et leurs conséquences sur leur dissipation et l'estimation des durées avant la récolte (DAR). Dans ce qui suit, l'accent sera mis sur l'effet de types de matrices, de formulations, et des procédés du lavage et de conservation sur les teneurs en résidus.

4.1. Effet des types formulations des produits phytosanitaires

Dans les formulations de pesticides, des composés dits « inertes » sont ajoutés aux substances actives pour améliorer l'efficacité des substances actives, optimiser et performer leur épandage. Ces composés "inertes" jouent un rôle crucial dans la dilution, la dispersion, la stabilité et la dégradation des molécules (Fader et Bukovac 1997; Leifer 1997; Tann 1997; Cox et Sorgan 2006). Une légère modification de la composition en inertes peut entraîner ou

non un changement dans les d'effets de formulations en matière résiduelle et par la suite dans la fixation des délais avant récoltes (Fader et Bukovac 1997).

Montemurro et al. (2002) ont signalé des dégradations plus ou moins rapides de la substance active chlorpyrifos quand elle est appliquée sous forme de formulations concentrée émulsifiable (EC) et granulés mouillables (WG) en comparaison à la formulation microcapsules (ME) où les teneurs de résidus demeurent stables dans les oranges, les feuilles et le sol. De même, Buzzetti (2017) a mis en évidence que la formulation influence considérablement l'efficacité et les dépôts initiaux des substances actives de l'acétamipride (WP et SP), de l'imidaclopride (SL, SC et WP) et de la diazinon (EW et WP), à l'exception des deux formulations du lambda-cyhalothrine (CS et EC). Zhou et al. (2014) ont constaté que le type de formulation affecte les dépôts initiaux et les résidus de rotenone sur le chou et le sol mais ne modifie pas la demi-vie de la substance active. A l'inverse, Abou Zeid et al. (2020) ont montré qu'il n'y a pas de différence significative entre les taux de dissipation du penconazole 10% EC de différents fabricants commerciaux appliqués sur les tomates. Mais Abdel-Hamid, El-Sayed, et Ahmed (2013) ont mis en évidence que la fenpyroximate persiste davantage dans les tomates lorsqu'elle est pulvérisée sous forme formulée EC que sous forme de formulation SC. Il en est de même pour l'imidaclopride où la dégradation a été plus rapide lorsqu'elle est appliquée sous forme SC en comparaison à d'autres types de formulations (SL, WP, WDG). Par ailleurs, Alister et al. (2017) ont constaté que les formulations de pesticides tels que le buprofézine (SC et WP), la fenhexamid (SC et WP), et l'acétamipride (SL et WP) n'avaient aucun effet significatif sur le dépôt initial et le taux de dissipation des substances actives dans les raisins et les pommes. Cependant, ils ont mis en évidence que c'est le stade de croissance du fruit au moment de l'application et le type de matrice qui ont une influence notable sur les taux de dissipation des pesticides.

4.2. Effet du type de matrice sur les résidus de pesticides

Selon Fernández et Eichert (2009) et Lichiheb et al. (2015), la pénétration des pesticides dans les plantes est influencée par la perméabilité de la cuticule des feuilles et la lipophilie des pesticides. De même, Possingham et al. (1967) ont montré que la composition chimique et la structure de la cire cuticulaire à la surface des plantes de *Vitis vinifera* influencent également la pénétration des pesticides et peuvent varier en fonction du type de fruit et du stade de croissance. Ces auteurs ont mis en évidence que la cire cuticulaire joue un rôle dans la

régulation de la pénétration des insecticides en limitant la mobilité des solutés en fonction de leur lipophilie et de leur solubilité. De même, Edwards (1975) a montré la nature et la morphologie des plantes influencent la distribution, la rétention et l'absorption des pesticides dans les tissus végétaux. En examinant en temps réel *in situ* la pénétration et la persistance des fongicides, en particulier le thiabendazole et le ferbam, sur les feuilles de basilic, Yang et al. (2017) ont révélé que les feuilles vivantes présentaient un schéma de pénétration des pesticides plus rapide et plus profond par rapport aux feuilles récoltées due à la transpiration plus élevée dans les feuilles vivantes. De plus, les feuilles de basilic plus grandes et plus anciennes (45 jours) ont permis une pénétration plus profonde des pesticides que les petites et jeunes feuilles (15 jours). Aussi, les feuilles intactes ont permis une plus grande pénétration des pesticides que les feuilles endommagées en raison de la transpiration altérée et de l'augmentation des activités enzymatiques dans ces dernières.

4.3. Effet du lavage et du mode de conservation sur les résidus de pesticides

De nombreuses études se sont penchées sur la dissipation des résidus de pesticides dans les denrées alimentaires, de manière à aboutir à une méthode de conservation industrielle efficace pouvant réduire la demi-vie de ces résidus. Bajwa et Sandhu (2014) ont réalisé une revue sur l'impact du conservation, de la transformation et du lavage sur les résidus de pesticides dans les denrées alimentaires. Ces chercheurs ont conclu que la quantité de résidus de pesticides dans le produit final dépendait de plusieurs facteurs dont le type de molécule, son emplacement, la denrée concernée, la préparation et les étapes de conservation du produit (Bajwa et Sandhu 2014).

Corrias et al. (2020) ont mis en évidence que l'élimination des résidus dépend des propriétés physico-chimiques des molécules et du type de cuticule de la matrice. En comparant les différents procédés de lavage, Lozowicka et al. (2016) ont montré une élimination des 16 résidus de pesticides dans les fraises par l'ozonation (36-75%) et par ultrasons (90%), en comparaison aux méthodes conventionnelles du lavage à l'eau du robinet (20-68%) et à l'ébullition (42-92%). Une autre étude pertinente est celle de Nowowi et al. (2016), qui ont examiné l'efficacité de cinq solutions de nettoyage (une solution de soda-sel, solution de vinaigre, solution de jus de tamarin, solution de farine filtrée et eau du robinet) pour éliminer le chlorpyrifos du chou-fleur. Pour les feuilles de vigne, Shokr, Nasr, et Mahmoud (2015) ont révélé que le processus d'ébullition était très efficace pour éliminer les résidus de fénarimol

dans les feuilles de vigne par rapport aux résidus de flusilazole. La réduction des résidus de fénarimol et de flusilazole dans les feuilles due au processus d'ébullition a été de 47,06 et 24,76 % pour les feuilles cueillies un jour après la pulvérisation. Les teneurs en résidus ont passé de 0,34 et 0,21 dans les feuilles fraîches à 0,180 et 0,158 mg/kg dans les feuilles bouillies, pour les 2 molécules respectivement. De même, les résultats de l'expérience de Maestroni et al. (2018) incluant le lavage des feuilles de vigne, à l'eau froide et chaude, ont révélé que les propriétés physico-chimiques des pesticides étudiés (chlorpyrifos, chlorpyrifos-methyl, diazinon and diméthoate) influençaient principalement l'ampleur de la réduction des pesticides. Le lavage à l'eau chaude s'est avéré très efficace pour le diméthoate, un pesticide polaire et soluble dans l'eau, entraînant une réduction significative de 92 % des niveaux de résidus. Cependant, pour le chlorpyrifos, le composé le plus apolaire de l'étude, aucun effet significatif de réduction n'a été observé. Autrement, l'étude menée par Cangı, Yanar, et Dülgeroğlu (2019) en Turquie a examiné l'impact de deux méthodes de préservation : l'une consistait à placer directement les feuilles de vigne dans des bocaux avec une solution saline (8,0 % de sel + 0,25 % d'acide lactique) et l'autre à laver les feuilles de vigne à l'eau chaude avant de les placer dans les bocaux avec la saumure. Au cours de la recherche, les cinq fongicides étudiés étaient l'azoxystrobine, le triadimenol, l'hexaconazole, le chlorure de cuivre et le métalaxyl + mancozèbe. Les techniques de préservation ont entraîné une réduction des niveaux de résidus de fongicides et de cuivre, le marinage après lavage à l'eau chaude entraînant une réduction des niveaux de résidus de fongicides entre 75,2 % et 99,2 %. L'étude déconseillait l'utilisation de fongicides systémiques dans les vignobles où sont produites des feuilles de vigne marinées, recommandant à la place l'utilisation de fongicides de contact en vue d'adapter les pratiques agricole pour produire les raisins et les feuilles de vigne en même temps.

Pour choisir le procédé le plus approprié, de nombreux autres auteurs ont testés plusieurs méthodes d'élimination de résidus de pesticides et ont étudié les divers facteurs pouvant influencer les teneurs en résidus dans diverse matrices : Hwang, Seok, et Kim (2019) ont exploré le rôle des cires cuticulaires dans l'élimination des résidus de pesticides des pommes. Wu et al. (2019) ont étudié l'élimination des résidus de pesticides dans le Kumquat, les épinards et les concombres. Gao et al. (2020) sur les feuilles de thé, Yildirim Kumral et al. (2020) sur les olives noires, Acoglu et Omeroglu (2021) sur les oranges et Reddy et al. (2022) sur les raisins. D'autres études (tableau I-1) ont souligné l'importance de recourir à diverses

stratégies de traitement et de manipulation, seule ou en combinaison, pouvant réduire les teneurs de résidus de pesticides dans diverses matrices.

Tableau I- 1: effet du traitement sur les résidus de pesticides dans diverses matrices

| Matrice | Type de traitement | Molécule | % de réduction des résidus | Conclusions | Références |
|-------------------------|---|-------------------------|--|---|--|
| Fraises | (I) lavage à l'eau du robinet | Azoxystrobine | (I) ~45% (II) 45% | <ul style="list-style-type: none"> - Aucune corrélation entre la solubilité de la molécule et la diminution des résidus. - Le détergent dissout les cires, réduisant ainsi les résidus en dissolvant les pesticides à l'intérieur des cires épicuticulaires. | (Angioni et al. 2004) |
| | (II) lavage avec une solution de détergent végétal | Fenhexamide | (I) 43% (II) 60% | | |
| | | Pyriméthanil | (I) 0% (II) 47% | | |
| Olives | Lavages hebdomadaires à l'eau pendant 64 jours | Diuron | diuron, l' α -endosulfane et la térbuthylazine 85%. β -endosulfane 100 % simazine 48 %. | <ul style="list-style-type: none"> - Les résidus deviennent de plus en plus retenus par les cires cuticulaires avec le temps. - L'efficacité du lavage ne dépend pas de la solubilité des molécules, mais de leur coefficient Kow. - Les coefficients de partition entre la cuticule et l'eau présentent une corrélation significative avec les coefficients de partition octanol/eau. | (Guardia-Rubio, Ayora-Cañada, et Ruiz-Medina 2007) |
| | | Térbuthylazine | | | |
| | | Simazine | | | |
| | | α -endosulfane | | | |
| | | β -endosulfane | | | |
| Feuilles de piment fort | (I) Lavage à l'eau (II) Lavage + blanchiment (III) Lavage + blanchiment + séchage à l'air chaud | Dichlofluanide | (I) 84% (II) 100% (III) 100% | <ul style="list-style-type: none"> - Différents types de pesticides demeurent dans les feuilles de poivre séchées à des concentrations très faibles après le traitement - Il est nécessaire de déterminer les pesticides résistants à l'élimination dans les conditions de traitement. - Les propriétés lipophiles et systémiques d'une molécule influencent leur élimination. - Le changement de poids doit être pris en compte lors du calcul des ratios d'élimination. | (Lee et Jung 2009) |
| | | Flusilazole | (I) 76% (II) 88% (III) 13% | | |
| | | Folpet | (I) 91% (II) 100% (III) 100% | | |
| | | Iprodione | (I) 80% (II) 88% (III) 21% | | |
| | | λ -Cyhalothrine | (I) 70% (II) >91% (III) 85% | | |
| | | Lufénuron | (I) 27% (II) 52% (III) 0% | | |
| Fruits d'okra | (I) Lavage à l'eau (II) Ébullition (III) Lavage + ébullition | Fenazaquin | (I) 8 - 32% (II) 9 - 40% (III) 16- 61% | <ul style="list-style-type: none"> - La combinaison de lavage et d'ébullition est plus efficace. - Avec le temps, moins de résidus peuvent être | (Duhan et Kumari 2011) |

| | | | | | |
|------------|--|-----------------|------------------------------|---|------------------------|
| | (depuis le jour 0 jusqu'au jour 15 après le traitement à dose unique et double) | | | éliminés car ils pénètrent à l'intérieur du fruit. | |
| Courgettes | (I) Lavage (II) Épluchage (III) Cuisson | Azoxystrobine | (I)80% (II)90% (III) 0% | - L'azoxystrobine pénètre plus facilement dans la chair de la courgette, tandis que l'acrinathrine et le kresoxim-méthyl restent dans la pelure. - Il est important de prendre en compte la perte d'eau/concentration de la courgette pendant la cuisson dans les calculs de facteur de réduction. | (Aguilera et al. 2012) |
| | | Acrinathrine | (I)70% (II)100% (III)30-40% | | |
| | | Kresoxim-méthyl | (I)100% (II)100% (III)30-40% | | |
| Tomates | (I) Lavage à l'eau (II) Lavage avec du bicarbonate (III) Lavage avec du vinaigre | Acétamipride | (I)59% (II)67% (III)93% | - Aucune corrélation entre la solubilité et l'efficacité du nettoyage. - Les groupes ionisables ont plus d'influence que la solubilité sur l'efficacité du lavage. - Les coefficients de partition entre la cuticule et les traitements de lavage sont bien corrélés avec le Kow des pesticides. -Le lavage à l'eau ou avec d'autres solutions, ainsi que l'épluchage avant la consommation, sont recommandés pour réduire les résidus de pesticides dans les tomates. | (Andrade et al. 2015) |
| | | Azoxystrobine | (I)73% (II) 46% (III) 69% | | |
| | | Diflubenzurone | (I)74% (II)35% (III)41% | | |
| | | Diméthoate | (I)53% (II) 62% (III) 43% | | |
| | | Fipronil | (I)9% (II)3% (III) 0% | | |
| | | Imidaclopride | (I)58% (II)62% (III)71% | | |
| | | Procymidone | (I)0% (II)15% (III)44% | | |
| | | Thiaméthoxame | (I)65% (II)72% (III)78% | | |

5. Cinétiques de dissipation des résidus de pesticides

Les taux de dégradation des substances actives et de leurs métabolites figurent parmi les paramètres les plus importants pour évaluer la persistance des pesticides et l'exposition environnementale. Les études sur le terrain utilisent couramment le modèle cinétique de dissipation du premier ordre et les demi-vies associées pour estimer les niveaux de résidus dans les cultures récoltées (Li 2023). En effet, de nombreuses études sur le terrain ont montré que la dissipation des résidus suit le modèle du premier ordre. Gajbhiye et al. (2011) ont montré que la dissipation de l'azoxystrobine dans le sol suivait une cinétique de premier ordre avec une demi-vie moyenne de 8,1 jours à la dose recommandée d'application. Maestroni et al. (2018) ont démontré que la dissipation des résidus de chlorpyrifos, chlorpyrifos-méthyl,

diazinon et diméthoate sur les feuilles de vigne suivait une cinétique de premier ordre. Dans les feuilles de vigne, Balkan et Kara (2023) ont montré que la dissipation des résidus de cyperméthrine, boscalide, deltaméthrine, kresoxim-méthyl, lambda-cyhalothrine, metalaxyl-M, métrophénone et triadiménol suivait une cinétique de premier ordre. De plus, dans l'étude de Mohapatra et al. (2019) sur les grenades, une cinétique de réaction de premier ordre a été observée pour trois insecticides, avec des demi-vies de dégradation de 8 à 11,1 jours pour l'imidaclopride, 7,4 à 8,4 jours pour l'indoxacarbe et 9,8 à 14,2 jours pour le thiaméthoxame. Les cinétiques de premier ordre sont généralement décrites par l'équation suivante :

$$C_t = C_0 e^{-kt} \quad (1)$$

où C_t désigne la concentration résiduelle au temps d'échantillonnage t , C_0 indique la concentration initiale, et k représente le taux de dissipation de la molécule ainsi que la pente de la courbe de régression exponentielle utilisée pour calculer la demi-vie. Ensuite, la demi-vie des molécules peut être dérivée à partir de celle-ci en utilisant l'équation suivante:

$$DT_{50} = \frac{\ln 2}{k} \quad (2)$$

Il est crucial de noter que la dissipation des pesticides ne suit pas systématiquement des cinétiques du premier ordre dans les études sur le terrain. Il a également été démontré que cela s'applique aux dissipations dans des conditions post-récolte, y compris pendant le traitement et le stockage des aliments. Dans son travail sur la cinétique chimique dans le traitement des aliments, Singh (1996) a affirmé que de nombreuses réactions chimiques qui se produisent pendant le traitement et le stockage des aliments suivent des cinétiques du premier ordre. La constante de vitesse pour une réaction du premier ordre peut être obtenue en prenant d'abord le logarithme naturel des concentrations, puis en traçant contre le temps sur une échelle ordinaire. Lu et al. (2013) ont également appliqué la cinétique du premier ordre pour suivre la dissipation des molécules (éthoprophos, diméthoate, sumithion, malathion, chlorpyrifos, isocarbophos, méthidathion, tétrachlorvinphos, profénofos et triazophos) dans les choux marinés durant la période de conservation. De même, Rahimi et al. (2015) ont étudié la dissipation du malathion dans des extraits aqueux à 5 % de divers fruits et légumes. Leur travail a révélé que la dissipation du malathion suivait une décroissance exponentielle double du premier ordre dans les échantillons de fruits/légumes, tandis qu'un modèle cinétique du premier ordre simple décrivait sa dissipation dans le tampon de contrôle. Bo, Zhang, et Zhao (2011) ont utilisé la cinétique du premier ordre pour étudier la

cinétique de dégradation des pesticides organophosphorés dans le lait pendant le traitement du yaourt. Autrement, Shokoohi et al. (2021) ont exploré la dissipation des résidus de chlorpyrifos dans les tomates dans des conditions domestiques à température ambiante, révélant que la dissipation suivait une cinétique du premier ordre. Ces études diverses soulignent collectivement l'applicabilité des cinétiques du premier ordre pour élucider la dynamique des réactions chimiques sous divers scénarios liés aux aliments.

6. Conclusion du chapitre

Cette partie a mis en évidence la diversité des résidus de pesticides et a abordé les facteurs qui influencent leurs teneurs dans les différentes matrices. On y a exposé les travaux de recherche effectués sur l'effet des types de formulations et les taux d'applications de pesticides sur leurs teneurs dans les feuilles de vigne ainsi que les méthodes de leur élimination par les différents procédés de lavage et de conditionnement. Les chapitres suivants chercheront à élucider le mécanisme de dissipation des résidus dans les feuilles de vigne dans des conditions variables, notamment sur le terrain, pendant la conservation et après différents processus de lavage.

CHAPITRE II

Surveillance des niveaux de résidus de pesticides dans les raisins de table libanais de 2012 à 2014 et évaluation des risques pour la santé humaine

Les résultats obtenus dans ce chapitre feront l'objet de l'article suivant à soumettre à la revue « Food Additives & Contaminants: Part B » - Taylor & Francis Online : Majed L., Hayar S. & Dousset S. 2024. Monitoring of pesticide residue levels in Lebanese table grapes from 2012-2014 and human health risk assessment. Food Additives & Contaminants: Part B, X-X. <https://doi.org/10.1080/XXXXX>

Résumé synthétique

L'absence d'études sur les teneurs en résidus de pesticides dans les raisins et les feuilles de vigne produits au Liban constitue un défi important, limitant notre compréhension des pratiques phytosanitaires en vigne au Liban. Ce chapitre présente une analyse qualitative et quantitative des données de surveillance nationale sur les teneurs en résidus de 80 pesticides dans les raisins de 2012 à 2014 obtenues par le ministère de l'Agriculture libanais. Ces données proviennent d'échantillons de raisins prélevés des principales régions du Liban (Bekaa, Nord, Mont-Liban et Sud). En 2012, 2013 et 2014, respectivement, un total de 588, 425 et 581 échantillons ont été collectés. Les résidus de pesticides des raisins ont été extraits par la méthode QuEChERS puis analysés par LCMSMS et GCMSMS au laboratoire de phytopharmacie de Kfarchima du ministère de l'agriculture.

Parmi les 1594 échantillons analysés, des résidus de pesticides ont été détectés dans 1166 échantillons (soit dans 73 % des échantillons) 48 molécules distinctes ont été identifiées avec des teneurs comprises entre 0,001 et 11,2 mg. kg⁻¹. La présence de résidus de pesticides a été observée dans 97%, 63% et 56% des échantillons en 2012, 2013 et 2014, respectivement. Parmi ces échantillons, 33%, 17% et 16% contenaient une ou plusieurs molécules de pesticides dépassant les LMR du Codex Alimentarius. La médiane des concentrations de l'ensemble des molécules s'est avérée significativement plus élevée en 2012 (0,18 mg.kg⁻¹) comparée à celle de 2013 (0,06 mg.kg⁻¹) et 2014 (0,07 mg.kg⁻¹). L'analyse des 48 pesticides détectés révèle une prédominance de pesticides au mode d'action systémique, représentant 78 % des pesticides appliqués en 2012, 89 % en 2013 et 86 % en 2014. De même, la plupart des molécules détectées appartenaient à la classe fonctionnelle des fongicides, 73 % en 2012, 84 % en 2013 et 76 % en 2014, ciblant principalement l'oïdium, le mildiou et la pourriture grise.

L'évaluation des risques pour la santé révèle que les quotients de danger (HQ) calculés pour les diverses molécules étaient inférieurs à 1, tant pour les adultes que pour les enfants, indiquant un risque négligeable pour la santé humaine. Cependant, les HQs ne reflètent que le risque individuel des résidus. Ainsi, il faut prendre en compte que ces résidus contribuent en effet à l'exposition générale du consommateur en fonction de son régime

alimentaire et alors ne sont pas sans risques. Il est également important de noter que les HQs ne tiennent pas compte de l'effet cocktail des pesticides. Une présence de cocktails de plus de deux résidus a été observée (60 %) avec des mélanges jusqu'à neuf résidus identifiés (1%) en 2014. De plus, parmi les molécules détectées, beaucoup sont suspectées d'avoir des répercussions sur la santé humaine. Plus de 89 % sont des toxines du développement et de la reproduction (carbendazime, carbofuran, chlorpyrifos...), plus de 43 % classées comme cancérigènes (boscalid, carbendazime, cyperméthrine, thiametoxam...) et plus de 36 % comme génotoxiques (azoxystrobine, carbendazime, carbofuran, krésoxim-méthyl...). Ces résultats soulignent la nécessité d'une gestion plus stricte des pratiques phytosanitaires dans la viticulture libanaise par le ministère pour garantir la conformité aux normes internationales et la protection de la santé humaine et de l'environnement.

Monitoring of pesticide residue levels in Lebanese table grapes from 2012-2014 and human health risk assessment

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Abstract

In many countries, pesticide residue monitoring is regularly conducted in food by health organizations. In Lebanon, the lack of monitoring data on grapes has significant drawbacks for human health and economic impact. To address this, the Ministry of Agriculture in Lebanon conducted a monitoring campaign of pesticide residue levels in Lebanese table grapes between 2012 and 2014. For this study, 1,594 Lebanese table grape samples were collected from the main wine-growing regions. Among the 80 pesticides analyzed, 48 molecules were detected. Over 56% of grape samples contained pesticide residues, and 16% exceeded Codex Alimentarius MRLs. The median concentration of pesticide residues in 2012 (0.18 mg.kg⁻¹) was significantly higher than in 2013 (0.06 mg.kg⁻¹) and 2014 (0.07 mg.kg⁻¹). Pesticides with a systemic mode of action dominated (>78%), and fungicides were the prevalent functional class (>73%). The risk assessment indicated a low risk for adults and children (HQ < 1), however our HQ calculation did not take into account the presence of these molecules in foods other than grapes. Furthermore, the frequent detection of pesticide cocktails (>27%) in grapes could result in potential antagonistic and synergistic effects, and therefore a higher risk than that assessed by the HQ.

Key-words: *Fungicide, Insecticide, LCMSMS, GCMSMS, QuEChERS, Grapevine Disease, Pest Management*

1. Introduction

Grapevine (*Vitis vinifera* L.) is a significant global crop, covering 7.4 million hectares worldwide in 2018 and producing 77.8 million tons of grapes (Arkam et al., 2021). Mediterranean countries account for the largest viticultural area, with 4.2 million hectares (Capon et al., 2014), where viticulture is essential for economic growth and cultural heritage (Daniele and Paolo, 2023; João et al., 2024). However, the sector faces growing threats from climate change, which increase vineyards' vulnerability to diseases, especially cryptogamic ones (Bois et al., 2017; Jaume et al., 2022; Lia-Tânia et al., 2022).

To combat these threats, vineyards often undergo frequent pesticide treatments, especially with fungicides, sometimes up to 10 times per season (Zubrod et al. 2019). For example, in 2020, Italian vineyards received an average of 24.89 kilograms of pesticides per hectare (ISTAT, 2023), while Spain's usage was even higher at 34.2 kg/ha (Alonso González et al., 2021; Alonso González et al., 2022). In France, the average was 17 kg/ha (Fouillet et al., 2022). Consistently, studies have reported high levels of pesticide residues in grapes. For instance, 2.6% of grape samples imported from Italy exceeded European Union Maximum Residue Limits (MRLs) (Poulsen et al., 2007), while in Egypt, 87% of tested grape samples contained pesticide residues, with 39% above Codex Alimentarius MRLs (Elmarsafy and Kadah, 2018). Similar findings were observed in Tunisia (Bouagga et al., 2019) and Turkey (Golge and Kabak, 2018).

In Lebanon, table grapes are a major crop, with an annual production of 120,000 tons (CBI, 2018) and exports exceeding 21 million dollars in 2020 (Trade, 2022). However, a study revealed that grapes sold in the capital -Beirut- were among the most contaminated plant-based foods, with 3.1% of 387 samples testing positive for pesticide residues (Khazaaal et al., 2022). Nearly 50% of agricultural farms in Lebanon overuse pesticides, leading to significant environmental and health risks (Akkouch and Halwani, 2023), with some areas applying doses up to 100 times higher than recommended (Akkouch and Halwani, 2023). The excessive use of pesticides is driven by weak regulatory enforcement, limited resources, poverty, and lack of knowledge. Additionally, the continued use of banned pesticides (Koubeissy, 2014; Abou Zeid et al., 2020) and fragmented regulatory oversight further complicate effective control (Abou Zeid et al., 2020).

Despite the importance of viticulture in Lebanon, limited data exists on pesticide

residue levels in table grapes and the associated health risks. To address these gaps, Lebanon's Ministry of Agriculture implemented the "Activating Production Chains" strategy from 2010 to 2014, aiming to improve production quality and enhance competitiveness (Hobeika and El Khouri, 2011). As part of this initiative, a pesticide residue monitoring campaign for grapes was launched in 2012, accompanied by training workshops on Good Agricultural Practices (GAPs). This campaign enabled the collection of unprecedented data on pesticide residues in 1,594 table grape samples across Lebanon's four wine regions (Bekaa, North, Mount Lebanon, and South) over three years (2012 to 2014). The study was authorized for research and publication by the Minister of Agriculture (authorization number 3/7804, October 19, 2018). The primary objectives of this study were to monitor pesticide residue levels in Lebanese table grapes from 2012 to 2014 and assess the associated human health risks across the four wine-growing regions.

2. Material and Methods

Grape sampling across Lebanese Regions

Grape samples were collected from 2012 to 2014, covering the four main viticultural regions in Lebanon: Bekaa (including Bekaa and Baalbeck-El Hermel Governorates; total area: 444,200 ha; table grape area: 10,000 ha), the North (North and Akkar Governorates; total area: 202,400 ha; table grape area: 213 ha), Mount Lebanon (including Mount Lebanon and Jbeil-Keserwan Governorates; total area: 195,800 ha; table grape area: 500 ha), and the South (including South and Nabatieh Governorates; total area: 198,800 ha; table grape area: 761 ha) (Figure II-1).

Sampling and samples handling were realized according to FAO/WHO guidelines (FAO/WHO 2023) regarding the methods of sampling [CAC/GL 33-1999], the amounts of grapes to be analyzed [CAC/GL 41-1993] and the good laboratory practice in pesticide residue analysis [CAC/GL 40-1993]. Two-kilogram samples were collected from each monitored vineyard regardless its surface area by employees of the Ministry of Agriculture in the regional departments and/or by grape producers, following clauses 2, 3, and 4 of Decision No. 380 of the Ministry of Agriculture (MoA 2012). From each 2 kg grape sample, a 1 kg subsample was placed in a sealed polyethylene bag with proper labeling and a specific ID, and then sent directly to the Kfarshima Laboratory for pesticide analysis. The total number of grape samples

collected was 588 in 2012, 425 in 2013, and 581 in 2014.

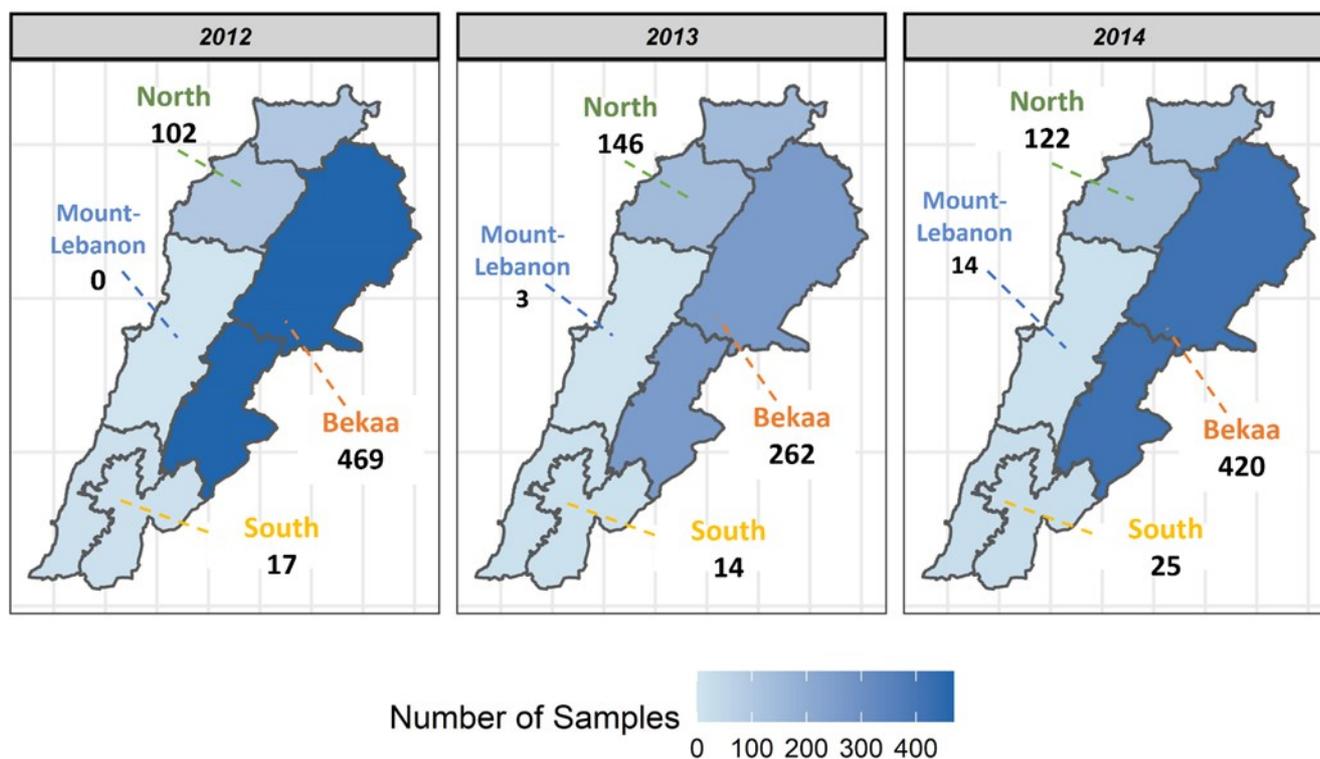


Figure II- 1: Distribution of the number of samples across the Lebanese Regions

Samples handling, pesticide residues extraction and analysis

All pesticide extraction then analyses were done within 3 days from the arrival of the samples to Kfarshima Laboratory. The 1 kg grape sample was grinded and homogenized using a VCM4 Waring Vertical Cutter Blender/Mixer 309 (Halde, Sweden) and 10 grams were taken and put in 50 ml polypropylene centrifuge tubes. The pesticide residues QuEChERS extraction and analysis of 80 pesticides (Annexe I - Table SII-1) by LCMSMS and GCMSMS was done as previously reported by Khazaal et al. 2022. In regards to the method performance criteria, during the period of residues analysis (2012-2014), SANCO/12495/2011 guidelines (EC 2023) were officially adopted by the Reference ministry accredited Laboratory Kfarchima. Thus, the performance of the method was assessed according to the following criteria: linearity ($R^2 > 0.98$), recovery (RM%) between 70–120%, within-laboratory repeatability ($RSDr\% \leq 20\%$) and reproducibility ($RSDRW\% \leq 20\%$). The Limits of Detection (LOD) and Quantification (LOQ) were established at 0.01 mg/kg for all the molecules.

Pesticide properties

The mode of action for pesticides was obtained from the Global Resistance Management

Mode of Action (IRAC 2023), and their properties were sourced from the PPDB database (Lewis et al., 2016). The PPDB (2023) alert system categorizes pesticides as high, moderate, or low risk based on their environmental fate (including persistence in soil, leaching potential, potential for loss via drain flow, and potential for particle-bound transport), ecotoxicity (acute and chronic toxicity to birds, fish, daphnia, bees, and earthworms), and human health effects (including acute and chronic mammalian toxicity, carcinogenicity, genotoxicity, endocrine disruption, reproductive and developmental effects, acetylcholinesterase inhibition, and neurotoxicity). A pesticide is assigned a high alert if any of its evaluated properties is classified as "High" (PPDB 2023).

Data Analysis

The data was analyzed using the R free software and multiple relevant packages. The dataset was summarized using descriptive statistical techniques like mean, median, standard deviation (SD), range (min-max), and count (N). Since the sample sizes were unequal across the years and regions, the Kruskal-Wallis test was employed to assess the significance of the concentration differences among 2012, 2013, and 2014. To conduct pairwise comparisons, the Dunn-test was utilized. The Dunn-test is particularly suitable for situations where sample sizes are unequal.

Health Risk Assessment

In order to evaluate the risk of pesticide residues in grapes to human health, calculations for HQ (hazard quotient) were performed. HQ estimations are utilized to assess the chronic effects of residues. A value exceeding 1 for HQ would indicate a potential risk to human health. To calculate HQ, the estimated daily intake (EDI) was determined using the following equation (1):

$$EDI = \sum \frac{F_i \times MR_i}{Bw} \quad (1)$$

where EDI = the estimated daily intake (in milligrams per kilogram per day)

F_i = the daily grape consumption data (kg)

MR_i = the mean residual pesticide concentration (mg/kg)

Bw = the body weight of the consumer (kg).

For adults, a body weight of 63 kg and grape consumption of 0.00635 kg/day were assumed (EFSA 2023). For children, a body weight of 16 kg and grape consumption of 0.0131 kg/day were considered (EFSA 2023).

HQ values were obtained using equation (2):

$$HQ = (EDI/ADI) \quad (2).$$

ADI = the acceptable daily intake (in milligrams per kilogram per day) (obtained from the PPDB database ,Lewis et al., 2016).

3. Results and Discussion

Grape Samples Collected

The number of grape samples collected varied significantly across the four Lebanese regions, with a notable focus on the Bekaa and North regions. The Bekaa region, being the center of Lebanese agriculture and hosting the largest viticultural area in Lebanon (10,000 ha), had the highest number of samples collected: 469 in 2012, 262 in 2013, and 420 in 2014. In contrast, the North region, despite having a smaller viticultural area of 213 ha compared to the South (761 ha) and Mount Lebanon (500 ha), also had a considerable number of samples collected: 102 in 2012, 146 in 2013, and 122 in 2014 (see Fig. II-1). The lower number of samples from South Lebanon, with 17, 14, and 25 samples in 2012, 2013, and 2014 respectively, and from Mount Lebanon, with 0, 3, and 14 samples over the same years, may be attributed to different levels of engagement among grape producers. Producers in the Bekaa and North regions may have been more proactive in submitting samples for analysis and reporting their intentions to export their grape production, thereby falling under the monitoring activities of the Ministry of Agriculture.

Pesticide Residue Occurrences and levels in grapes

Across all 4 Lebanese regions, the presence of pesticide residues was observed in 97%, 63%, and 56% of samples in 2012, 2013, and 2014, respectively (Table II-1). Among these samples, 33%, 17%, and 16% showed levels of one or more pesticides above the Codex Alimentarius MRLs, and therefore were classified as rejected by the Ministry of Agriculture (Table II-1). The number of grape samples with residues and without residues across Lebanese regions in 2012, 2013 and 2014 is given in Annexe I - Table SII-2. In addition, the range of concentrations, mean concentration with standard deviation, median concentration, as well as the incidence of MRL exceedances for each molecule detected during the years 2012, 2013, and 2014 are given in Annexe I - Table SII-3.

Table II- 1: Frequency of detection of pesticide residues across the four regions in 2012, 2013, and 2014

| | | | 2012 | 2013 | 2014 |
|--|--|-----------------------------|------|------|------|
| Samples | Frequency of detection of pesticide residues | <i>Bekaa</i> | 810 | 302 | 592 |
| | | <i>North</i> | 270 | 205 | 150 |
| | | <i>South</i> | 4 | 21 | 31 |
| | | <i>Mount-Lebanon</i> | 0 | 2 | 20 |
| | Status* (%) | <i>RD</i> | 97.5 | 63.1 | 55.9 |
| | | <i>NRD</i> | 2.5 | 36.9 | 44.1 |
| | | <i>Accepted</i> | 67.0 | 82.6 | 83.8 |
| | | <i>Rejected</i> | 33.0 | 17.4 | 16.2 |
| Molecules** | Mode of action (%) | <i>Contact</i> | 21.3 | 9.1 | 12.4 |
| | | <i>Systemic</i> | 77.9 | 89.4 | 86.4 |
| | | <i>Contact and systemic</i> | 0.7 | 1.5 | 1.3 |
| | Functional class (%) | <i>Fungicide</i> | 73.0 | 84.5 | 76.2 |
| | | <i>Insecticide</i> | 26.7 | 15.5 | 23.8 |
| | | <i>Acaricide</i> | 0.2 | 0.0 | 0.0 |
| | | <i>Herbicide</i> | 0.1 | 0.0 | 0.0 |
| | Legal status in Lebanon in 2023 (%) | <i>Approved</i> | 72.2 | 78.1 | 78.8 |
| | | <i>Not approved</i> | 27.0 | 21.1 | 21.2 |
| | | <i>Pending</i> | 0.7 | 0.7 | 0 |
| | Legal status in EU in 2023 (%) | <i>Approved</i> | 60.6 | 66.2 | 75.2 |
| | | <i>Not approved</i> | 39.4 | 33.8 | 24.8 |
| | Classification according to the World Health Organization (WHO) (%) | <i>Ib</i> | 0.5 | 0 | 0.1 |
| | | <i>II</i> | 40.1 | 39.4 | 39.2 |
| | | <i>III</i> | 8.6 | 4.5 | 5.5 |
| | | <i>U</i> | 39.6 | 47.4 | 44.9 |
| | | <i>Not Listed</i> | 11.2 | 8.7 | 10.2 |
| | Recommended (%) | <i>Yes</i> | 80.7 | 78.7 | 74.0 |
| | | <i>No</i> | 19.3 | 21.3 | 26.0 |
| | *RD : residues detected ; NRD: no residues detected; Accepted samples are samples with residues < Codex MRLs; Rejected samples are samples with residues > Codex MRLs. | | | | |
| **Legal status in 2023; To be used on grapes according to the European phytosanitary index; For WHO classification: Ib: highly hazardous, II: moderately hazardous, III: slightly hazardous, U: unlikely to present an acute hazard, Not Listed: Not listed in WHO classification; Recommended: by the Ministry of Agriculture in Lebanon according to its 2010-2014 strategy guide for table grape producers. | | | | | |

Among the 80 molecules investigated, the analysis showed the presence of 48 pesticides (Annexe I -Table SII-3). The median concentration in 2012, was significantly higher (0.18 mg.kg⁻¹) than the median concentrations in 2013 (0.06 mg.kg⁻¹) and 2014 (0.07 mg.kg⁻¹), with no significant difference between the latter two, as determined by Kruskal-Wallis - Dunn tests (Figure II-2). Details of the pesticides concerned and their levels are shown in the Annexe I - Figure SII-1. The observed decline in median pesticide concentration is likely due to improved

practices encouraged by the issued guide by the Ministry of Agriculture, enhanced awareness and training for viticulturists, and stricter regulatory enforcement and monitoring. However, the percentage of residues from banned molecules increased to reach 26% of the detected molecules in 2014 (table II-1). This increase in the occurrence of banned molecules may be attributed to the war in Syria (2011-2021), which has resulted in loose border controls. These conditions have facilitated the smuggling of forbidden and unregistered pesticide products across the border, allowing them to be sold at cheaper prices.

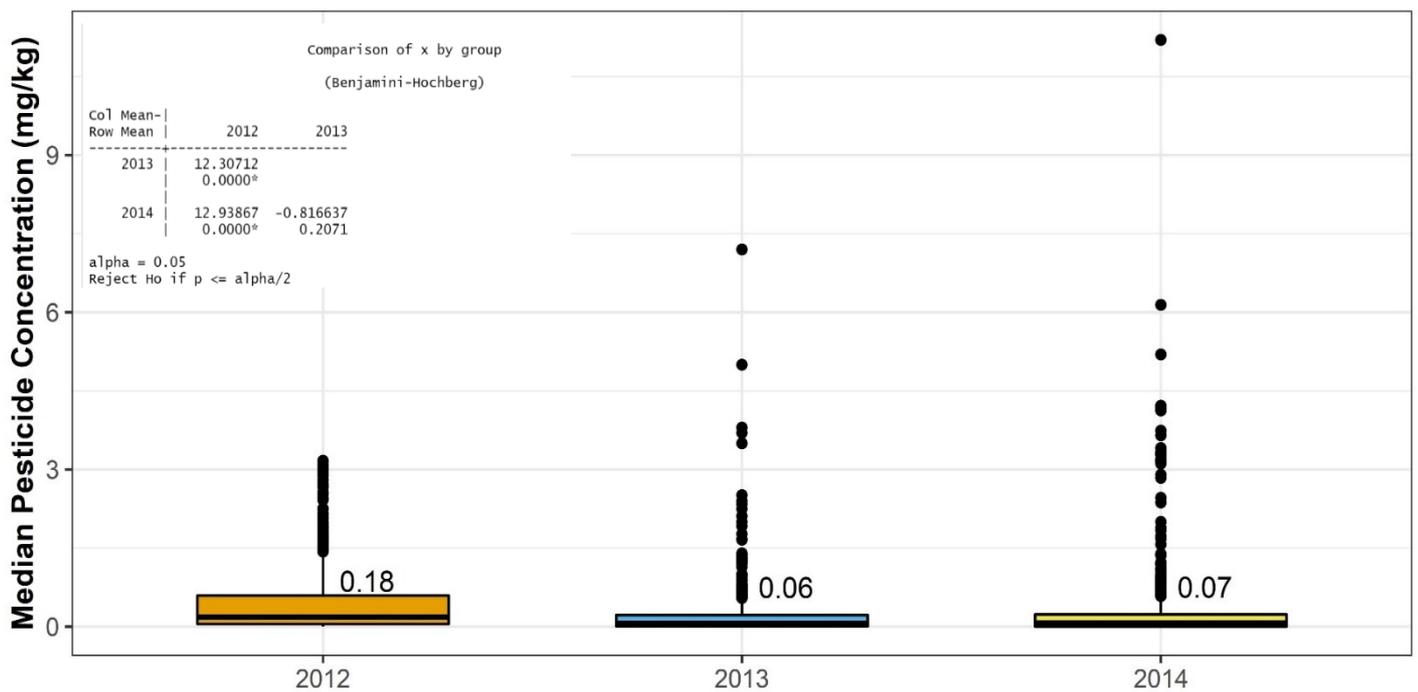


Figure II- 2: Median pesticide concentrations (mg/kg) in grapes for the three years and Kruskal-Wallis - Dunn tests results

Figure II-3 illustrates molecules detected more than 20 times per year from 2012 to 2014. Among these frequently detected molecules, Thiophanate-methyl, Cypermethrin, and Difenoconazole, pesticide recommended in the Ministry of Agriculture's guide consistently exceeded MRLs over the three years. Specifically, Thiophanate-methyl exceeded Codex MRLs in 76%, 79%, and 71% of instances, while Cypermethrin exceeded MRLs in 64%, 81%, and 97% of instances in 2012, 2013, and 2014 respectively. Similarly, Difenoconazole exceeded MRLs in 18%, 33%, and 38% of instances over the same period. Yet it is speculated that viticulturists did not adhere to the recommended dose per hectare specified on the product label and/or to pre-harvest intervals, the minimum required time prior to harvest that allows residues to fall below MRLs, resulting in inadequate adoption of GAPs to avoid MRL exceedances. Additionally, forbidden molecules such as Acetamiprid and Tetraconazole were frequently

detected with MRL exceedances. Acetamiprid showed MRL exceedances in 44% and 35% of instances in 2012 and 2013 respectively, this molecule was also frequently detected in 2014 (73 occurrences) although at levels below MRLs. Tetraconazole was only detected in 2014 with MRL exceedances in 31% of instances. Chlorpyrifos also exhibited MRL exceedances in 52% of instances, but only in 2014. These results highlight the non-compliance with regulations in terms of authorized molecules and doses applied. The implementation of agricultural logbooks could be a way to improve the traceability of the molecules used as well as the doses applied.

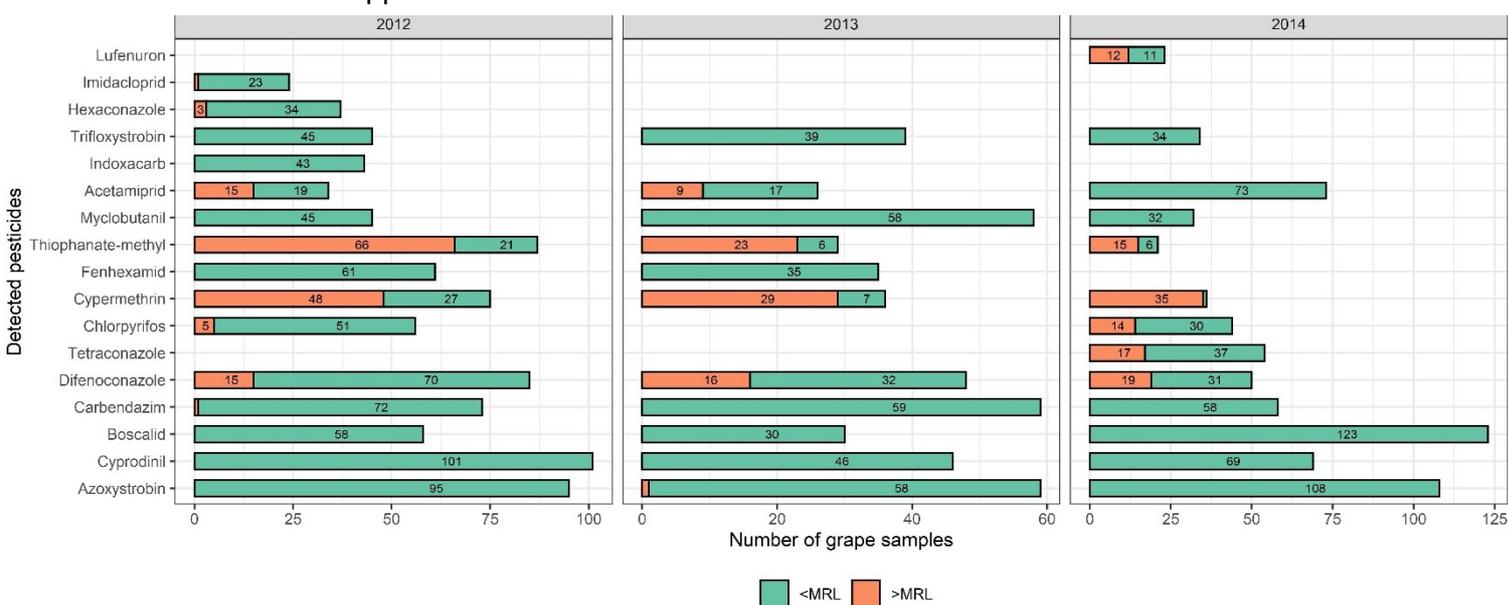


Figure II- 3: Frequently detected pesticides in grapes samples (>20 times) and their MRLs exceedances in 2012, 2013 and 2014

Types of the Molecules, Functional Class and Chemical Family

Regarding the types of the molecules according to their mechanism of action it becomes evident that systemic pesticides were consistently prevalent across the study years, accounting for 78% in 2012, 89% in 2013, and 86% in 2014 (Table II-1). This observation is of concern since systemic pesticides are suspected to have sublethal health effects on humans and contribute to the widespread contamination on the environment (Sánchez-Bayo, Goka, and Hayasaka 2016; Odewale et al. 2022).

According to their functional class, there was a noticeable predominance of fungicides, constituting 73% in 2012, 85% in 2013, and 76% in 2014, compared to insecticides (24-27 %); acaricides and herbicides were only present in 2012 although in less than 0.2 % of incidences (Table II-1). These findings align with results from table grape monitoring in other Mediterranean countries. In Tunisia, 60.7% of the detected molecules in table grape samples were fungicides (Bouagga et al. 2019). In Turkey, 87.5% of the quantifiable molecules were

fungicides (Golge and Kabak 2018). Similarly, in the Czech Republic, 89.7% of the detected pesticide residues were fungicides, aligning with the results of this study in terms of the nature and functional class of the molecules detected (Schusterova et al. 2021). The estimated mean concentration of fungicides in table grape was the highest in 2012 (0.46 mg/kg) followed by 2014 (0.36 mg/kg) and 2013 (0.28 mg/kg) (Table II-2). The total concentration of insecticide in table grape was significantly lower than fungicides with average concentrations from 0.26 to 0.30 mg/kg. The detected fungicides were employed primarily targeted powdery mildew, followed by downy mildew and grey mold. In the case of insecticides, aphids were the primary target. Annexe I - Figure SII-3 illustrates the frequency of pesticide treatments.

Table II- 2: Average pesticide residue levels (mg/kg) in table grapes according to their functional class, chemical family, sampling region and sampling year

| | | 2012 | | | | 2013 | | | | 2014 | | | |
|--|----------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| | | Mean Concentration in mg/kg \pm SD | | | | | | | | | | | |
| Functional Class | Chemical Family | Bekaa | North | South | Mount-Lebanon | Bekaa | North | South | Mount-Lebanon | Bekaa | North | South | Mount-Lebanon |
| Fungicides | Anilinopyrimidine | 0.38 \pm 0.28 | 0.47 \pm 0.34 | 0.07 | NS | 0.17 \pm 0.21 | 0.15 \pm 0.11 | 0.20 | <LOD | 0.27 \pm 0.36 | 0.37 \pm 0.34 | 0.64 \pm 0.48 | 0.47 \pm 0.39 |
| | Benzimidazole | 0.82 \pm 0.70 | 0.68 \pm 0.60 | <LOD | NS | 0.85 \pm 1.31 | 0.42 \pm 0.81 | 0.18 \pm 0.11 | 0.30 \pm 0.14 | 0.54 \pm 1.68 | 0.52 \pm 0.69 | 0.74 \pm 0.49 | 1.21 |
| | Carboxamide | 1.33 \pm 1.08 | 0.21 \pm 0.21 | 0.07 | NS | 0.09 \pm 0.05 | 0.11 \pm 0.07 | 0.12 \pm 0.03 | <LOD | 0.32 \pm 0.43 | 0.27 \pm 0.18 | 0.63 \pm 0.57 | 0.42 \pm 0.59 |
| | Cyanoacetamide oxime | 0.01 | <LOD | <LOD | NS | <LOD |
| | Dicarboximide | <LOD | 0.01 \pm 0.00 | <LOD | NS | <LOD |
| | Hydroxyanilide | 0.49 \pm 0.49 | 0.93 \pm 0.54 | <LOD | NS | 0.45 \pm 0.60 | 0.53 \pm 0.74 | <LOD | <LOD | 0.50 \pm 0.63 | <LOD | 0.26 \pm 0.11 | <LOD |
| | Morpholine | 0.04 | <LOD | <LOD | NS | 0.03 \pm 0.01 | <LOD |
| | Phenylamide | 0.19 \pm 0.18 | 0.15 \pm 0.13 | <LOD | NS | <LOD | <LOD | <LOD | <LOD | 0.13 \pm 0.18 | <LOD | <LOD | <LOD |
| | Strobilurin | 0.31 \pm 0.33 | 0.35 \pm 0.35 | 0.04 \pm 0.01 | NS | 0.21 \pm 0.41 | 0.11 \pm 0.15 | 0.11 \pm 0.12 | <LOD | 0.11 \pm 0.13 | 0.14 \pm 0.13 | 0.20 \pm 0.17 | <LOD |
| | Triazole | 0.13 \pm 0.21 | 0.17 \pm 0.32 | <LOD | NS | 0.15 \pm 0.19 | 0.12 \pm 0.14 | 0.12 \pm 0.04 | <LOD | 0.53 \pm 1.25 | 0.38 \pm 0.91 | 0.53 \pm 1.14 | 1.68 \pm 2.04 |
| Mean fungicides level by region | | 0.46 \pm0.62 | 0.50 \pm0.53 | 0.052 \pm0.02 | NA | 0.33 \pm0.72 | 0.21 \pm0.42 | 0.14 \pm0.08 | 0.30 \pm0.14 | 0.34 \pm0.88 | 0.33 \pm0.58 | 0.50 \pm0.66 | 0.96 \pm1.41 |
| Mean fungicides level by year | | 0.46 \pm0.60 | | | | 0.28 \pm0.61 | | | | 0.36 \pm0.85 | | | |
| Insecticides | Benzoylurea | 0.15 \pm 0.17 | 0.27 | <LOD | NS | <LOD | 0.39 \pm 0.01 | <LOD | <LOD | 0.17 \pm 0.29 | 0.43 \pm 0.28 | 1.36 | <LOD |
| | Carbamate | 0.09 | <LOD | <LOD | NS | <LOD | <LOD | <LOD | <LOD | 0.004 | <LOD | <LOD | <LOD |

| | | | | | | | | | | | | | |
|--|--------------------------|---------------------------|---------------------------|---------------------------|----|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | ± 0.10 | | | | | | | | | | | |
| | Diphenyl oxazoline | <LOD | 0.09 | <LOD | NS | <LOD |
| | Neonicotinoid | 0.37 ± 0.31 | 0.25 ± 0.25 | <LOD | NS | 0.20 ± 0.27 | 0.31 ± 0.25 | <LOD | <LOD | 0.14 ± 0.12 | 0.17 ± 0.14 | 0.07 ± 0.07 | 0.05 |
| | Organophosphate | 0.23 ± 0.20 | 0.22 ± 0.19 | <LOD | NS | 0.29 ± 0.51 | 0.20 ± 0.05 | <LOD | <LOD | 0.46 ± 0.42 | 0.27 ± 0.22 | 0.13 | <LOD |
| | Oxadiazine | 0.24 ± 0.27 | 0.43 ± 0.38 | <LOD | NS | 0.15 ± 0.01 | 0.11 ± 0.02 | <LOD | <LOD | 0.33 ± 0.23 | <LOD | <LOD | <LOD |
| | Pyrethroid | 0.38 ± 0.35 | 0.81 ± 0.52 | <LOD | NS | 0.33 ± 0.25 | 0.25 ± 0.14 | 0.30 ± 0.08 | <LOD | 0.35 ± 0.22 | 0.46 ± 0.26 | <LOD | 0.30 |
| | Pyridazinone | 0.02 ± 0.04 | 0.05 ± 0.06 | <LOD | NS | <LOD | 0.01 | 0.003 | <LOD | 0.01 | 0.01 ± 0.00 | <LOD | <LOD |
| | Sulphite ester | 2.40 ± 0.57 | <LOD | <LOD | NS | <LOD |
| Mean insecticides level by region | | 0.30 ± 0.36 | 0.30 ± 0.35 | NA | NA | 0.26 ± 0.30 | 0.26 ± 0.20 | 0.23 ± 0.16 | NA | 0.27 ± 0.30 | 0.26 ± 0.23 | 0.34 ± 0.57 | 0.17 ± 0.17 |
| Mean insecticides level by year | | 0.30 ± 0.35 | | | | 0.26 ± 0.24 | | | | 0.27 ± 0.29 | | | |
| Herbicides | Aryloxyphenoxypropionate | 0.01 | <LOD | <LOD | NS | <LOD |
| Mean herbicide level by region | | 0.01 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Mean herbicide level by year | | 0.01 $\pm NA$ | | | | NA | | | | NA | | | |
| Mean pesticide level by region | | 0.41 ± 0.56 | 0.45 ± 0.50 | 0.01 ± 0.02 | NA | 0.24 ± 0.61 | 0.17 ± 0.35 | 0.13 ± 0.11 | 0.20 ± 0.20 | 0.24 ± 0.70 | 0.23 ± 0.46 | 0.34 ± 0.60 | 0.68 ± 1.24 |
| Mean pesticide level by year | | 0.41 ± 0.54 | | | | 0.21 ± 0.51 | | | | 0.25 ± 0.67 | | | |

*SD : Standard Deviation ; NS : No Samples; LOD: Limit of Detection; NA: Not Applicable

It is of significance to note that, in the year 2012, the residues of fungicides exhibited a clear predominance of the triazole chemical family (223 residues), followed by the benzimidazole chemical family (167 residues) and the strobilurin chemical family (159 residues). Similarly, in 2013, these same three chemical family continued to dominate, with 140 residues attributed to the triazole family, 104 to the strobilurin family, and 88 to the benzimidazole family. However, in the subsequent year, 2014, a noticeable shift occurred as the carboxamide family emerged as the third most prevalent among fungicides, comprising 123 residues. The strobilurin group retained its prominence with 160 residues, while the triazole family accounted for 153 residues. Regarding insecticides, the residues primarily belonged to three key chemical family: organophosphates, neonicotinoids, and pyrethroids (Annexe I - Figure SII-4).

Application repeated from one year to the next of the same chemical family, particularly benzimidazole and triazoles, contributes to the emergence of resistant fungal populations, especially *B. cinerea* (grey mold) that is one of the main diseases attacking grape vines in Lebanon. Shao, Zhao, and Ma (2021) reported that benzimidazole-resistant *B. cinerea* (grey

mold) populations became dominant in China. A similar situation applies to triazoles, with resistant populations of *Uncinula necator* (powdery mildew) being reported since 2000 in South Africa vineyards (Halleen and Holz 2017).

In addition, for the 3 years and all regions, the insecticide contents were the highest for the organophosphates and pyrethroids chemical family (Annexe I - Table SII-4) which are frequently detected in surface and ground-water in Lebanon (Akkouch and Halwani 2023). These repeated treatments of fungicides and insecticides therefore generate not only risks of pest resistance but also risks to human health. In Lebanon, a study revealed that many agricultural workers are unaware of the safety rules regarding the use of pesticides (Salameh et al. 2004). This lack of awareness exposes both agricultural workers and consumers to unsafe levels of pesticide residues.

Occurrence of Multiresidue Samples

Figure II-4 provides clear evidence of the presence of samples containing multiple residues (reaching 9 residues) throughout the years 2012, 2013, and 2014. The majority of these samples consisted of two residues, representing 27% in 2012, 32 % in 2013, and 29% in 2014. Less than 3% of the samples contained as many as nine residues in 2014. For instance, one of the cocktails (9 residues) obtained in 2014 contained 2 molecules with a mode of action targeting acetylcholinesterase (Acetamiprid, Chlorpyrifos), 2 molecules targeting the tubulin polymerization (Carbendazim, Thiophanate-methyl), 3 DeMethylation Inhibitors (Difenoconazole, Penconazole, Tetraconazole), 1 sodium channel inhibitor (Cypermethrine), and 1 targeting the respiration - Quinone outside Inhibitors (Azoxytrobine). The presence of several molecules with the same mode of action class in these cocktails contributes to resistance in agents causing grapevine diseases, leading to increased usage of phytosanitary products (IRAC 2023). In pests, the use of pesticides with the same mode of action leads to resistance through various mechanisms, including behavioral, penetration, metabolic, and altered target-site resistances (Massi, Torriani, Borghi, and Toffolatti, 2021). These findings highlight the need to train Lebanese viticulturists about pesticide to avoid repetitive and simultaneous use of pesticides with the same mode of action. Furthermore, the implementation of integrated pest control techniques would make it possible to reduce the quantities of pesticides applied to vineyard plots.

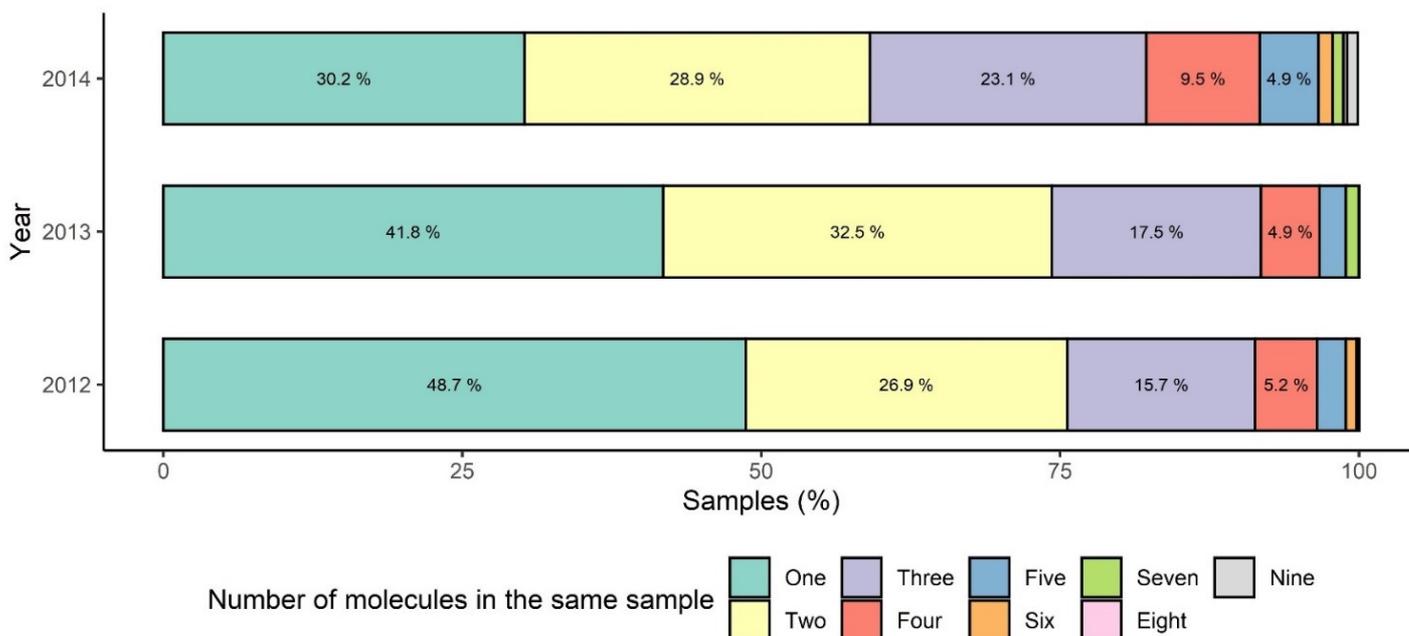
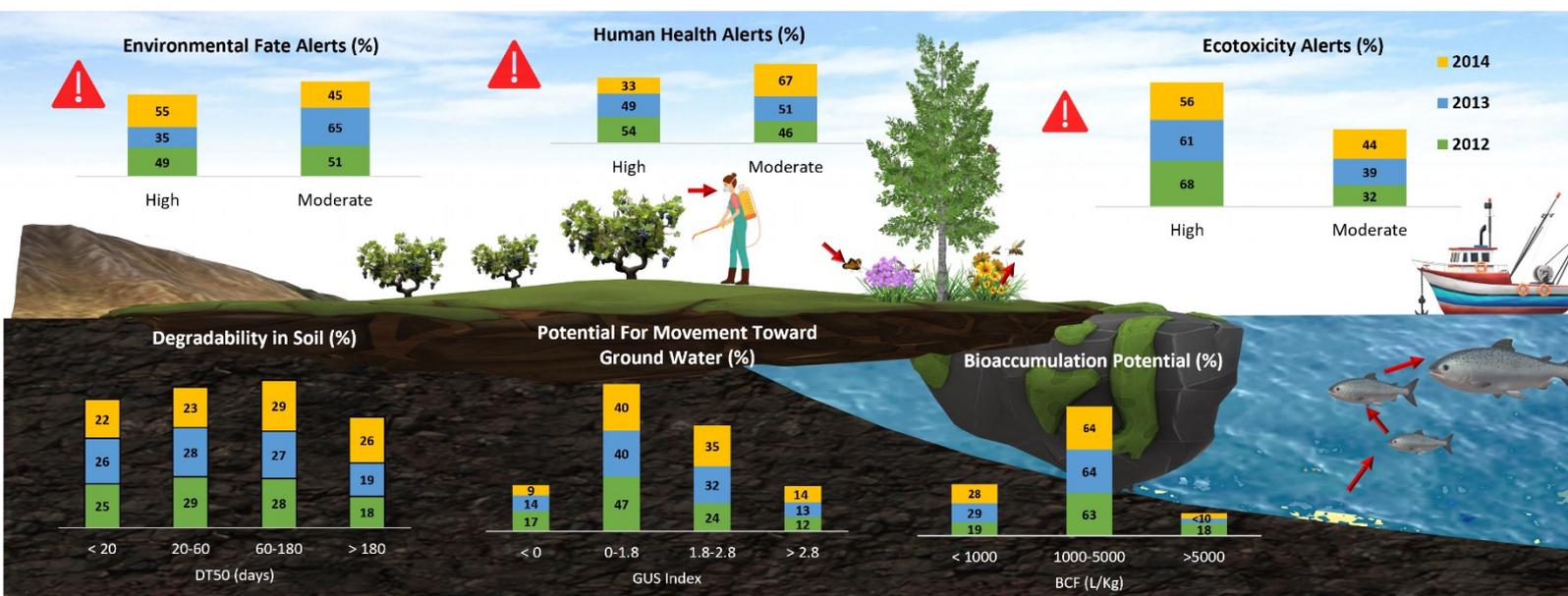


Figure II- 4: Percentage of grape samples containing one or more pesticide residues in 2012,2013, and 2014

Toxicity of the detected pesticides

In our study, around 43% of the detected molecules over the three years were found to be unlikely to present an acute hazard according to the WHO hazard classification (category U), whereas 39% of those fell under the category II – moderately hazardous - which refers to molecules having a lethal dose fifty (LD50) between 50 and 500 mg/kg; meanwhile 6% fell under the slightly hazardous category (III), 0.2 % under the highly hazardous category (Ib) and around 10 % were not listed in the WHO classification (WHO 2019) (Table II-1).

Additionally, an in-depth analysis of the molecules detected in the grape samples revealed that more than 89% of them are developmental and reproductive toxins, with over 43% classified as carcinogenic and more than 36% as genotoxic (Annexe I - Figure SII-5). Indeed , the teratogenicity, carcinogenicity and potential to act as an endocrine disrupter as well as other health impacts of the detected molecules (Annexe I - Figure SII-5), explain the high and moderate human health alerts obtained in around 45 and 55 % of the instances respectively as shown in Figure II-5. Moreover, the occurrence of pesticide cocktails as discussed aggravate the notorious effect of these residues on human health. Since pesticides with analogous functions can exacerbate their deleterious effects through mechanisms such as dose/concentration addition, response addition, or cumulative biological effects, including synergistic augmentation and antagonistic effects (WHO 2018, Gamet-Payrastre 2019).



Degradability in Soil: DT50 (days) <20 : RD, Readily Degradable; 20–60 : FD, Fairly Degradable; 60–180 :SD, Slightly Degradable, >180: VSD, Very Slightly Degradable (FAO 2000; Lewis et al. 2016)
Potential for Movement Toward Groundwater: GUS Index < 0: Extremely Low; 0 – 1.8: Low; 1.8 – 2.8: Moderate; > 2.8 High; NA (Not Applicable) (Goss 1992; Lewis et al. 2016)
Bioaccumulation Potential: BCF (L/Kg) < 1000: NB, Not Bioaccumulative; 1000-5000 B, Bioaccumulative; >5000 VB, Very Bioaccumulative (Lewis et al. 2016; Rosioru et al. 2016)
 **Environmental Fate Alerts, Human Health Alerts and Ecotoxicity Alerts as per Lewis et al. 2016.

Figure II- 5: Assessment of alert levels in terms of environmental fate, human health and ecotoxicity for the detected pesticides in 2012, 2013, and 2014

Human Risk Assessment: Adults and Children

The health risk assessment reveals that all Hazard Quotients (HQs) obtained for each of the detected molecules across a three-year period were below 1 for both adults and children (Table II-3), indicating a negligible risk to human health. Notably, in the years 2013 and 2014, there was a general decline in HQ values for most molecules when compared to those recorded in 2012 excepting for chlorpyrifos, difenocazole, dimethoate, Hexaconazole, Indoxacarb, and thiophanate methyl. It is worth emphasizing the considerably high HQ values for adults and children associated with chlorpyrifos, surpassing those of all other molecules. Aligning to these results, Khazaal et al. (2022) showed that the majority of foods of plant origin in Beirut were safe with residues having HQs <1 except for chlorpyrifos residues in cucumber (1.8). El Hawari et al. (2019) also calculated high HQ for chlorpyrifos on apples. It is, however, important to note that our HQ calculations take into account the quantities of pesticides provided by the ingestion of grapes, without taking into account other foodstuffs which may also provide these same molecules.

Table II- 3: Assessment of health risk parameters: estimated daily intake (EDI), acceptable daily intake (ADI) and hazard quotient (HQ) for adults and children

| Molecule | Year | Mean (mg.kg ⁻¹) | ADI* (mg.kg ⁻¹ .day ⁻¹) | EDI _{adult} ** (mg.kg ⁻¹ .day ⁻¹) | EDI _{children} ** (mg.kg ⁻¹ .day ⁻¹) | HQ _{adult} | HQ _{children} |
|---------------------|------|-----------------------------|--|---|--|---------------------|------------------------|
| Acetamidrid | 2012 | 0.28 | 0.025 | 2.78E-05 | 2.24E-04 | 0.0011 | 0.009 |
| | 2013 | 0.24 | | 2.46E-05 | 1.98E-04 | 0.0010 | 0.0079 |
| | 2014 | 0.13 | | 1.35E-05 | 1.09E-04 | 0.0005 | 0.0043 |
| Azoxystrobin | 2012 | 0.46 | 0.2 | 4.59E-05 | 3.69E-04 | 0.0002 | 0.0018 |
| | 2013 | 0.19 | | 1.95E-05 | 1.57E-04 | 0.0001 | 0.0008 |
| | 2014 | 0.12 | | 1.19E-05 | 9.57E-05 | 0.0001 | 0.0005 |
| Boscalid | 2012 | 1.27 | 0.04 | 1.28E-04 | 1.03E-03 | 0.0032 | 0.0258 |
| | 2013 | 0.10 | | 1.01E-05 | 8.15E-05 | 0.0003 | 0.002 |
| | 2014 | 0.33 | | 3.31E-05 | 2.66E-04 | 0.0008 | 0.0067 |
| Carbendazim | 2012 | 0.95 | 0.02 | 9.58E-05 | 7.71E-04 | 0.0048 | 0.0385 |
| | 2013 | 0.31 | | 3.12E-05 | 2.51E-04 | 0.0016 | 0.0126 |
| | 2014 | 0.30 | | 3.03E-05 | 2.44E-04 | 0.0015 | 0.0122 |
| Carbofuran | 2012 | 0.01 | 0.00015 | 8.06E-07 | 6.49E-06 | 0.0054 | 0.0433 |
| Chlorpyrifos | 2012 | 0.24 | 0.001 | 2.45E-05 | 1.97E-04 | 0.0245 | 0.1972 |
| | 2013 | 0.33 | | 3.35E-05 | 2.69E-04 | 0.0335 | 0.2693 |
| | 2014 | 0.43 | | 4.30E-05 | 3.46E-04 | 0.0430 | 0.3459 |
| Chlorpyrifos-methyl | 2012 | 0.14 | 0.01 | 1.43E-05 | 1.15E-04 | 0.0014 | 0.0115 |
| Cymoxanil | 2012 | 0.01 | 0.013 | 8.97E-07 | 7.22E-06 | 0.0001 | 0.0006 |
| Cypermethrin | 2012 | 0.44 | 0.05 | 4.47E-05 | 3.60E-04 | 0.0009 | 0.0072 |
| | 2013 | 0.28 | | 2.83E-05 | 2.28E-04 | 0.0006 | 0.0046 |
| | 2014 | 0.37 | | 3.73E-05 | 3.00E-04 | 0.0007 | 0.006 |
| Cyproconazole | 2012 | 0.08 | 0.02 | 8.47E-06 | 6.81E-05 | 0.0004 | 0.0034 |
| | 2013 | 0.01 | | 7.84E-07 | 6.31E-06 | 0.0000 | 0.0003 |
| Cyprodinil | 2012 | 0.39 | 0.03 | 3.89E-05 | 3.13E-04 | 0.0013 | 0.0104 |
| | 2013 | 0.17 | | 1.69E-05 | 1.36E-04 | 0.0006 | 0.0045 |
| | 2014 | 0.30 | | 3.04E-05 | 2.45E-04 | 0.0010 | 0.0082 |
| Deltamethrin | 2012 | 0.16 | 0.01 | 1.65E-05 | 1.33E-04 | 0.0017 | 0.0133 |
| Difenoconazole | 2012 | 0.08 | 0.01 | 8.24E-06 | 6.63E-05 | 0.0008 | 0.0066 |
| | 2013 | 0.12 | | 1.18E-05 | 9.48E-05 | 0.0012 | 0.0095 |
| | 2014 | 1.47 | | 1.48E-04 | 1.19E-03 | 0.0148 | 0.1189 |
| Diflubenzuron | 2012 | 0.01 | 0.1 | 8.06E-07 | 6.49E-06 | 0.0000 | 0.0001 |
| Dimethoate | 2012 | 0.08 | 0.001 | 7.72E-06 | 6.21E-05 | 0.0077 | 0.0621 |
| | 2013 | 0.08 | | 8.52E-06 | 6.85E-05 | 0.0085 | 0.0685 |
| | 2014 | 0.13 | | 1.35E-05 | 1.09E-04 | 0.0135 | 0.1087 |
| Dimethomorph | 2012 | 0.04 | 0.05 | 4.03E-06 | 3.24E-05 | 0.0001 | 0.0006 |
| | 2013 | 0.03 | | 2.55E-06 | 2.05E-05 | 0.0001 | 0.0004 |
| Etozazole | 2012 | 0.09 | 0.04 | 9.07E-06 | 7.30E-05 | 0.0002 | 0.0018 |
| Fenhexamid | 2012 | 0.64 | 0.2 | 6.47E-05 | 5.20E-04 | 0.0003 | 0.0026 |
| | 2013 | 0.48 | | 4.86E-05 | 3.91E-04 | 0.0002 | 0.002 |
| | 2014 | 0.46 | | 4.66E-05 | 3.75E-04 | 0.0002 | 0.0019 |
| Fluzifop-butyl | 2012 | 0.01 | NA | 9.07E-07 | 7.30E-06 | NA | NA |
| Flufenoxuron | 2012 | 0.27 | 0.01 | 2.71E-05 | 2.18E-04 | 0.0027 | 0.0218 |
| Flusilazole | 2012 | 0.19 | 0.002 | 1.91E-05 | 1.54E-04 | 0.0095 | 0.0768 |
| | 2013 | 0.11 | | 1.06E-05 | 8.54E-05 | 0.0053 | 0.0427 |
| | 2014 | 0.05 | | 5.39E-06 | 4.34E-05 | 0.0027 | 0.0217 |
| Hexaconazole | 2012 | 0.02 | 0.005 | 1.75E-06 | 1.41E-05 | 0.0004 | 0.0028 |
| | 2013 | 0.01 | | 6.79E-07 | 5.47E-06 | 0.0001 | 0.0011 |
| | 2014 | 0.10 | | 9.90E-06 | 7.97E-05 | 0.0020 | 0.0159 |
| Imidacloprid | 2012 | 0.42 | 0.06 | 4.25E-05 | 3.42E-04 | 0.0007 | 0.0057 |
| | 2013 | 0.28 | | 2.86E-05 | 2.30E-04 | 0.0005 | 0.0038 |
| | 2014 | 0.24 | | 2.45E-05 | 1.98E-04 | 0.0004 | 0.0033 |

| | | | | | | | |
|--------------------|------|------|--------|----------|----------|--------|--------|
| Indoxacarb | 2012 | 0.26 | 5.0E-3 | 2.59E-05 | 2.08E-04 | 0.0052 | 0.0417 |
| | 2013 | 0.13 | | 1.28E-05 | 1.03E-04 | 0.0026 | 0.0206 |
| | 2014 | 0.33 | | 3.36E-05 | 2.71E-04 | 0.0067 | 0.0541 |
| Kresoxim-methyl | 2012 | 0.65 | 0.4 | 6.57E-05 | 5.29E-04 | 0.0002 | 0.0013 |
| | 2013 | 0.54 | | 5.41E-05 | 4.36E-04 | 0.0001 | 0.0011 |
| | 2014 | 0.28 | | 2.85E-05 | 2.29E-04 | 0.0001 | 0.0006 |
| Lufenuron | 2012 | 0.14 | 0.015 | 1.39E-05 | 1.12E-04 | 0.0009 | 0.0074 |
| | 2013 | 0.39 | | 3.93E-05 | 3.16E-04 | 0.0026 | 0.0211 |
| | 2014 | 0.29 | | 2.94E-05 | 2.37E-04 | 0.0020 | 0.0158 |
| Metalaxyl | 2012 | 0.18 | 0.08 | 1.79E-05 | 1.44E-04 | 0.0002 | 0.0018 |
| | 2014 | 0.13 | | 1.29E-05 | 1.04E-04 | 0.0002 | 0.0013 |
| Metalaxyl-M | 2012 | 0.20 | | 2.00E-05 | 1.61E-04 | 0.0002 | 0.002 |
| Methamidophos | 2012 | 0.15 | 4.0E-3 | 1.51E-05 | 1.22E-04 | 0.0038 | 0.0304 |
| Methiocarb | 2014 | 0.00 | 1.2E-2 | 4.23E-07 | 3.41E-06 | 0.0000 | 0.0003 |
| Methomyl | 2012 | 0.18 | 0.0025 | 1.80E-05 | 1.45E-04 | 0.0072 | 0.0581 |
| Myclobutanil | 2012 | 0.30 | 0.025 | 3.07E-05 | 2.47E-04 | 0.0012 | 0.0099 |
| | 2013 | 0.20 | | 2.02E-05 | 1.63E-04 | 0.0008 | 0.0065 |
| | 2014 | 0.23 | | 2.27E-05 | 1.83E-04 | 0.0009 | 0.0073 |
| Penconazole | 2012 | 0.10 | 0.03 | 1.05E-05 | 8.44E-05 | 0.0003 | 0.0028 |
| | 2013 | 0.06 | | 6.45E-06 | 5.19E-05 | 0.0002 | 0.0017 |
| | 2014 | 0.09 | | 8.92E-06 | 7.18E-05 | 0.0003 | 0.0024 |
| Pirimiphos-methyl | 2012 | 0.00 | 0.004 | 3.02E-07 | 2.43E-06 | 0.0001 | 0.0006 |
| Procymidone | 2012 | 0.01 | 0.0028 | 6.18E-07 | 4.98E-06 | 0.0002 | 0.0018 |
| Propargite | 2012 | 2.40 | 7.0E-3 | 2.42E-04 | 1.95E-03 | 0.0346 | 0.2781 |
| Propiconazole | 2013 | 0.00 | 0.04 | 1.61E-07 | 1.30E-06 | 0.0000 | 0 |
| | 2014 | 0.01 | | 5.09E-07 | 4.10E-06 | 0.0000 | 0.0001 |
| Pyraclostrobin | 2012 | 0.24 | 0.03 | 2.45E-05 | 1.97E-04 | 0.0008 | 0.0066 |
| | 2014 | 0.08 | | 7.79E-06 | 6.27E-05 | 0.0003 | 0.0021 |
| Pyridaben | 2012 | 0.03 | 0.01 | 3.48E-06 | 2.80E-05 | 0.0003 | 0.0028 |
| | 2013 | 0.01 | | 6.49E-07 | 5.22E-06 | 0.0001 | 0.0005 |
| | 2014 | 0.01 | | 9.64E-07 | 7.76E-06 | 0.0001 | 0.0008 |
| Pyrimethanil | 2012 | 0.59 | 0.17 | 5.90E-05 | 4.75E-04 | 0.0003 | 0.0028 |
| | 2013 | 0.09 | | 9.53E-06 | 7.67E-05 | 0.0001 | 0.0005 |
| | 2014 | 0.40 | | 4.05E-05 | 3.26E-04 | 0.0002 | 0.0019 |
| Tebuconazole | 2013 | 0.09 | 0.03 | 8.67E-06 | 6.98E-05 | 0.0003 | 0.0023 |
| Tetraconazole | 2012 | 0.16 | 0.004 | 1.65E-05 | 1.33E-04 | 0.0041 | 0.0332 |
| | 2013 | 0.15 | | 1.49E-05 | 1.20E-04 | 0.0037 | 0.0299 |
| | 2014 | 0.08 | | 8.08E-06 | 6.50E-05 | 0.0020 | 0.0163 |
| Thiamethoxam | 2013 | 0.46 | 2.5E-2 | 4.68E-05 | 3.76E-04 | 0.0018 | 0.0145 |
| Thiophanate-methyl | 2012 | 0.62 | 0.08 | 6.21E-05 | 5.00E-04 | 0.0008 | 0.0062 |
| | 2013 | 1.44 | | 1.45E-04 | 1.17E-03 | 0.0018 | 0.0146 |
| | 2014 | 1.23 | | 1.24E-04 | 1.00E-03 | 0.0016 | 0.0125 |
| Tolclofos-methyl | 2012 | 0.52 | 0.064 | 5.21E-05 | 4.20E-04 | 0.0008 | 0.0066 |
| Triadimenol | 2012 | 1.40 | 0.05 | 1.41E-04 | 1.14E-03 | 0.0028 | 0.0227 |
| Trifloxystrobin | 2012 | 0.04 | 0.1 | 4.17E-06 | 3.35E-05 | 0.0000 | 0.0003 |
| | 2013 | 0.07 | | 6.67E-06 | 5.36E-05 | 0.0001 | 0.0005 |
| | 2014 | 0.11 | | 1.09E-05 | 8.79E-05 | 0.0001 | 0.0009 |

*ADI : Acceptable Daily Intake retrieved from Lewis et al. 2016; **EDI: Estimated Daily Intake = $\sum \frac{F_i \times MR_i}{Bw}$;
MRi =mean residual pesticide concentration; Fi : daily grape consumption data. Bw corresponds to the body weight of the consumer. For adults, a body weight of 63 kg and grape consumption of 0.00635 kg/day (EFSA). For children, a body weight of 16.15 kg and grape consumption of 0.0131 kg/day (EFSA).
 $HQ = \left(\frac{EDI}{ADI} \right)$; HQ >1 =Potential risk for human health.

Environmental Impact

In our study, molecules with DT50s in soils greater than 180 days were found in 18, 19 and 26 % of instances in 2012, 2013 and 2014, respectively (Figure II-5). This high persistency, has an adverse impact on the soil fertility by affecting its integrity, beneficial macrofauna and microflora and making it a repository to these chemicals (Aktar, Sengupta, and Chowdhury 2009). That further contributes to the emergence of resistances to pesticides. Additionally, our results indicated that throughout the three years, in 12 – 35 % of instances, the detected molecules had moderately to high potential to move toward groundwaters with GUS Index values varying from 1.8 to 2.8 or exceeding 2.8 in 12-14 % of the instances (Figure II-5). These findings are further showcased in the work of Youssef et al. in 2015 in Lebanon's South Litani River, their research brought attention to the long-lasting presence of prohibited pesticides, particularly organochlorines such as aldrin, heptachlor epoxide, and Dichlorodiphenyltrichloroethane (DDT), within both surface water and groundwater (Youssef et al. 2015). The leaching of these residues into ground and surface waters poses significant concerns, as it exacerbates toxic residues bioaccumulation through food cycle. This phenomenon is particularly alarming when these waters are utilized for irrigation purposes or when these residues become accumulated within aquatic organisms, such as fish. Similarly, Abou Zeid et al. (2020) and Akkoush and Halawani (2023) reported the presence of multiple residues in soils, surface waters, ground and drinking waters as well as honey samples.

Additionally, our results show that, throughout the three years the detected molecules had the potential to bioaccumulate in around 64 % of instance with bioconcentration factors (BCF) of 1000-5000 L/Kg. Additionally, in 18, 7 and 8 % of instances, the detected molecules had BCFs greater than 5000 L/Kg in 2012, 2013 and 2014, respectively, indicating a very bioaccumulative potential. Altogether, these results are reflected by the fact that the environmental fate alerts of the detected molecules as per Lewis et al. (2016)'s alert system, was high in around 46 % of instances and moderate in around 54 % of instances throughout the years. Similarly, for the ecotoxicity alerts that were considered as high in 62 % of instances and moderate in 38% of instances. These findings underscore the pressing necessity for more stringent environmental regulations in Lebanon, with a focus on mitigating the widespread use of pesticides. Specifically, banning the utilization of phytosanitary molecules characterized by high persistence in the environment.

4. Conclusion

The 2012-2014 monitoring data showed high frequency of pesticide residues in table grape, 56 to 97 % of positive samples with 15-33 % exceeding MRLs for one or more pesticide residues additionally to the usage of unrecommended molecules on grapes (>19%). Although phytosanitary practices may have improved nowadays, the findings of this study reflected the importance to train viticulturists in Lebanon to apply good agricultural practices and ensure safe products. Although the human risk assessment indicated no immediate health risks for adults and children, the consumption of grapes contributes to the overall exposure to residues of these molecules, in particular chlorpyrifos which presents the highest HQ values. Nevertheless, the HQ calculations only considered the exposure to residues through grapes consumption and do not encompass the exposure of individuals to these molecules from other food and non-food sources, as well as the exposure of viticulturists and/or pesticide applicators, thus do not reflect individuals' overall exposure to these residues or the potential hazards of synergistic, analogous, and additive effects from multiple residue exposures. . To add, the high incidences of residue cocktails raise concerns over human health and environmental impacts (pest resistance). The findings of this study showed that stringent regulations of pesticide use in Lebanon in particular in vineyards are imperative in order to reduce the risks for the human and environment health.

References

- Abou Zeid, Mohamad I, Adla M Jammoul, Khalil C Melki, Yusuf Abou Jawdah, and Mireille Kallassy Awad. 2020. "Suggested Policy and Legislation Reforms to Reduce Deleterious Effect of Pesticides in Lebanon." *Heliyon* 6 (12): e05524. <https://doi.org/10.1016/j.heliyon.2020.e05524>
- Akkouch, Nisreen, and Jalal Halwani. 2023. "Pesticide Occurrence in Food, Water, and Human Biomonitoring in Lebanon-A Health Repercussion." *Annals of Epidemiology and Public Health* 6 (1): 1101.
- Aktar, Md. Wasim, Dwaipayan Sengupta, and Ashim Chowdhury. 2009. "Impact of Pesticides Use in Agriculture: Their Benefits and Hazards." *Interdiscip Toxicol* 2 (1): 1–12. <https://doi.org/10.2478/v10102-009-0001-7>
- Arkam, M., Alves, A., Lopes, A. et al. Diversity of Botryosphaeriaceae causing grapevine trunk diseases and their spatial distribution under different climatic conditions in Algeria. *Eur J Plant Pathol* 161, 933–952 (2021). <https://doi.org/10.1007/s10658-021-02377-7>

- Bois, Benjamin, S. Zito, and A. Calonnec. 2017. "Climate vs Grapevine Pests and Diseases Worldwide: The First Results of a Global Survey." *OENO One* 51 (2): 133–39. <https://doi.org/10.20870/OENO-ONE.2017.51.2.1780>
- Bouagga, A., H. Chaabane, K. Toumi, A. Mougou Hamdane, B. Nasraoui, and L. Joly. 2019. "Pesticide Residues in Tunisian Table Grapes and Associated Risk for Consumer's Health." *Food Additives & Contaminants: Part B* 12 (2): 135–44. <https://doi.org/10.1080/19393210.2019.1571532>
- Capone, R., el Chami, D., Cardone, G., Mai Bari, C., Adinolfi, F., and di Pasquale, J. 2014. Trade and Logistics: The case Of the Wine Industry. In CIHEAM (Ed.), *Logistics and Agro-Food Trade – A Challenge for the Mediterranean Area (Mediterra)*. Presses de Sciences Po.
- CBI. 2018. "Value Chain Analysis Lebanon Fresh Fruit and Vegetables." <https://www.cbi.eu/market-information/fresh-fruit-vegetables/vca-lebanon-fresh-fruit-vegetables-2018>
- Costa, J. Miguel, Ricardo Egipto, Francisca C. Aguiar, Paulo Marques, Amaia Nogales, and Manuel Madeira. 2023. "The Role of Soil Temperature in Mediterranean Vineyards in a Climate Change Context." *Frontiers in Plant Science* 14 (May): 1145137. <https://doi.org/10.3389/FPLS.2023.1145137/BIBTEX>
- Daniele, Penna., Paolo, Benettin. (2023). Ecohydrological dynamics and temporal water origin in European Mediterranean vineyards: a case study in Tuscany, Central Italy. doi: 10.5194/egusphere-egu23-16318
- Dumitriu (Gabur), G.-D., Teodosiu, C., & V. Cotea, V. (2022). Management of Pesticides from Vineyard to Wines: Focus on Wine Safety and Pesticides Removal by Emerging Technologies. IntechOpen. doi: 10.5772/intechopen.98991
- EC. 2023. "SANCO/12495/2011 EURL Residues of Pesticides Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed." <https://www.eurl-pesticides.eu/docs/public/tmpl/article.asp?CntID=727&LabID=100&Lang=EN>
- EFSA. 2023. "Pesticide Evaluation: Tools." 2023. <https://www.efsa.europa.eu/en/applications/pesticides/tools>
- Elmarsafy, Ashraf M., and Taher M. Kadah. 2018. "Measuring the Economic Impact and Health Risks of Pesticide Residues in Potatoes and Grapes Crops." *Egyptian Journal of Agricultural Research* 96 (3): 1203–28. <https://doi.org/10.21608/EJAR.2018.141160>
- FAO-WHO. 2023. "Guidelines | CODEXALIMENTARIUS FAO-WHO." 2023.

<https://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/>

- Fouillet, E., Delière, L., Chartier, N., Munier-Jolain, N., Cortel, S., Rapidel, B., & Merot, A. (2022). Reducing pesticide use in vineyards: Evidence from the analysis of the French DEPHY network. *European Journal of Agronomy*, 136, 126503. <https://doi.org/10.1016/j.eja.2022.126503>
- Gamet-Payrastre, Laurence. 2019. « Impact des mélanges de pesticides. » . *Innovations Agronomiques* 73 : 51-59. <https://doi.org/10.15454/3WKNA9.hal-02282298>
- Golge, Ozgur, and Bulent Kabak. 2018. “Pesticide Residues in Table Grapes and Exposure Assessment.” *Journal of Agricultural and Food Chemistry* 66 (7): 1701–13. https://doi.org/10.1021/ACS.JAFC.7B05707/ASSET/IMAGES/MEDIUM/JF-2017-05707A_0004.GIF
- Halleen, F., and G. Holz. 2017. “An Overview of the Biology, Epidemiology and Control of *Uncinula Necator* (Powdery Mildew) on Grapevine, with Ref Ere Nee to South Africa.” *South African Journal of Enology & Viticulture* 22 (2). <https://doi.org/10.21548/22-2-2205>
- Hawari, Khaled El, Samia Mokh, Mohamad Al Iskandarani, Wafaa Halloum, and Farouk Jaber. 2019. “Pesticide Residues in Lebanese Apples and Health Risk Assessment.” *Food Additives & Contaminants: Part B* 12 (2): 81–89. <https://doi.org/10.1080/19393210.2018.1564370>
- Hobeika, Charbel, and Dani El Khouri. 2011. “CGP/LEB/021/ITA - A Grower’s Guide to Good Agricultural Practices for Table Grapes.” Ministry of Agriculture - Lebanon. 2011.
- IRAC. 2023. “Mode of Action Classification Insecticide Resistance Management.” IRAC. <https://irac-online.org/mode-of-action/classification-online/>
- ISTAT. 2011. “The Use of Phytosanitary Products in Wine Grape.” 2011. <https://www.istat.it/en/archivio/20219>
- Jaume, Gil., Elena, Alter., Maria, José, La, Rota., Enric, Tello., Vittorio, Galletto., Roc, Padró., Teresa, Martínez., Soazig, Darnay., Joan, Marull. (2022). Towards an agroecological transition in the Mediterranean: A bioeconomic assessment of viticulture farming. *Journal of Cleaner Production*, 380:134999-134999. doi: 10.1016/j.jclepro.2022.134999
- João, Prada., Lia-Tânia, Dinis., Elia, Soriato., Elodie, Vandelle., Oğuzhan, Soltekin., Şener, Uysal., Abdelhi, Dihazi., Conceição, Santos., João, A., Santos. (2024). Climate change impact on Mediterranean viticultural regions and site-specific climate risk-reduction strategies. *Mitigation and Adaptation Strategies for Global Change*, 29(6) doi: 10.1007/s11027-024-10146-0
- Khazaal, Salma, Nada El Darra, Amal Kobeissi, Rola Jammoul, and Adla Jammoul. 2022. “Risk

- Assessment of Pesticide Residues from Foods of Plant Origin in Lebanon.” *Food Chemistry* 374 (April): 131676. <https://doi.org/10.1016/j.foodchem.2021.131676>
- Koubeissy, Amal. 2014. “The Challenge of Facilitating Exports of Table Grapes In Agriculture in Lebanon.” Dekweneh, Lebanon: Lebanese University.
- Lelay, G., Roger, T., Delhomme, B., and Roby, J.-P. (2003). La filière viticole au Liban : Analyse et propositions pour une évolution. ENITA de Bordeaux.
- Lewis, Kathleen A, John Tzilivakis, Douglas J Warner, and Andrew Green. 2016. “An International Database for Pesticide Risk Assessments and Management.” *Human and Ecological Risk Assessment: An International Journal* 22 (4): 1050–64. <https://doi.org/10.1080/10807039.2015.1133242>
- Li, Changjian, Huimin Zhu, Changyan Li, He Qian, Weirong Yao, and Yahui Guo. 2021. “The Present Situation of Pesticide Residues in China and Their Removal and Transformation during Food Processing.” *Food Chemistry* 354: 129552. <https://doi.org/10.1016/j.foodchem.2021.129552>
- Lia-Tânia, Dinis., Sara, Bernardo., Chenyao, Yang., Helder, Fraga., Aureliano, C., Malheiro., José, Moutinho-Pereira., João, A., C., Santos. (2022). Mediterranean viticulture in the context of climate change. *Ciencia E Tecnica Vitivinicola*, 37(2):139-158. doi: 10.1051/ctv/ctv20223702139
- Limon-Miro, Ana Teresa, Maria Lourdes Aldana-Madrid, Gerardo Alvarez-Hernandez, Lesley Evelyn Antunez-Roman, Guillermo Rodriguez-Olibarria, and Mauro E Valencia Juillerat. 2017. “Breast Milk Intake and Mother to Infant Pesticide Transfer Measured by Deuterium Oxide Dilution in Agricultural and Urban Areas of Mexico.” *Chemosphere* 181 (August): 682–89. <https://doi.org/10.1016/j.chemosphere.2017.04.110>
- Massi, Federico, Torriani, Stefano F.F., Borghi, Lorenzo, & Toffolatti, Silvia L. 2021. Fungicide Resistance Evolution and Detection in Plant Pathogens: Plasmopara viticola as a Case Study. *Microorganisms*, 9(1), 1–18. <https://doi.org/10.3390/MICROORGANISMS9010119>
- MoA. 2012. “Decision No. 380, Dated March 5, 2012, Subjects Table Grapes and Apples to Pesticide Residue Testing.” *The Lebanese University - Center for Legal Informatics*. <http://77.42.251.205/LawView.aspx?opt=view&LawID=231764>
- Mostafalou, Sara, and Mohammad Abdollahi. 2016. “Pesticides: An Update of Human Exposure and Toxicity.” *Archives of Toxicology* 2016 91:2 91 (2): 549–99. <https://doi.org/10.1007/S00204-016-1849-X>

- Odewale, Gideon Oluwasogo, Mosudi Babatunde Sosan, John Adekunle Oyedele Oyekunle, and Adeoluwa Oluwaseyi Adeleye. 2022. "Assessment of Systemic and Carcinogenic Health Risks of Persistent Organochlorine Pesticide Residues in Four Fruit Vegetables in South-Western Nigeria." *British Food Journal* 124 (5): 1755–74. <https://doi.org/10.1108/BFJ-05-2021-0573/FULL/XML>
- Pathak, Vinay Mohan, Vijay K. Verma, Balwant Singh Rawat, Baljinder Kaur, Neelesh Babu, Akansha Sharma, Seeta Dewali, et al. 2022. "Current Status of Pesticide Effects on Environment, Human Health and It's Eco-Friendly Management as Bioremediation: A Comprehensive Review." *Frontiers in Microbiology* 13 (August): 962619. <https://doi.org/10.3389/FMICB.2022.962619/BIBTEX>
- Poulsen, M. E., H. K. Hansen, J. J. Sloth, H. B. Christensen, and J. H. Andersen. 2007. "Survey of Pesticide Residues in Table Grapes: Determination of Processing Factors, Intake and Risk Assessment." *Food Additives and Contaminants* 24 (8): 886–95. <https://doi.org/10.1080/02652030701245320>
- PPDB. 2023, November. Rules for generating data alerts. Accessed 22/02/2024 https://sitem.herts.ac.uk/aeru/ppdb/en/docs/Data_alerts_rules.pdf
- Salameh, Pascale R., Isabelle Baldi, Patrick Brochard, and Bernadette Abi Saleh. 2004. "Pesticides in Lebanon: A Knowledge, Attitude, and Practice Study." *Environmental Research* 94 (1): 1–6. [https://doi.org/10.1016/S0013-9351\(03\)00092-6](https://doi.org/10.1016/S0013-9351(03)00092-6)
- Sameut Bouhaik, Azzeddine. 2009. "Modélisation de dépôt des pesticides sur des plans d'eau." 5, boulevard Descartes Champs-sur-Marne 77454 Marne-la-Vallée Cedex 2: Université Paris-Est Marne-la-Vallée - Laboratoire de Géo matériaux et Environnement. https://www.generations-futures.fr/wp-content/uploads/2019/09/rapportfinalazzed_repaired.pdf
- Sánchez-Bayo, Francisco, Koichi Goka, and Daisuke Hayasaka. 2016. "Contamination of the Aquatic Environment with Neonicotinoids and Its Implication for Ecosystems." *Frontiers in Environmental Science* 4. <https://www.frontiersin.org/articles/10.3389/fenvs.2016.00071>
- Schusterova, Dana, Jana Hajslova, Vladimir Kocourek, and Jana Pulkrabova. 2021. "Pesticide Residues and Their Metabolites in Grapes and Wines from Conventional and Organic Farming System." *Foods* 2021, Vol. 10, Page 307 10 (2): 307. <https://doi.org/10.3390/FOODS10020307>
- Shao, Wenyong, Youfu Zhao, and Zhonghua Ma. 2021. "Advances in Understanding Fungicide

- Resistance in *Botrytis Cinerea* in China.” *Phytopathology* 111 (3): 455–63.
<https://doi.org/10.1094/PHYTO-07-20-0313-IA/ASSET/IMAGES/LARGE/PHYTO-07-20-0313-IAT1.JPEG>
- Smadi, Nadia, Adla Jammoul, and Nada El Darra. 2019. “Assessment of Antibiotic and Pesticides Residues in Breast Milk of Syrian Refugee Lactating Mothers.” *Toxics* 7 (3): 39.
<https://doi.org/10.3390/toxics7030039>
- Tang, Fiona H M, Manfred Lenzen, Alexander McBratney, and Federico Maggi. 2021. “Risk of Pesticide Pollution at the Global Scale.” *Nat. Geosci.* 14 (4): 206–10.
<https://doi.org/10.1038/s41561-021-00712-5>
- Trade, Ministry of Economy&Trade. 2022. “LEBTRADE-Table Grapes.” *LEBTRADE*.
<https://lebtrade.gov.lb/>.
- Tucker, Samuel, Georgiana Diana Dumitriu, and Carmen Teodosiu. 2022. “Pesticides Identification and Sustainable Viticulture Practices to Reduce Their Use: An Overview.” *Molecules* 2022, Vol. 27, Page 8205 27 (23): 8205. <https://doi.org/10.3390/MOLECULES27238205>
- Tudi, Muyesaier, Huada Daniel Ruan, Li Wang, Jia Lyu, Ross Sadler, Des Connell, Cordia Chu, and Dung Tri Phung. 2021. “Agriculture Development, Pesticide Application and Its Impact on the Environment.” *Int J Environ Res Public Health* 18 (3): 1112.
<https://doi.org/10.3390/ijerph18031112>
- WHO. 2018. “Generic Risk Assessment Model for Insecticides Used for Larviciding and Mollusciciding — Second Edition.” <https://www.who.int/publications-detail-redirect/9789241515047>
- WHO. 2019. “The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification, 2019 Edition.” <https://www.who.int/publications-detail-redirect/9789240005662>
- World Weather. 2023. “Local Weather Forecast.” 2023. <https://en.tutiempo.net/>
- Youssef, Lobna, Ghassan Younes, Abir Kouzayha, and Farouk Jaber. 2015. “Occurrence and Levels of Pesticides in South Lebanon Water.” *Chemical Speciation & Bioavailability* 27 (2): 62–70.
<https://doi.org/10.1080/09542299.2015.1023092>
- Zubrod, J. P., Bundschuh, M., Arts, G., Brühl, C. A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J. J., Rohr, J., Scharmüller, A., Smalling, K., Stehle, S., Schulz, R., & Schäfer, R. B. (2019). Fungicides: An Overlooked Pesticide Class?. *Environmental science & technology*, 53(7), 3347–3365. <https://doi.org/10.1021/acs.est.8b04392>

CHAPITRE III

Effets de la formulation sur la dissipation de l'imidaclopride dans les raisins et les feuilles de vigne ainsi que sur les durées avant récolte nécessaires dans les conditions climatiques libanaises

Les résultats de ce chapitre ont été publiés ainsi :

Majed L., Hayar S., Zeitoun R., Maestroni B.M. & Dousset S. 2022. The effects of formulation on imidacloprid dissipation in grapes and vine leaves and on required pre-harvest intervals under Lebanese climatic conditions. Molecules, 27, 252. <https://doi.org/10.3390/molecules27010252> (Annexe II-1)

Résumé synthétique

Les teneurs en résidus de pesticides dans les différentes matrices sont influencées par divers facteurs parmi lesquels les propriétés physico-chimiques de la matière active du pesticide, le type de formulation de la spécialité commerciale, etc. Ce dernier facteur a été peu étudié. En effet, une spécialité commerciale correspond à un mélange de matières actives et d'adjuvants de divers ordres. L'objectif de ces adjuvants est d'améliorer l'efficacité de la matière active en fonction de divers paramètres : conditions météorologiques, etc. Des résultats contradictoires peuvent être trouvés dans la littérature. Ainsi, les diverses études réalisées sur l'effet de la formulation sur des matrices telles que les pommes, les oranges, les tomates et les raisins ont montré que les formulations pouvaient affecter soit les dépôts initiaux des résidus, soit la dissipation des résidus, soit les deux, ou bien qu'elles n'affectaient pas ces paramètres.

Dans cette étude, l'imidaclopride, un insecticide systémique, a été appliqué sur un vignoble, situé à Tamnine-El-Tahta, Gouvernorat de Baalbeck Hermel au Liban comprenant 1200 pieds de vigne, couvrant une superficie de 5000 m², divisée en deux parcelles (2500 m²) dont chacune a été traitée par une des formulations commerciales : Diclean 20% sous forme de liquide soluble (SL) ou Pilarcking Plus 70% sous forme de granulés dispersables dans l'eau (WDG). L'objectif de ce travail était d'étudier l'effet de la matrice (feuilles et raisins) sur la dissipation des pesticides pour les deux formulations et d'estimer les durées avant la récolte (DAR). Ainsi, des échantillonnages aléatoires de raisins et de feuilles de vigne ont été effectués du 2^{ème} au 18^{ème} jours après le traitement. Les extractions de résidus de pesticides ont été réalisées selon la méthode QuEChERS et les quantifications des teneurs en résidus par LC-MS-MS.

Bien que la formulation SL ait présenté un dépôt initial significativement plus élevé que la formulation WDG sur les raisins et les feuilles de vigne, l'étude de la cinétique de dissipation a révélé des demi-vies de 0,5 jour pour les deux formulations dans les deux matrices. Aussi, indépendamment du type de formulation, les résidus d'imidaclopride sont de 20 à 70 fois plus élevés dans les feuilles de vigne que dans les raisins à tous les moments d'échantillonnage pour l'ensemble des échantillons analysés. Le mode de conduite de la vigne

en pergola, les différences morphologiques et physiologiques entre les feuilles de vigne et les raisins et le fait que les feuilles recouvrent les raisins expliqueraient ces résultats.

Par conséquent, aucune DAR ne semble nécessaire sur les raisins (< 24 heures) pour les deux formulations. Sur les feuilles de vigne, pour une LMR de 2 mg. kg⁻¹, des DARs de 3,7 jours pour la formulation SL et de 2,8 jours pour la formulation WDG a été estimée. En revanche, pour une LMR de 0,01 mg.kg⁻¹, des DARs de 7 et 6 jours ont été estimées pour les formulations SL et WDG, respectivement. L'importance de spécifier une LMR pour les feuilles de vigne a été démontrée puisqu'avec une LMR de 2 mg.kg⁻¹, il était envisageable de procéder à la cueillette quotidienne/bihebdomadaire des feuilles tout en respectant les normes. Cependant, pour des LMR à 0,01 mg.kg⁻¹, les DARs correspondantes ne reflétaient pas la fréquence de récolte pendant la saison d'effeuillage gastronomique.

The effects of formulation on imidacloprid dissipation in grapes and vine leaves and on required pre-harvest intervals under Lebanese climatic conditions

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Abstract: In this study, imidacloprid, a systemic insecticide, currently having a specified European Commission MRL value for vine leaves (2 mg.kg⁻¹), was applied on a Lebanese vineyard under different commercial formulations: as a soluble liquid (SL) and water dispersible granules (WDG). In Lebanon many commercial formulations of imidacloprid are subject to the same critical good agricultural practice (cGAP). It was, therefore, important to verify the variability in dissipation patterns according to matrix nature and formulation type. Random samplings of grapes and vine leaves were performed starting 2 days until 18 days after treatment. Residue extractions were performed according to the QuEChERS method and the analytical determination using liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS). The SL formulation yielded significantly higher initial deposit than the WDG formulation on grapes and vine leaves. The formulation type did not significantly affect the dissipation rates; the estimated half-lives in grapes and vine leaves were 0.5 days for all imidacloprid formulations. No pre-harvest intervals were necessary on grapes. PHIs of 3.7 days for the SL formulation and 2.8 days for the WDG formulation were estimated on vine leaves. The results showed that the type of formulation and the morphological and

physiological characteristics of the matrix had an effect on the initial deposits, and thus residue levels, but not on the dissipation patterns.

Keywords: *Imidacloprid; Vine leaves; Grape; QuEChERS; SL and WDG Formulation; Dissipation; Half-life; Pre-harvest Intervals*

1. Introduction

Grapevines are cultivated all over the world, yielding a wide range of products that are part of our daily diet. Grapes, the most economically important product, can be used to make juice, jellies, wine, and pies, and the leaves can be used in cooking [1–3]. In Lebanon and nearby countries, vine leaves are commonly used in the preparation of a number of traditional dishes, especially the famous dishes in “Lebanese Mezze”. As with any other plant, grapevines are vulnerable to fungal and pest infestation and thus the use of phytosanitary products may be unavoidable in order to prevent and control any occurring disease to increase the yield [1,3].

However, the use of these products may be harmful to final consumers since they could be exposed to residues of phytosanitary molecules through their daily diet [4]. To overcome this challenge and make good use of pesticides without compromising human and environmental health, national and international bodies, mainly the European Commission (EC) and Codex Alimentarius, have specified legal limits for residues in food, i.e., maximum residues limits (MRLs).

Ensuring that residues are below MRLs is of high importance for producers to meet regulatory and market requirements. Yet such a goal may be impossible to attain for some crops due to lack of specific MRLs. This the case for vine leaves, for which no specified legal limits for pesticide residue levels have been set by national and international organizations, and, as a consequence, a MRL that corresponds to the limit of detection (LOD) of the analytical method for the molecules applied on grapevines is assigned for this commodity, i.e. at the European Union level [5–9].

In the field, MRLs are the benchmark against which it is possible to set the preharvest interval values (PHI). PHI corresponds to the time gap between pesticide applications and the crop harvest in order to yield a healthy product that is in compliance to the legal limit [3]. Many studies concluded that the molecules’ physiochemical properties, the formulation properties, the local climatic conditions, and the plant physiology could affect the main two

parameters used in pesticide residues studies, i.e., dissipation rates and PHIs [3,10–12]. That is why any possible factor affecting these two previously mentioned parameters must be investigated in order to identify the different variables involved and to gain a better understanding of their interactions.

Formulating a pesticide is about combining an active ingredient with compatible “inerts” or “inactive ingredients”. Inerts are present to achieve specific results; they can be emulsifiers, petroleum solvents, wetting agents or UV-light blocking chemicals, etc. that increase the persistence of active ingredients and enhance their application and performance [13]. Throughout the industry, pesticide products are marketed as emulsifiable concentrates (EC), microencapsulated formulations (ME), flowable (F), water dispersible granules (WDG), sprayable (S), wettable powders (WP), among others. The type and amount of inert ingredients give the phytosanitary product its uniqueness and thus allows distinction between phytosanitary product lines and markets. Therefore, when selecting which formulation to use, farmers have to take into consideration the potential influence of formulants on pesticide efficacy, and more importantly, their potential impact on residues level in crops [14], which is one of the biggest concerns for producers. In fact, non-conclusive results can be found throughout the literature pertaining to the effect of formulation type on residues dissipation. Cabras et al. [15] stated that liquid formulations yield more residues compared to granulated ones and Abdel-Hamid et al. [11] correlated the initial deposit of pesticides on tomato fruits to the variation of physical and chemical properties among pesticide formulations. They demonstrated that EC formulations of fenpyroximate showed higher persistence compared to suspension concentrate (SC) formulations on tomato fruits, the same for imidacloprid where they compared four different formulations (SC, WDG, SL and WP) and found lower initial deposits with higher degradation rates for the SC formulation compared to the others [11]. Buzzetti [16] also demonstrated that the pesticide formulation of acetamiprid, imidacloprid and diazinon had an effect on the initial pesticide deposit and persistence on apple samples, but not in case of I-cyhalothrin. Montemurro et al. [17] compared three different formulations of chlorpyrifos and showed different dissipation rates for EC and WG formulations as compared to ME formulation in orange fruits, but surprisingly they observed a similar behavior for the three formulations (EC, WG and ME) in orange leaves and soil. In contrast, after conducting four comparative dissipation studies of 3 commercial formulations of penconazole 10% EC on four varieties of tomatoes Abou Zeid et al. [18]

concluded that there was no statistically significant difference in rate of dissipation among the 3 evaluated EC formulations. Similarly, Alister et al. [12] concluded that formulation type (SC, SL and WP) did not have a significant effect on initial deposit and dissipation rates of acetamiprid, buprofazine and fenhexamid on apple fruits and grape berries.

Imidacloprid, 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, a predominantly systemic insecticide, is extensively used for the control of a wide range of insects and pests at various stages of grape cultivation especially thrips and mealybug [19]. It is important to note that imidacloprid is no longer approved for use by the European Commission since 1/12/2020 according to EU resolution EU/2020/1643 [20]. Starting June 2022 import tolerances will be applied, and the applicable MRLs will be 0.7 mg.kg⁻¹ and 0.01 mg.kg⁻¹ for grapes and vine leaves respectively; the latter value corresponds to the lower limit of analytical determination for vine leaves [20]. These new MRLs will be replacing the currently approved MRLs of 1 mg.kg⁻¹ and 2 mg.kg⁻¹ for grapes and vine leaves respectively (Reg. (EU) No 491/2014) [21]. As stated by the EU pesticide data base these modifications were not implemented due to toxicological concerns but due to unavailability of data. In Lebanon, 16 commercial formulation products, registered under different trade names, contain imidacloprid as the main active ingredient. They are subject to the same critical good agricultural practice (cGAP), that is the same PHIs and the same application rates despite the fact they differ in the composition of co-formulants [18]. It was therefore important to study the variability of the imidacloprid formulation type as soluble liquid concentrate (SL) or as water dispersible granules (WDG) on the dissipation rates and the PHIs on grapes and vine leaves under Lebanese climatic conditions.

2. Results and Discussion

2.1. SL- and WDG-Imidacloprid dissipation kinetics

The statistical analysis of imidacloprid residues concentration data showed that the first order decay model according to eq. (1) was a useful approximation of the data up to day 12 (Figure III- 1 (a)). The data relative to day 18 data did not fit the first order decay model. Therefore, a two-compartment model was formulated; however, there were insufficient data to establish the point of change from the first to the second compartment as well as the rate of decline in the second compartment. Therefore, an alternative statistical model, called the

continuous change model, was proposed to fit the data. In this model the half-life is steadily increasing with time. The rate of imidacloprid dissipation after day 12 is very slow but still occurring according to the model. Such a model was considered satisfactory (Figure III- 1 (b)), see also Figure SIII 1; SIII 2 and Table SIII 1; SIII 2 in the supplementary materials (Annexe II- 2). In this model the slopes of the fitted lines did not differ significantly from each other. The pooled slope of the regression lines (corresponding to the K_{diss}) was -1.269 ± 0.068 . Significant differences were found for the regression intercepts as shown in Table 1.

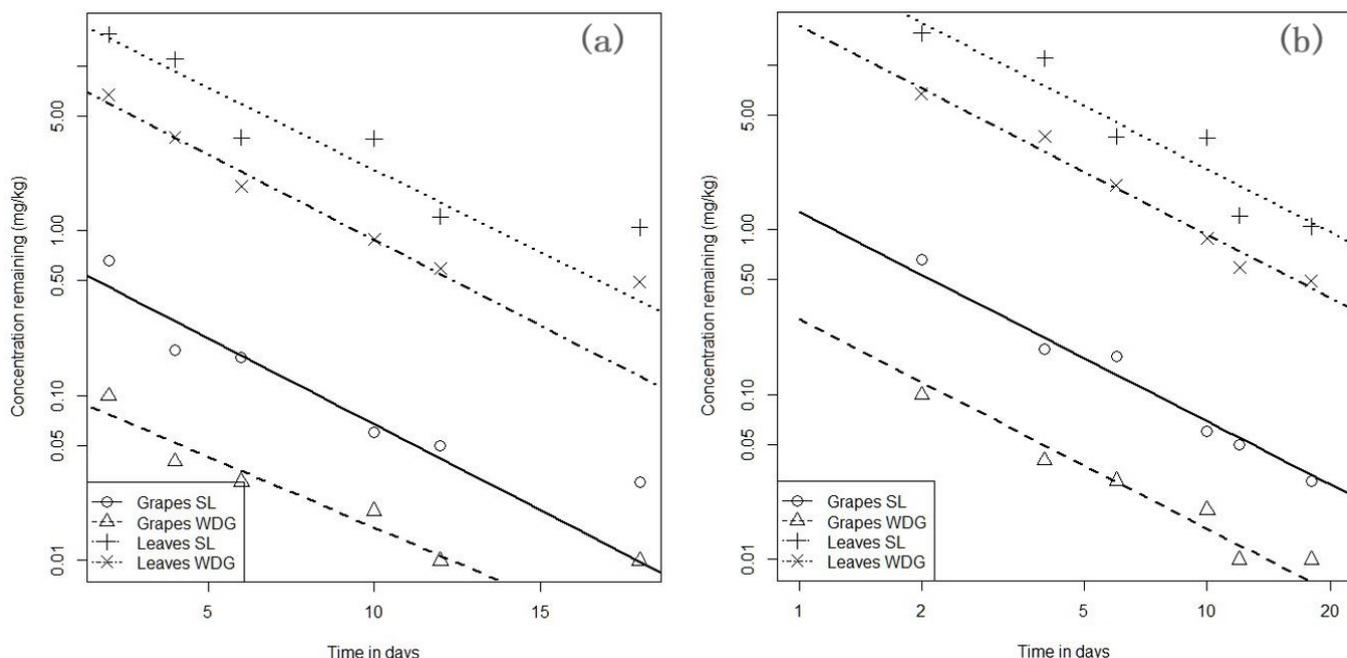


Figure III- 1: (a) Plot of residual Imidacloprid on a linear-log scale using a first order decay model. (b) Continuous change model of imidacloprid degradation with pooled slope but individual intercepts.

Table III- 1: Regression equations, dissipation rates, half-life and estimated PHI according to European Union 2021 MRLs (EU 2021) and to European Union 2022 MRLs (EU 2022) for grapes and vine leaves.

| Grapes | | | | | | | | |
|--------------------------|------------------------|---------------------|---------------|-------------------------|------------|---------|----------------------------|---------|
| Imidacloprid Formulation | Regression equation | Slope (k) | Intercept (b) | DT ₅₀ (days) | PHI (days) | | MRL (mg.kg ⁻¹) | |
| | | | | | EU 2021 | EU 2022 | EU 2021 | EU 2022 |
| SL | $y = 1.28e^{-1.269x}$ | -1.269 (± 0.068) | 0.249 | 0.546 | 0.196 | 0.477 | 1 | 0.7 |
| WDG | $y = 0.29e^{-1.269x}$ | -1.269 (± 0.068) | -1.249 | 0.546 | -0.984 | -0.703 | 1 | 0.7 |
| Vine Leaves | | | | | | | | |
| Imidacloprid Formulation | Regression equation | Slope (k) | Intercept (b) | DT ₅₀ (days) | PHI (days) | | MRL (mg.kg ⁻¹) | |
| | | | | | EU 2021 | EU 2022 | EU 2021 | EU 2022 |
| SL | $y = 43.55e^{-1.269x}$ | -1.269 (± 0.068) | 3.774 | 0.546 | 2.428 | 6.603 | 2 | 0.01* |
| WDG | $y = 17.37e^{-1.269x}$ | -1.269 (± 0.068) | 2.855 | 0.546 | 1.704 | 5.879 | 2 | 0.01* |

*Indicates the lower limit of detection.

2.2. Matrix and Residue levels

Regardless the formulation type, imidacloprid residues were found to be 20 to 70 times higher in vine leaves than in grapes at all sampling times for all of the analyzed samples (Table III - 2). The literature relates these finding to the morphological and physiological differences between vine leaves and grapes and to the fact that grapes are covered by the leaves i.e., greater contact surface.

Table III- 2: Residues of imidacloprid (SL and WDG) in grapes and vine leaves (n= 5).

| Imidacloprid Formulation | Grapes | | | | | |
|--------------------------|---|---------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| | Mean Concentration (\pm SD) in mg.kg ⁻¹ | | | | | |
| | T ₂ | T ₄ | T ₆ | T ₁₀ | T ₁₂ | T ₁₈ |
| SL* | 0.66 (\pm 0.031) ^a (0) ^b | 0.19 (\pm 0.020) (64.1) | 0.17 (\pm 0.012) (67.9) | 0.06 (\pm 0.003) (88.7) | 0.05 (\pm 0.006) (90.5) | 0.03 (\pm 0.008) (94.3) |
| WDG** | 0.10 (\pm 0.004) (0) | 0.04 (\pm 0.008) (50) | 0.03 (\pm 0.001) (62.5) | 0.02 (\pm 0.018) (75) | 0.01 (\pm 0.001) (87.5) | 0.01 (\pm 0.003) (87.5) |
| Imidacloprid Formulation | Vine Leaves | | | | | |
| | Mean Concentration (\pm SD) in mg.kg ⁻¹ | | | | | |
| | T ₂ | T ₄ | T ₆ | T ₁₀ | T ₁₂ | T ₁₈ |
| SL | 15.60 (\pm 0.960) (8.77) | 11.00 (\pm 0.780) (35.7) | 3.69 (\pm 0.510) (78.4) | 3.64 (\pm 0.501) (78.7) | 1.22 (\pm 0.300) (92.8) | 1.05 (\pm 0.230) (93.8) |
| WDG | 6.71 (\pm 0.148) (0) | 3.68 (\pm 0.580) (42.5) | 1.87 (\pm 0.019) (70.8) | 0.89 (\pm 0.090) (86.1) | 0.59 (\pm 0.210) (90.8) | 0.49 (\pm 0.111) (92.3) |

^a Mean \pm standard deviation of five replications

^b Figures in parentheses indicate cumulative % dissipation through time

*SL: soluble liquid **WDG: water dispersible granules

According to Edwards [22], the distribution, retention and ab/adsorption of pesticides in/on plant tissues are greatly influenced by plant morphological and physiological characteristics. Also, Maclachlan and Hamilton [10] stated that complex factors dictate the quantity of pesticide initially deposited and retained on leaves i.e., their nature, the phytosanitary molecules' proprieties and abiotic factors such as wind speed, temperature and humidity. They, [10], also underlined the importance to take canopy density and crop leaf surface into consideration when it comes to spray deposits, given that the canopy acts as a filter of spray droplets and thus deeper parts of the plant far from spray nozzle may receive less spray.

Furthermore, Lichiheb et al. [23] and Fernández and Eichert [24] mentioned that leaf cuticle (permeability of leaf surface) and pesticide lipophilicity are two of the main factors

influencing pesticide penetration in plants. Possingham et al. [25] studied wax structure and composition of leaves and fruit of *Vitis vinifera* and found a “considerable qualitative difference between the waxes of leaves and fruits” where grapes’ cuticular wax consisted of “hard” wax component (70%); i.e., oleanolic acid; and “soft” wax component; i.e., mixture of long chain acid, alcohols, aldehydes, ester and hydrocarbons; meanwhile leaves had only the “soft” fraction.

Since diffusion is the main process for insecticide penetration [26], the fact that cuticular waxes affect that process by reducing solutes mobility [27] and that pesticide transfer are driven by its lipophilicity and concentration [3]. It is thus harder for molecules with low K_{ow} ($\log P = 0.57$) and high water solubility (610 mg.kg^{-1}) like imidacloprid [28] to move through grapes’ than through leaves’ cuticular waxes, which explains higher residues found in leaves compared to grapes regardless of leaves’ density and vines’ conducting system (pergola).

Hence, our results underline the impact of plants’ nature and morphology on the amount and distribution of residues across plant parts and are in agreement with results obtained by Alister et al. [12], Bletsou et al. [29], Abdallah [30] and Hanafi et al. [31]. Bletsou et al. [29] showed the effect of leaf density, where they used higher application rates of bifenthrin in beans (2.9 kg.ha^{-1}) than in peas (2.2 kg.ha^{-1}), and found 2.5 times less initial deposit on green beans compared to peas. This result was related to morphological structure differences as green beans did not receive most of the spraying solution due to coverage by their leaves, while pea pods, having smaller leaves, were almost totally exposed to spraying [29].

As in our paper, Abdallah [30] also found higher residues of chlorfenapyr and difenoconazole in vine leaves compared to grapes. Cuticular wax chemistry and structural arrangement, which influence pesticide penetration [26,32], change according to fruit type and growth stage. Alister et al. [12] endorsed the effect of cuticular wax on pesticide penetration, where they concluded that fruit growth stage was the predominant parameter affecting pesticide initial deposit and dissipation rate, and that the effect of environmental parameters, such as rain, are important to consider but ultimately is the fruit type that determines the amount of pesticide penetration. Finally, Hanafi et al. [31] used the same application rate of imidacloprid (0.625 kg.ha^{-1}) and oxamyl (1.8 kg.ha^{-1}) on green beans and chili peppers, and found residue level for both molecules higher in green beans compared to chili peppers, similarly, they attributed these findings to morphological characteristic of each plant and to the so-called “dilution-effect” related to the growth stage.

2.3. Formulation and residues level

As shown in Table III- 2, despite a lower application amount per unit area ($0.07 \text{ kg}\cdot\text{ha}^{-1}$ for SL and $0.21 \text{ kg}\cdot\text{ha}^{-1}$ for WDG), higher initial residues levels were found in vine leaves and grapes treated with SL- imidacloprid compared to WDG- imidacloprid. The finding that the SL formulation yielded more residues than the WDG formulation is in accordance with the results obtained by Buzzetti [16], where in her work on apples, higher residue levels, initial and final deposits, of imidacloprid were found when applied as SL formulation (initial: $1.20 \text{ mg}\cdot\text{kg}^{-1}$, final: $0.47 \text{ mg}\cdot\text{kg}^{-1}$) compared to WP (initial: $0.90 \text{ mg}\cdot\text{kg}^{-1}$, final: $0.30 \text{ mg}\cdot\text{kg}^{-1}$) and soluble concentrate (SC) (initial: $0.89 \text{ mg}\cdot\text{kg}^{-1}$, final: $0.29 \text{ mg}\cdot\text{kg}^{-1}$) formulations. The author inferred that the variations of the ratio and nature of the other components of the formulated product (adjuvants, surfactant, inerts...) was behind the variation of the level of residues detected between SL, WP and SC formulations, despite the fact that all the treatments were performed in a way to obtain the same dose of active ingredient per hectare [16]. Also, Buzzetti [16] explained the similarity of the level of residues of the WP and SC formulations to be due to the fact that both have in common that they form suspensions on water compared to the SL formulation that forms a solution. This is a similar situation as the study described in this paper where a SL formulation and another suspension forming formulation (WDG) are compared.

Abdel-Hamid et al. [11] also reported a great influence of the formulation type on the residue level, more precisely on the initial deposits, when comparing 4 different formulations (SL, WDG, SC and WP) of imidacloprid in their two consecutive year study (2009 and 2010). However, and contrary to Buzzetti [16] and with the results presented in this study, among the 4 formulations they studied, they reported higher residues level in tomatoes for imidacloprid WDG formulation (initial: $4.55 \text{ mg}\cdot\text{kg}^{-1}$ in 2009 and $3.68 \text{ mg}\cdot\text{kg}^{-1}$ in 2010, final: $0.51 \text{ mg}\cdot\text{kg}^{-1}$ in 2009 and $0.30 \text{ mg}\cdot\text{kg}^{-1}$ in 2010) compared to SL formulation (initial: $3.11 \text{ mg}\cdot\text{kg}^{-1}$ in 2009 and $2.49 \text{ mg}\cdot\text{kg}^{-1}$ in 2010, final: $0.05 \text{ mg}\cdot\text{kg}^{-1}$ in 2009 and below the detection limit in 2010) [11]. Taken together, these findings support the hypothesis that the formulation type has an impact on the level of residues on vine leaves and grapes according to the Food and Agriculture Organization (FAO) statement “while having the same concentration of an active ingredient two products are not considered similar if they have different formulations or have different synthesizing methods” [16].

The dissipation rates (k) of SL and WDG formulations are shown in Table 1. Despite the previously discussed higher initial deposits of the SL formulation compared to WDG's, and the higher residues found on vine leaves than on grapes, the two formulations followed the same dissipation patterns and had quite similar dissipation rates of 1.269 day^{-1} on grapes and vine leaves, leading to similar half-lives of 0.5 day for the two formulations.

Pre-harvest intervals were estimated according to the MRLs set by the European Commission on vine leaves and grapes (Table III- 1). For grapes, no PHIs were necessary since all estimated PHIs values were less than one day for the two formulations, which could be related to the aforementioned low initial deposits of imidacloprid on grapes. In the case of vine leaves, when using the currently applicable EU MRL (2 mg.kg^{-1}) for the calculations, the calculated PHIs were 2.4 and 1.7 days for the SL and the WDG imidacloprid formulation, respectively. Whereas, when the new EU MRL (0.01 mg.kg^{-1}) that is approved for application starting June 2022, is used, the PHIs were 6.6 and 5.9 days for the SL and the WDG imidacloprid formulation, respectively.

Furthermore, it was noticeable that after only 12 days post-treatment nearly 90% of imidacloprid residues had dissipated in grapes and vine leaves for the two formulations (table III- 2), which is consistent with previous studies where 98% of imidacloprid dissipated after 6 days in sugar beet and where total imidacloprid dissipation was observed after 15 days in broad bean [11]. Likewise, imidacloprid rapid dissipation was widely discussed in the literature and short PHIs were reported in various matrix e.g., vine leaves and grapes, tomatoes, okra, rocket, parsley, green beans, chili peppers, zucchini, etc. [11,19,30,31,33–35]. It was found to be due to imidacloprid's high sensibility to photodegradation, even under low light intensity conditions[36,37]. Altogether, these studies demonstrated a significant PHI dependence on climatic conditions (sunlight, humidity, temperature, etc.) and they underlined the need to determine PHIs on a regional scale to ensure their accuracy and reliability.

3. Materials and Methods

3.1. Chemicals and Reagents

Analytical imidacloprid standard was purchased from Dr. Ehrenstorfer. Analytical grade solvents and reagents, acetonitrile, methanol and ammonium acetate were purchased from

Sigma-Aldrich International GmbH (Munich, Schnellendorf, Germany). Laboratory ultra-pure water was obtained using Milli-Q water purification system (Millipore, Billerica, MA, USA). NaCl, anhydrous MgSO₄, PSA, and GCB were purchased from Agilent technologies (Santa Clara, CA, USA). The two commercially formulated imidacloprid products used in field trials Diclean 20% (SL) and Pilarking Plus 70% (WDG) were officially registered in the Ministry of Agriculture of Lebanon and were purchased from Amalia, S.A.L., Verdun, Rabah Center 5th floor, Beirut, Lebanon and the National Development and General Trading Co., Bank Street, Tyr, Lebanon (Table III- 3).

Table III- 3: Pesticides active ingredients and phytosanitary commercial products used for the experimental treatment of vines.

| Trade name | Active substance (%) Formulation type | Recommended Dose (L.ha ⁻¹ - Kg.ha ⁻¹) | PHI (days) | Supplier Country | Importer |
|-----------------------------|--|--|---------------|--|---|
| Pilarking [®] Plus | Imidacloprid 70% WDG | 0.3 | 14 | Zhejiang Hisun Chemical Co., LTD China | Rmaily Trading Est. |
| Diclean | Imidacloprid 20% SL | 0.35 | 14 | Hailir Pesticides and Chemicals Group Co., LTD China | National Development and General Trading Co. |

3.2. Site location and specification

The vineyard of local Tfeifihi variety (*Vitis vinifera*) (1200 vines - 12 years old- conduction system: pergola), is located in Tamnine-El-Tahta, Governorate of Baalbeck Hermel [33°52'43.8"N 36°00'13.9"E] at an altitude of 960 m and has an area of 5,000 m². No imidacloprid treatments were performed on the target vineyard before the study.

3.3. Pesticide application and sampling

Imidacloprid was applied in the first week of July 2018, when the temperature was 31°C with passing clouds and the wind speed was 2 km/h blowing from 270° West to East with a relative humidity of 39%. During the sampling period, the temperature varied between 18 and 32°C, the relative humidity ranged between 27 and 65% and no precipitations were recorded.

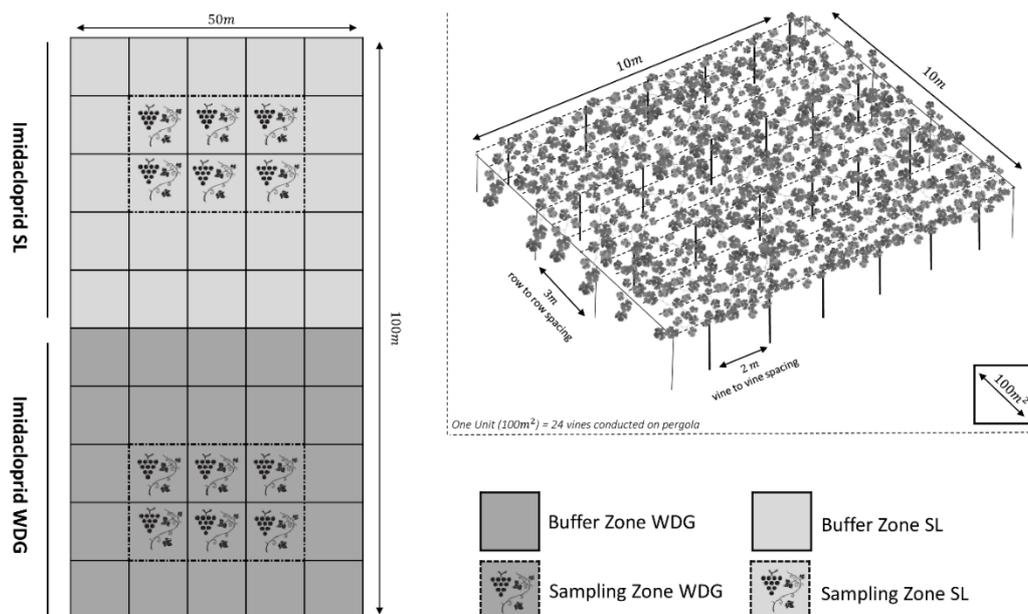


Figure III- 2: On the left: layout of the field experimental design showing the surface area treated with imidacloprid SL (light grey), the surface area treated with imidacloprid WDG (dark grey), buffer zones and sampling zones. On the top right: an overview of vines conduction system (pergola) and canopy density per unit.

As shown in Figure III- 2, the field was divided into two equal plots of 2500 m², each plot was treated according to the OECD guidelines for crop field trials [38], with one imidacloprid commercial product per plot, Diclean 20% (SL, rate 0.35 L.ha⁻¹) and Pilarcking Plus 70% (WDG, rate 0.30 L.ha⁻¹). A back sprayer calibrated according to FAO Guidelines on good practice for ground application of pesticides (nozzle calibrated to 200–400 µm with a spray pressure of 40 PSI) was used for applications [39]. A buffer zone of 2000 m² consisting of treated but unsampled vines was established to separate the sampling zones (1200 m² ~144 vines) delimited in each plot. Label indications were followed meticulously to prepare the imidacloprid formulations and extra care was taken to make sure the products were homogeneously dissolved. For WDG treatments continuous agitation of the tank was done during spraying to keep the imidacloprid ingredient suspended in water.

3.3.1. Sampling Procedure for grapes and vine leaves

Randomized sampling of vine leaves was conducted every 2 days, from 2 days up to 18 days after treatment. Sampling was implemented according to FAO guidelines (CAC/GL 33–1999) [40]. For each sampling date, one composite field sample of 2 kg of vine leaves and grapes was collected. From the composite sample a laboratory sample of 1 kg was subsampled and weighed, kept in polyethylene bag and sent directly to the laboratory for residue analysis. The laboratory sample (1 kg sample) was homogenized using a VCM4 Waring

Vertical Cutter Blender/Mixer 309 (Halldé, Sweden) and 5 replicates analytical portions of 10 g were taken for analysis.

3.4. Residue extraction and clean-up

Residue extraction was performed following the original unbuffered QuEChERS method which is widely used for pesticide residue extraction. QuEChERS is an abbreviation for Quick, Easy, Cheap, Effective, Rugged and Safe, and it was developed and first published by Anastassiades et al. [41]. Ten grams of a homogenized sample were weighed in a 50 ml polypropylene centrifuge tube, 10 ml of acetonitrile (ACN) were added. The mixture was shaken by hand for 1 minute, followed by addition of 4 g of MgSO₄ and 1 g of NaCl. The tube was manually shaken again for 1 min. Afterwards, the tube was subjected to centrifugation, for 10 minutes at 2066 g. One ml of the supernatant was isolated and put in a dispersive solid-phase extraction (d-SPE) tube containing 150 mg MgSO₄, 25 mg primary secondary amine (PSA) and, only for vine leaves samples, 50 mg graphitized carbon black (GCB). The tube was shaken for 1 minute and then subjected to centrifugation for 10 minutes at 3000 rpm. The extract was isolated in a 15 ml polypropylene tube and put in the refrigerator overnight. Finally, the supernatant was filtered using a 0.20 µm PTFE filter. Different levels of dilutions (100 times and 200 times dilution) were performed in acetonitrile in order to minimize the matrix effect and to reduce the concentration level to a level that would fall within the validated analytical range. The final extract was transferred into a glass vial to be directly analyzed by liquid chromatography mass spectrometry (LC-MS/MS).

3.5. Instrumentation and LC-MS/MS analytical conditions

The LC-MS/MS analysis was performed using Agilent Technologies 1200 Infinity Series Liquid chromatograph coupled to 3200 QTrap Triple Quadrupole Mass Spectrometer (AB Sciex, Dublin, CA, USA). The unit was equipped with a Phenomenex Analytical, C18 Synergi Fusion 150 X 0.25 mm x 2.5 µm, separation column and a guard column. The injection volume of 5 µl was delivered using an automatic injector with a flow rate of 0.4 ml/min. The eluent was composed of a solvent water (A)-methanol (B) gradient (MeOH), which was buffered with 5 mM ammonium acetate. The gradient program was as follows: 2% B to 100% of B over 12 min, held at 100% B until 20 min then decreased to 2% B at 25.01 min. The total run time was 30 min. The retention time of imidacloprid was 9.47 min. The equipped mass spectrometer provides the capability of combining positive and negative ionization modes by ESI. It was

operated in positive ion mode and MRM (multiple reaction monitoring) mode was used for data acquisition (Figure III- 3).

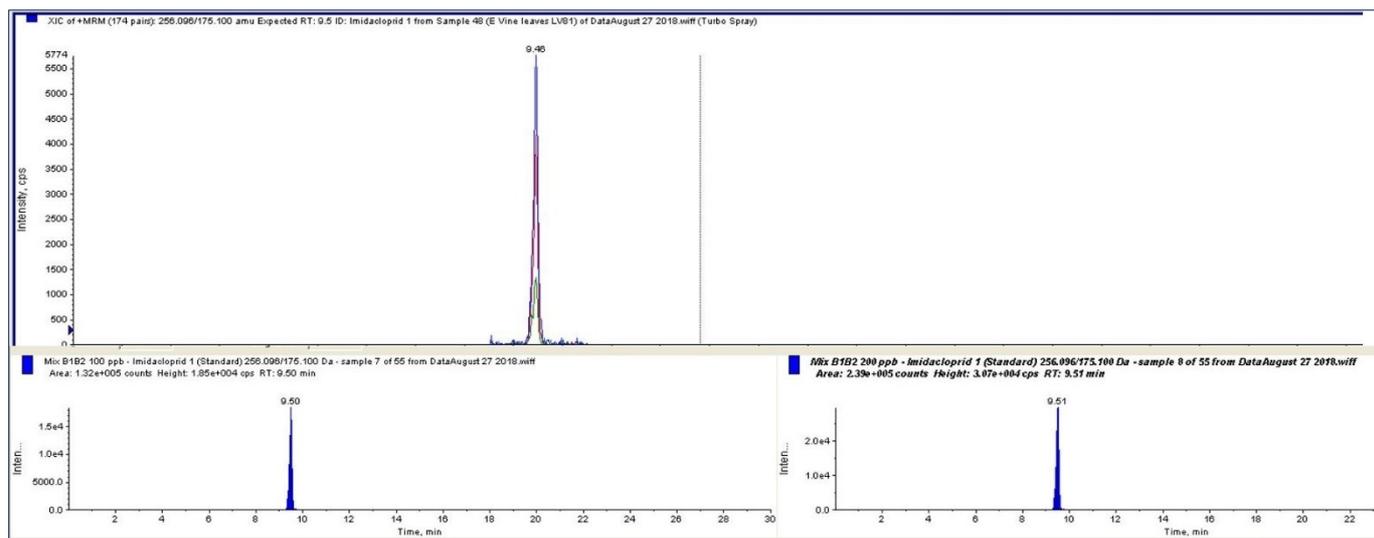


Figure III- 3: Total ion chromatogram (TIC) of the MRM of imidacloprid and the extracted ion chromatogram for imidacloprid in vine leaves at 100 µg/L (lower left figure) and 200 µg/L (lower right figure).

Table III- 4 shows the optimized parameters used for imidacloprid qualification and quantification. The source temperature and the ion spray voltages were 500 °C and 5000 v respectively. The ions underwent fragmentation by collisions with nitrogen (inert gas) that was also used as nebulizer curtain gas. Pre-configured iMethod™ Application (AB Sciex) and associated libraries designed for quantitative and qualitative screening using QTRAP® technology were used. EU SANTE/12682/2019 guidelines were followed for imidacloprid identification and quantification [42].

Table III- 4: Precursor, Transition ions and Source Parameters for imidacloprid residues analyzed by the LC-MS/MS method.

| Condition | | Content | | | | | | | | | |
|------------------------|----------|---|----|------------|-----|---------------------|----|------------|-----|------------|------------|
| Instrument : | | Model AB Sciex 3200 QTRAP LC-MS/MS SYSTEM | | | | | | | | | |
| Column : | | C ₁₈ column, Phenomenex Analytical Synergi, 150 x 2 mm, 2.5 µm particle size | | | | | | | | | |
| Column Flow : | | Gradient elution program at 0.4 ml.min ⁻¹ | | | | | | | | | |
| Source temperature : | | 500 °C – 5000 v | | | | | | | | | |
| Ion Spray- Potential : | | Electron Spray Ionization, | | | | | | | | | |
| Mode : | | Positive Mode | | | | | | | | | |
| Molecule | RT (min) | Transition Q1 (m/z) | DP | CE (Volts) | CXP | Transition Q2 (m/z) | DP | CE (Volts) | CXP | LOD (ng/g) | LOQ (ng/g) |
| Imidacloprid | 9.47 | 256.0 --> 209 | 51 | 21 | 7 | 256.0 --> 175.0 | 46 | 25 | 7 | 1.93 | 6.45 |

3.6. Method validation for grapes and vine leaves

Method validation was implemented according to Hayar et al. [7]. The following parameters, as required by EU SANTE/12682/2019 [42], were established: linearity (R^2), recovery (RM%), within-laboratory repeatability ($RSD_r\%$) and reproducibility ($RSD_{RW}\%$) and the limit of detection and quantification (LOD and LOQ, respectively).

As per Hayar et al. [7] linearity was performed by first preparing a stock solution of 1000 mg.kg^{-1} of imidacloprid standard in acetonitrile. Afterwards, aliquot solutions were obtained by serial dilution with 6 concentrations ranging from 5 to 500 $\mu\text{g.kg}^{-1}$. These solutions were later used to build standard calibration curves. Similarly, matrix-matched standard solutions were prepared by adding an imidacloprid standard to blank sample extracts, previously prepared, of grapes and vine leaves. Linear regression of all calibration curves had regression coefficient R^2 greater than 0.99. The limits of detection and quantification (LOD and LOQ) were 1.93 and 6.45 $\mu\text{g.kg}^{-1}$, respectively, in vine leave matrix. In grape matrix, the limits of detection and quantification (LOD and LOQ) were 1.08 and 5.03 $\mu\text{g.kg}^{-1}$, respectively.

Recovery and % RSD were determined by fortifying matrix blanks (10g) with three concentration levels (0.01; 0.05; and 0.1 mg.kg^{-1}) of imidacloprid standard mixture. Five replicates of each fortification level were prepared on three different days. After fortification the samples were left at room temperature for 30 min to allow the pesticide to be evenly incorporated into the matrix. Later, QuEChERS extraction procedure was performed and followed by LC-MS-MS analysis as described in Sections 2.3 and 2.4, respectively.

For the method to be satisfactory for imidacloprid analysis, EU SANTE/12682/2019 guidelines [42] require recovery values between 70 % and 120 % with a relative standard deviation (% RSD) less than 20 %, for samples tested on the same day (expressed as repeatability % RSD_r) and for samples analyzed on three different days (expressed as reproducibility % RSD_{RW}). In our study, recovery means were greater than 92 % and 80 % for grapes and vine leaves, respectively, with $RSD\% < 20\%$ for all values (Table III- 5).

Table III- 5: Method validation results showing the average of recovery data (RM%), repeatability ($RSD_r\%$) and reproducibility ($RSD_{RW}\%$) for imidacloprid at the three fortification levels, 0.01, 0.05 and 0.1 mg.kg^{-1} ($n = 5$ at each level) in grapes and vine leaves samp

| Matrix | Level of spiking ¹⁾ (mg.kg^{-1}) | Recovery Mean (RM%) | Repeatability ($RSD_r\%$) | Reproducibility ($RSD_{RW}\%$) |
|-------------|--|---------------------|-----------------------------|----------------------------------|
| Grapes | 0.01 | 96.5 | 16.6 | 12.1 |
| Vine leaves | | 92.0 | 17.0 | 19.0 |

| | | | | |
|--------------------|------|------|------|------|
| Grapes | | 92.6 | 13.3 | 9.5 |
| Vine leaves | 0.05 | 84.0 | 7.0 | 8.0 |
| Grapes | | 98.5 | 1.2 | 2.3 |
| Vine leaves | 0.1 | 82.0 | 11.0 | 13.0 |

3.7. Statistical Analysis

The data was subjected to statistical analysis using R free Software [43] to give regression equations and half-life (DT_{50}) (Table 1 and 2) and was fit to a first order kinetic dissipation model (Maclachlan and Hamilton [10]) according to equation (1):

$$C_t = C_0 e^{-kt} \quad (1)$$

Where C_t represents the residual concentration at sampling time t , C_0 represents the initial concentration and k represents the dissipation rate of the molecule and at the same time the slope of the exponential regression curve that is used for the determination of the half-life which is the time required for imidacloprid to decrease to half of its initial concentration after application [3]. The following equation was used:

$$DT_{50} = \frac{\ln 2}{k} \quad (2)$$

Pre-harvest intervals (PHI) were estimated as the time needed for the residues to fall to their specified EU MRL (see Table 1) and were derived from equation (3) PHIs were estimated as the time needed for the residues to dissipate to values equivalent to MRL after pesticide application (time 0) using an established regression model. The Equation used was:

$$PHI = \left[\frac{\text{intercept} - \ln(\text{MRL value})}{k} \right] \quad (3)$$

For data visualization, R 3.6.3 software packages were used [43].

4. Conclusions

The effect of formulation type on imidacloprid residues in vine leaves and grapes was investigated. Higher residue levels were detected when grapevines were treated with Diclean 20% (SL) than when treated with Pilarcking Plus 70% (WDG). The type of formulation and the morphological and physiological characteristics of the matrix were found to have an impact on initial deposits, and thus on residue levels, but not on the dissipation patterns.

Since each product formulation is unique, the designers of pesticide formulations have a wide territory to innovate out of the traditional basic roles of adjuvants as carriers,

penetrants, stickers, buffers, etc., and move towards more holistic approaches when developing new products that encompass all the legal, economical, ecological and safety challenges from farm to fork. Consequently, the improvement in formulation and inert compositions will enable new phytosanitary products to meet regulatory authorities' requirements, which are becoming more and more restrictive especially when it comes to pesticide residues in food products and safety to applicators.

Further field studies need to be conducted under Lebanese pedoclimatic conditions in order to set more accurate and reliable PHIs, specific to the local environmental conditions, and to provide farmers with the knowledge they need to choose the appropriate pesticide formulation for their crop variety (e.g. vines, apple), targeted matrix (e.g. berries or leaves), the plants' growth stage (e.g. grapes and leaves diameters) and local climatic conditions (e.g. temperature, humidity).

In this context, this work may be considered as a pilot study for other future ones that will involve other phytosanitary molecules used on grapevines and in which the effect of pesticide application frequencies will be evaluated.

Supplementary Materials: The following are available online, Table SIII1: Summary of two compartment models, Figure SIII1: Two compartmental model for each treatment combination, Table SIII2: Summary of model of imidacloprid decomposition with pooled slope but individual intercepts, Figure SIII2: Model of imidacloprid degradation with separate slopes and intercepts.

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References

1. Sannakki, S.; Rajpurohit, V.; Nargund, V.; Kulkarni, P. "Diagnosis and Classification of Grape Leaf Diseases Using Neural Networks"; 2013; p. 5; ISBN 978-1-4799-3926-8.
2. Lacerda, D.S.; Costa, P.C.; Funchal, C.; Gomez, C.D. and R. Benefits of Vine Leaf on Different Biological Systems. *Grape and Wine Biotechnology* 2016, doi:10.5772/64930.
3. Maestroni, B.M.; Ghanem, I.; Correll, R.; Alnaser, A.A.; Islam, M.; Cesio, V.; Heinzen, H.; Cannavan, A. Required Withholding Period for Vine Leaves Following Spraying with Pesticide. *Journal of Health and Environmental Research* 2018, 4, 140, doi:10.11648/j.jher.20180404.14.
4. Mdh, P.; Mw, A.; Sn, A. Determination of Pre-Harvest Interval for Quinalphos, Malathion, Diazinon and Cypermethrin in Major Vegetables. 2018, doi:10.4172/2161-0525.1000553.
5. EU Pesticides Database (v.2.1) Details Available online: <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/products/?event=details&p=163> (accessed on 4 April 2021).
6. Katalinic, V.; Mozina, S.S.; Generalic, I.; Skroza, D.; Ljubenkovic, I.; Klancnik, A. Phenolic Profile, Antioxidant Capacity, and Antimicrobial Activity of Leaf Extracts from Six *Vitis Vinifera* L. Varieties. *International Journal of Food Properties* 2013, 16, 45–60, doi:10.1080/10942912.2010.526274.
7. Hayar, S.; Zeitoun, R.; Maestroni, B.M. Validation of a Rapid Multiresidue Method for the Determination of Pesticide Residues in Vine Leaves. Comparison of the Results According to the Different Conservation Methods. *Molecules* 2021, 26, 1176, doi:10.3390/molecules26041176.
8. Hayar, S.; Al Hadi, J.; Merhi, A.; Majed, L.; Saba Ayoun, L. L'effet de 3 modes de conservation sur la dissipation des résidus de pesticides dans les feuilles de vigne *Vitis vinifera*. In *Proceedings of the 47ème Congrès du Groupe Français des Pesticides: Regards transfrontaliers sur les pesticides*; Nancy, France, May 15 2017; pp. 65–75.

9. Sandikly, N.; Hayar, S.; Millet, M. Monitoring Survey of Pesticide Residues in Table Grapes and Risk Assessment to Human in Lebanon. In Proceedings of the 49ème Congrès du Groupe Français des Pesticides; Montpellier, France, May 21 2019.
10. Maclachlan, D.J.; Hamilton, D. A New Tool for the Evaluation of Crop Residue Trial Data (Day-Zero-plus Decline). *Food Additives & Contaminants: Part A* 2010, 27, 347–364, doi:10.1080/19440040903403024.
11. Abdel-Hamid, M.; El-Sayed, W.; Ahmed, N. The Relationship Between Different Formulation Types And The Residue Levels Of Pesticides On Tomato Fruits. *RESEARCH JOURNAL OF AGRICULTURE AND BIOLOGICAL SCIENCES* 2013, 9(1), 8-16.
12. Alister, C.; Araya, M.; Becerra, K.; Saavedra, J.; Kogan, M. Preharvest Interval Periods and Their Relation to Fruit Growth Stages and Pesticide Formulations. *Food Chem* 2017, 221, 548–554, doi:10.1016/j.foodchem.2016.11.140.
13. Cress, D. Factors Affecting Pesticide Behavior and Breakdown Available online: <https://bookstore.ksre.ksu.edu/pubs/MF958.pdf> (accessed on 17 April 2021).
14. Ad Meijs, W.H.M. New Type of Formulations and New Application Techniques; Consequences for the Authorisation of Pesticides. *Environmentalist* 2008, 28, 5–8, doi:10.1007/s10669-007-9038-3.
15. Cabras, P.; Gennari, M.; Meloni, M.; Cabitza, F.; Cubeddu, M. Pesticide Residues in Lettuce. 2. Influence of Formulations. *J. Agric. Food Chem.* 1989, 37, 1405–1407, doi:10.1021/jf00089a043.
16. Buzzetti, K. Role of the Formulation in the Efficacy and Dissipation of Agricultural Insecticides. *Insectic Agric Toxicol* 2018, doi:<https://doi.org/10.5772/intechopen.72340>.
17. Montemurro, N.; Grieco, F.; Lacertosa, G.; Visconti, A. Chlorpyrifos Decline Curves and Residue Levels from Different Commercial Formulations Applied to Oranges. *J Agric Food Chem* 2002, 50, 5975–5980, doi:10.1021/jf0256687.
18. Abou Zeid, M.; Jammoul, A.; Melki, K.; Abou-Jawda, Y.; Kallassy, M. Dissipation of 3 Commercial Formulations of Penconazole 10% EC on Greenhouse Tomatoes in Lebanon. *RESEARCH JOURNAL OF CHEMISTRY AND ENVIRONMENT* 2020, 13, 2230.
19. Mohapatra, S.; Ahuja, A.; Sharma, D.; Manthirachalam, D.; Prakash, G.; Kumar, S. Residue Study of Imidacloprid in Grapes (*Vitis Vinifera* L.) and Soil. *Quality Assurance and Safety of Crops & Foods* 2011, 3, doi:10.1111/j.1757-837X.2010.00084.x.
20. Commission Implementing Regulation (EU) 2020/1643 of 5 November 2020 Amending Implementing Regulation (EU) No 540/2011 as Regards the Approval Periods of the Active Substances Calcium Phosphide, Denathonium Benzoate, Haloxyfop-P, Imidacloprid, Pencycuron and Zeta-Cypermethrin (Text with EEA Relevance); 2020; Vol. 370;.
21. EU Pesticides Database (v.2.2) Pesticide Residue(s) and Maximum Residue Levels (Mg/Kg) Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=details&pest_res_ids=326&product_ids=&v=1&e=search.pr (accessed on 19 October 2021).
22. Edwards, C.A. Factors that affect the persistence of pesticides in plants and soils. *Pure and Applied Chemistry* 1975, 42, 39–56, doi:10.1351/pac197542010039.
23. Lichiheb, N.; Bedos, C.; Personne, E.; Benoit, P.; Bergheaud, V.; Fanucci, O.; Bouhleb, J.; Barriuso, E. Measuring Leaf Penetration and Volatilization of Chlorothalonil and Epoxiconazole Applied on Wheat Leaves in a Laboratory-Scale Experiment. *Journal of Environmental Quality* 2015, 44, 1782–1790, doi:10.2134/jeq2015.03.0165.

24. Fernández, V.; Eichert, T. Uptake of Hydrophilic Solutes Through Plant Leaves: Current State of Knowledge and Perspectives of Foliar Fertilization. *Critical Reviews in Plant Sciences* 2009, 28, 36–68, doi:10.1080/07352680902743069.
25. Possingham, J.; Chambers, T.; Radler, F.; Grncarevic, M. Cuticular Transpiration and Wax Structure and Composition of Leaves and Fruit of *Vitis Vinifera*. *Aust. Jnl. Of Bio. Sci.* 1967, 20, 1149, doi:10.1071/BI9671149.
26. Chowdhury, A.; Jepson, P.; Ford, M.; Frampton, G. The Role of Cuticular Waxes and Surface Roughness in Determining the Insecticidal Efficacy of Deltamethrin and Dimethoate Applied as Emulsifiable Concentrates to Leaf Surfaces. *International Journal of Pest Management - INT J PEST MANAGE* 2005, 51, 253–263, doi:10.1080/09670870500404674.
27. Baur, P.; Marzouk, H.; Schönherr, J.; Bauer, H. Mobilities of Organic Compounds in Plant Cuticles as Affected by Structure and Molar Volumes of Chemicals and Plant Species. *Planta* 1996, 199, 404–412, doi:10.1007/BF00195732.
28. Lewis, K.A.; Tzilivakis, J.; Warner, D.J.; Green, A. An International Database for Pesticide Risk Assessments and Management. *Human and Ecological Risk Assessment: An International Journal* 2016, 22, 1050–1064, doi:10.1080/10807039.2015.1133242.
29. Bletsou, A.A.; Hanafi, A.H.; Dasenaki, M.E.; Thomaidis, N.S. Development of Specific LC-ESI-MS/MS Methods to Determine Bifenthrin, Lufenuron, and Iprodione Residue Levels in Green Beans, Peas, and Chili Peppers Under Egyptian Field Conditions. *Food Anal. Methods* 2013, 6, 1099–1112, doi:10.1007/s12161-012-9515-2.
30. Abdallah, O. Dissipation Behavior of Chlorfenapyr and Difenconazole Residues in/on Grapes (*Vitis Vinifera* L.). *nature and science* 2014, 12, 49–54.
31. Hanafi, A.; Dasenaki, M.; Bletsou, A.; Thomaidis, N.S. Dissipation Rate Study and Pre-Harvest Intervals Calculation of Imidacloprid and Oxamyl in Exported Egyptian Green Beans and Chili Peppers after Pestigation Treatment. *Food Chemistry* 2018, 240, 1047–1054, doi:10.1016/j.foodchem.2017.08.037.
32. Rogiers, S.Y.; Hatfield, J.M.; Jaudzems, V.G.; White, R.G.; Keller, M. Grape Berry Cv. Shiraz Epicuticular Wax and Transpiration during Ripening and Preharvest Weight Loss. 2004, 7.
33. Arora, P.K.; Jyot, G.; Singh, B.; Battu, R.S.; Singh, B.; Aulakh, P.S. Persistence of Imidacloprid on Grape Leaves, Grape Berries and Soil. *Bull Environ Contam Toxicol* 2009, 82, 239–242, doi:10.1007/s00128-008-9554-y.
34. Karthik, P.; Venugopal, S.; Datchina Murthy, K.; Lokesh, S.; Karthik, G.; Sharmila, U.; Paramasivam, M.; Senguttuvan, K.; Gunasekaran, K.; Kuttalam, S. Bioefficacy, Phytotoxicity, Safety to Natural Enemies and Residue Dynamics of Imidacloprid 70 WG in Okra (*Abelmoschus Esculenta* (L) Moench) under Open Field Conditions. *Crop Protection* 2015, 71, 88–94, doi:10.1016/j.cropro.2015.01.025.
35. Oliva, J.; Cermeño, S.; Cámara, M.A.; Martínez, G.; Barba, A. Disappearance of Six Pesticides in Fresh and Processed Zucchini, Bioavailability and Health Risk Assessment. *Food Chem* 2017, 229, 172–177, doi:10.1016/j.foodchem.2017.02.076.
36. Scholz, K.; Reinhard, F. Photolysis of Imidacloprid (NTN 33893) on the Leaf Surface of Tomato Plants. *Pesticide Science* 1999, 55, 652–654, doi:10.1002/(SICI)1096-9063(199906)55:6<652::AID-PS997>3.0.CO;2-I.
37. Dikshit, A.K.; Pachauri, D.C.; Jindal, T. Maximum Residue Limit and Risk Assessment of Beta-Cyfluthrin and Imidacloprid on Tomato (*Lycopersicon Esculentum* Mill). *Bull. Environ. Contam. Toxicol.* 2003, 70, 1143–1150, doi:10.1007/s00128-003-0101-6.

38. OECD Guidance Document on Crop Field Trials Available online: https://read.oecd-ilibrary.org/environment/guidance-document-on-crop-field-trials_2794fdd6-en (accessed on 19 October 2021).
39. Food and Agriculture Organization of the United Nations Guidelines on Good Practice for Ground Application of Pesticides Available online: <http://www.fao.org/3/y2767e/y2767e00.htm> (accessed on 19 March 2021).
40. FAO CAC/GL 33-1999 RECOMMENDED METHODS OF SAMPLING FOR THE DETERMINATION OF PESTICIDE RESIDUES FOR COMPLIANCE WITH MRLS Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?Ink=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXG%2B33-1999%252FCXG_033e.pdf (accessed on 19 March 2021).
41. Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D.; Schenck, F.J. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce. *Journal of AOAC INTERNATIONAL* 2003, 86, 412–431, doi:10.1093/jaoac/86.2.412.
42. Guidance SANTE/12682/2019 – Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed Available online: <https://www.accredia.it/en/documento/guidance-sante-12682-2019-guidance-document-on-analytical-quality-control-and-method-validation-procedures-for-pesticides-residues-analysis-in-food-and-feed/> (accessed on 19 October 2021).
43. R Core Team R: A Language and Environment for Statistical Computing.; R Foundation for Statistical Computing: Vienna, Austria, 2020;

CHAPITRE IV

Effet du traitement des feuilles de vigne sur la dissipation des résidus d'azoxystrobine, de fenazaquin et d'indoxacarbe : facteurs de traitement et évaluation de la sécurité des consommateurs

Les résultats de ce chapitre ont été publiés ainsi :

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Résumé synthétique

Après la récolte, les feuilles de vigne sont soit consommées directement, soit soumises à diverses étapes de préparation/transformation dont le lavage et la conservation qui sont les plus couramment utilisés dans les ménages et les industries. Ces techniques ont un effet sur l'élimination de contaminants tels que les résidus de pesticides, qui constituent un défi pour les agriculteurs et les industriels, surtout dans une culture orpheline comme les feuilles de vigne qui n'ont pas de LMR spécifique. Dans le Codex Alimentarius les LMR pour feuilles de vigne sont absentes, en revanche des LMRs qui correspondent à la limite inférieure de détermination analytique, retenue par principe de précaution, sont adoptées par la Commission Européenne/EFSA pour 98% des pesticides. Étant donné qu'en 2020 un décret ministériel libanais (Décision 260 du 02/08/2020 -Ministère d'agriculture) a imposé de se référer aux normes européennes, ces LMR non évaluées d'un point de vue toxicologique empêchent les industriels de l'agro-alimentaire d'exporter cette denrée alimentaire vers les pays européens.

Ainsi dans cette étude, une parcelle de variété "Grenache" (Kefraya, Liban) a été divisée en trois sous-parcelles, chacune traitée par une des molécules suivantes : l'indoxacarbe, la fenazaquin et l'azoxystrobine. Douze heures après traitement, des échantillons de feuilles de vigne ont été prélevés pour étudier l'effet des pratiques post-récolte, le mode de conservation et de lavage sur la dissipation des résidus des trois pesticides. La conservation industrielle des feuilles a été réalisée à sec et en saumure. Les teneurs en résidus de pesticides ont été suivies au cours du temps dans les feuilles et les eaux de saumure (0, 2, 4, 8, 12, 20, 28, 44 et 60 jours). Les procédures de lavage ont simulé les pratiques des consommateurs et de l'industrie, en utilisant de l'eau du robinet, du vinaigre à 10 % et de l'eau bouillante. Les extractions des résidus de pesticides ont été réalisées selon la méthode QuEChERS, et les analyses ont été effectuées par LC-MS-MS (laboratoire CCIAT- Tripoli). Les résultats obtenus ont permis de calculer les demi-vies, les pourcentages de réduction des teneurs en résidus de pesticides et d'évaluer le risque pour la santé humaine en calculant les quotients de danger (HQ).

Les résultats ont montré que les modes d'action et les propriétés physico-chimiques des molécules sont les principaux facteurs dictant l'efficacité des méthodes de conservation

et de lavage dans l'élimination des résidus. Pour la conservation à sec, les demi-vies ont été estimées à 210, 16 et 100 jours pour l'azoxystrobine, le fenazaquin et l'indoxacarbe, respectivement tandis que pour la conservation en saumure, les demi-vies étaient de 164, 8 et 644 jours. La conservation à sec pourrait être recommandée puisqu'après 60 jours de conservation, les teneurs en résidus de pesticides ont diminués de 60 % comparé à 48% pour la conservation en saumure, indépendamment de la nature des molécules. Par ailleurs, l'analyse des eaux de saumure a démontré le transfert d'une fraction de ces résidus dans ces eaux (2 %). Ainsi, il est recommandé de considérer ces eaux comme contaminées par les industriels et les consommateurs. Concernant la méthode de lavage, le lavage à l'eau bouillante a été préféré pour l'azoxystrobine (-40,34 %) et l'indoxacarbe (-22,39 %). Pour le fenazaquin, le lavage à l'eau du robinet a montré la plus grande réduction des résidus (-43,38 %). De manière globale, à partir de ces résultats, le lavage à l'eau bouillante pourrait être recommandée, puisque les teneurs en résidus sont réduits de 30 % en moyenne, contre 26% pour l'eau robinet et 18% pour la solution vinaigrée indépendamment de la nature des molécules.

L'évaluation des risques pour la santé a indiqué que la consommation de feuilles de vigne, même dans le pire cas (récolte des feuilles 12 h après le traitement) et avec une consommation surestimée (30 g/jour/personne), ne présentait aucun risque pour les consommateurs (HQ <1). Toutefois, pour le calcul du HQ, nous n'avons pas pris en compte la présence de ces pesticides dans d'autres denrées alimentaires contribuant également à l'exposition du consommateur. Des études ultérieures devraient être réalisées pour la prise en compte de l'ensemble des sources de résidus de ces 3 molécules.

Étant donné que les procédures de conservation ne garantissent pas l'élimination totale des résidus pendant le stockage ni lors des procédures de lavages, il pourrait être recommandé d'éviter les traitements phytosanitaires pendant la période de récolte des feuilles de vigne, qui s'étend de mai à juin. En cas de pression fongique pendant la saison de croissance des feuilles, il est conseillé d'utiliser des molécules ayant des demi-vies courtes et d'avoir recours à des applications curatives au lieu de traitements préventifs.

Effect of vine leaves processing on Azoxystrobin, Fenazaquin and Indoxacarb residues dissipation: processing factors and consumer safety assessment

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Abstract:

The effect of vine leaves processing techniques on Azoxystrobin, Fenazaquin, and Indoxacarb residues was investigated. Residue extraction following field application of pesticides and leaf processing was carried out using the QuEChERS method, with analysis conducted by LC-MS/MS. In dry conservation, Azoxystrobin's half-life was estimated to exceed a year, Fenazaquin's was 18 days, and Indoxacarb's was 142 days. Azoxystrobin had a half-life of 261 days, Fenazaquin had a half-life of 9 days, and Indoxacarb's half-life exceeded a year in brine conservation. It is recommended to use dry conservation because it results in an average 60% reduction in residue levels for the three pesticides. Boiling water significantly reduced pesticide residues (Azoxystrobin -40.3%, Indoxacarb -22.4%, and Fenazaquin -28.8%). It is recommended to use boiling water for washing, as it shows an average removal rate of approximately 30%. The health risk assessment indicated that consuming vine leaves posed no health risk for consumers, but overall exposure to residues must be considered.

Keywords: pesticide residues, washing, vine leaves, dry conservation, brine conservation, half-lives, MRLs, QuEChERS

Chemical compounds studied in this article:

Azoxystrobin (PubChem CID: 3034285); Fenazaquin (PubChem CID: 86356) ; Indoxacarb (PubChem CID: 107720)

1. Introduction

Being the main ingredient of one of the most iconic Mediterranean dishes, vine leaves are consumed throughout the year by the local populations. Whether stuffed with meat, rice or vegetables, vine leaves entered the diet of many countries and were subject to diverse culinary practices to enhance their organoleptic proprieties and to extend their shelf life (Cantwell, Hong, Albornoz & Berlanga, 2022). Therefore, many conservation methods were traditionally used to preserve vine leaves' appealing sensory proprieties: e.g., the dry, the brining, the pickling, and the blanching/freezing conservation methods (Cantwell et al., 2022). As a result, this commodity is marketed in various forms to meet its worldwide growing demand and expanding market.

Still, vine leaves may contain harmful residues from pesticides used in vineyards, posing a challenge for international trade. Notably, specific phytosanitary regulations, such as Maximum Residue Limits (MRLs), have not been established for this commodity leading to border rejections of vine leaves exports when residues are quantified in this commodity (Hayar, Zeitoun & Maestroni, 2021). Thus reducing the amount of these residues or completely removing the residues is of outmost importance to protect human health and facilitate vine leaves trade. In fact, the marginalization of vine leaves as a minor crop on the international level, resulted in a limited number of accessible studies on pesticide residues levels or removal methods in this food (Cangi, Yanar & Dülgeroğlu, 2019). Most of these studies have primarily focused on the effects of brining on residue reduction and have suggested potential benefits of brining and hot water washings in reducing residues (Maestroni et al., 2018; Cangi et al. 2019; Hayar et al. 2021). Where more than 97 and 82 % of Azoxystrobin and Triadimenol residues respectively were removed by hot water brining in Cangi et al. (2019) work. Hayar et al. (2021) reported lower residue contaminations in brined vine leaves compared to dry or stuffed from samples collected from the Lebanese market. Indeed, in their review on the effect of processing and washing on residues in food, Bajwa and Sandhu (2014) have demonstrated that nearly all food processing techniques (e.g., washing, peeling, pasteurization, boiling, cooking, pickling and drying) altered pesticide residue levels. However, the amount of pesticide residue in the finished product depends on factors such as the type of pesticide molecule, location, commodity, processing steps, and

product preparation (Bajwa et al., 2014; Gao et al., 2020).

Since the existing literature do not comprehensively investigate and compare the effectiveness of various processing/washing methods for vine leaves, the aim of this study was to investigate whether two industrial preservation methods (in brine and dry) and three commonly used industrial/household washing methods (tap water, boiling water or blanching, and a 10% vinegar solution) have varying effects on pesticide residue levels. Three pesticides (Azoxystrobin, Fenazaquin, and Indoxacarb) were chosen for this study due to the frequent detection of their residues in national grape monitoring, which reported MRLs exceedances in Lebanon (unpublished results). This selection is further supported by documentation from the Rapid Alert System for Food and Feed (RASFF) of the European Union, which identifies these pesticide residues in rejected exports of vine leaves (RASFF 2021). Azoxystrobin, Fenazaquin and Indoxacarb have no Codex Alimentarius MRL for vine leaves (Codex Online Databases | CODEXALIMENTARIUS FAO-WHO) , meanwhile, in the European Union the MRLs on vine leaves are set at the limits of detection (LODs), for Azoxystrobin and fenazaquin (0.01 mg.kg⁻¹) and for indoxacarb (0.02 mg.kg⁻¹) (EU Pesticides Database) .

Additionally, this study sought to determine if any of the studied methods could be recommended as a universal approach for effectively reducing the residues of all three molecules, thereby facilitating their removal by industrialists for pesticide residue control. Processing factors and health risk assessments were estimated to support this objective.

2. Materials and Methods

2.1. Chemicals and Reagents

The analytical standards for the three compounds —Indoxacarb, Fenazaquin, and Azoxystrobin—were bought from Dr. Ehrenstorfer. Acetonitrile, methanol, and ammonium acetate were acquired from Sigma-Aldrich International GmbH as analytical grade solvents and reagents (Munich, Schnellendorf, Germany). The Milli-Q water purification system was used to obtain ultra-pure laboratory water (Millipore, Billerica, MA, USA). These items were purchased from Agilent Technologies: NaCl, Anhydrous MgSO₄, and PSA (Santa Clara, CA, USA). The purchase of GCB from Phenomenex (Long Beach, CA, USA). The commercial phytosanitary products used in the treatments (Annexe III-2 - Table SIV-1) are officially registered with the Lebanese Ministry of Agriculture.

2.2. Pesticide Application and Sampling of Vine Leaves

Vine leaves were obtained from a vineyard (44404 m² ~ 111010 vines) of the “Grenache” variety, located in Kefraya, a wine-growing village, renowned for its quality wine production, in the Western Bekaa District of the Bekaa Governorate of the Republic of Lebanon [33°38' 20.8"N 35°44'24.6"E]. No phytosanitary treatments had ever been done before this study commenced, so the vineyard can be considered “pristine.”

The treatments were conducted in the second week of June 2021, in the afternoon period. According to Time and Date online platform the temperatures in Kefraya ranged between 26-27 °C with passing clouds and the wind speed was 13 km/h (3.6 m/s) with a relative humidity of 57% (Steffen Thorsen 2021). Although the wind speed slightly exceeded the recommended limit set by Good Agricultural Practices (GAP) (< 10.8 km/h ~ 3 m/s), Cypress windbreaker trees surround the treated vineyard (Figure IV- 1), further reduces the wind speed. It should be mentioned that the accuracy of the application was not affected. Indeed, residue levels in vine leaves at T0 were measured across the plots, and the average concentrations from three replications were uniform (Azoxystrobin: 12.82 ± 0.002 mg.kg⁻¹; fenazaquin: 3.69 ± 0.002 mg.kg⁻¹; and indoxacarb: 3.39 ± 0.177 mg.kg⁻¹). As shown in the map of Figure 1, the land was divided into 3 equal plots of 1500 m². Each plot was treated according to the OECD guidelines for field trials (OECD 2016) with only one of the commercial products (Supporting information: Annexe III-2 - Table SIV-1). The three plots were treated with Indoxacarb, Fenazaquin and Azoxystrobin respectively as shown in Figure IV-1. The label instructions were carefully followed for preparing the pesticide formulations and extra precautions were taken to ensure that the products were evenly and properly dissolved. For wettable dust granulate (WDG) treatments, vigorous shaking was applied during the mixture preparation to keep the Indoxacarb active ingredient fully suspended in the water. The pesticide formulations were applied using a 1600-liter capacity tank, with nozzles calibrated at 400 microns, delivering a spray pressure of 40 PSI and an application amount of 150 L every 1500 m². The active substance amounts applied to the vines were 0.036 Kg.ha⁻¹ of Indoxacarb, 0.08 Kg.ha⁻¹ of Fenazaquin and 0.25 Kg.ha⁻¹ of Azoxystrobin. Buffer zones of 20-40 meters were established in each plot to ensure no drift was present in the sampling zones (1500 m² ~ 350 vines).

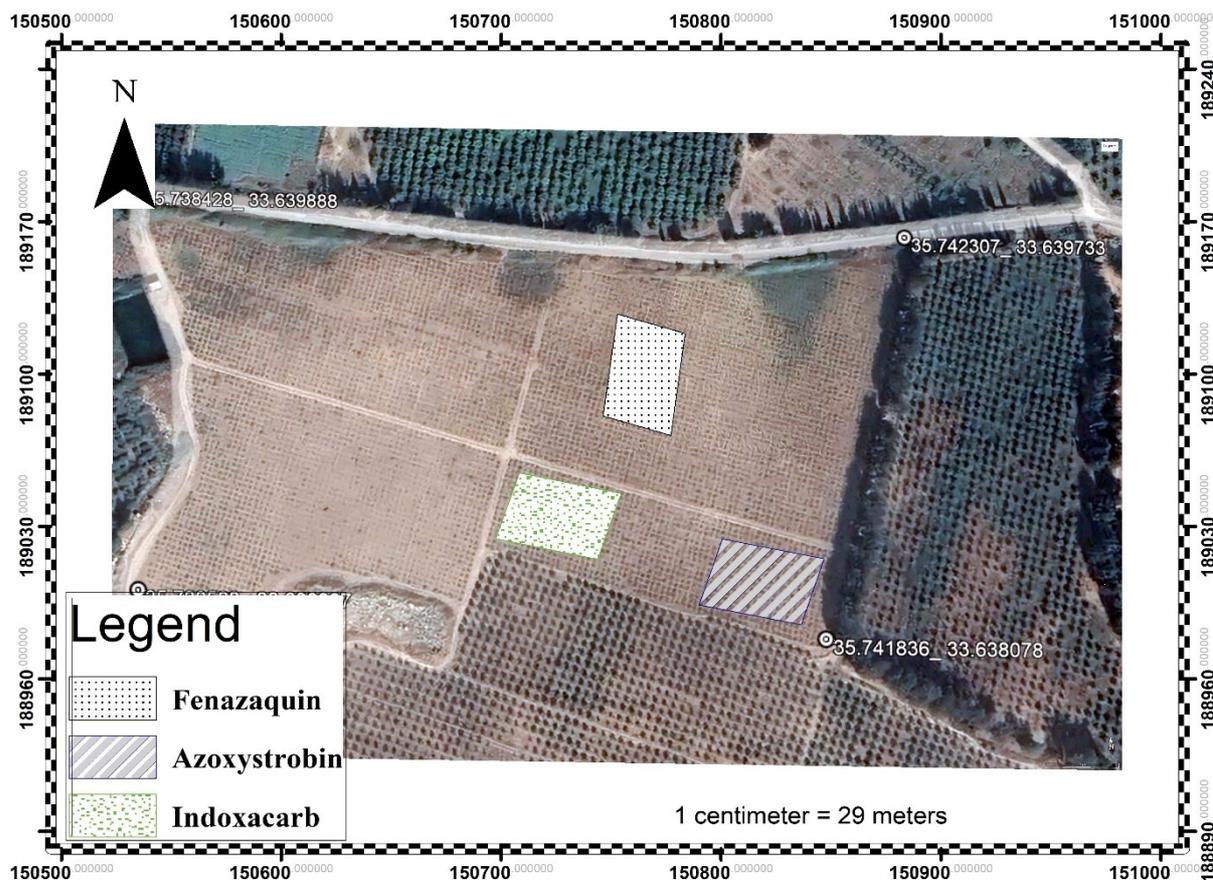


Figure IV- 1: Map showing the study site divided into plots of 1500 m² (georeferenced satellite image)

The sampling procedure was implemented in accordance with the FAO guidelines (CAC/GL 33–1999) (Joint FAO/WHO Food Standards 1999). As shown in Figure IV-2, composite samples of vine leaves, totaling 12 kg per plot, were randomly collected 12 hours after the pesticide application, adhering to the necessary waiting period before entering the plot for workers' safety, i.e., restricted entry intervals (REI). This choice was made to align with current harvest practices for vine leaves that can be harvested either daily or biweekly. Additionally, PHIs for grapes were not adopted since that implies waiting 10, 21, and 30 days for Indoxacarb, Azoxystrobin, and Fenazaquin, respectively (Annexe III-2 - Table SIV-1), making the short vine leaf harvesting season (from May to June) not economically profitable with vine leaves becoming tough or old during this time and no longer suitable for culinary use. Therefore, we selected the 12-hour post-treatment period to assess risks in a worst-case scenario and to replicate real harvest practices while adhering to REI (Annexe III-2 - Table SIV-1). Thus, sub-samples of 6750 g/plot were sent to the Conserves Modernes Chtaura Factory – West Bekaa,

Lebanon for direct preservation (dry and brine). Sub-samples of 1350 g/plot were sent to the laboratory (EDST-PRASE) sealed in polythene bags and, at arrival, they were kept in a cold room at 4°C. From the remaining 3900 g/plot, 450 g were used as control group to assess the concentration at the time of harvesting (3 samples of 150 g/plot were directly put under -20 °C) and 3450 g/plot were kept as reserve samples and stored at -80 °C (Figure IV- 2).

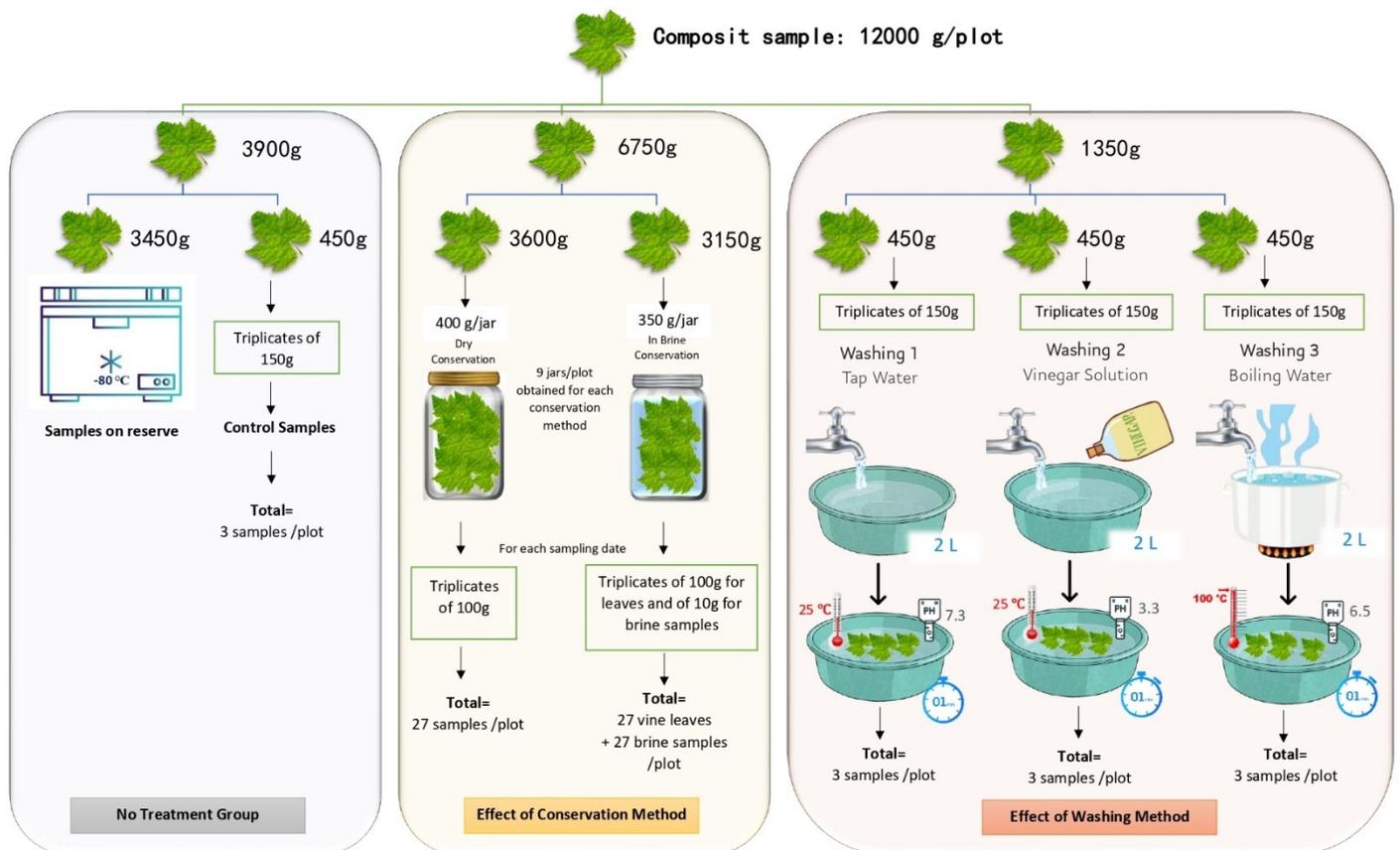


Figure IV- 2: Schematic diagram of the entire experimental procedures

2.3. Vine Leaves Processing

2.3.1. Industrial Conservation Procedure

The preservation of the vine leaves was conducted at the industry Conserves Modernes Chtaura – Lebanon, in accordance with the standards of the Lebanese Institute of Normalization Libnor (n° 699/2003 and 139/2004). The vine leaves’ petioles were removed, and the leaves were sorted by size. For the dry storage, 400 g of fresh vine leaves were put in a jar and sealed. For preservation in brine, 350 g of fresh leaves were placed in a jar and then hot brine was poured over them. The brine composition was water (89.12%), sodium chloride (10.7%), citric acid (0.15%) and EDTA (0.03%); the pH was set at 3.5. The glass jars were sealed

to prevent the entry of air and pasteurized in boiling water at 90°C for 20 minutes.

To monitor the pesticides dissipation rates, a jar from each conservation method and from each molecule was sampled at different storage time (0, 2, 4, 8, 12, 20, 28, 44 and 60 days). The decision to sample for 60 days was based on a preliminary laboratory study (unpublished results), where the dissipation of pesticide residues across various conservation methods reached a plateau at 60 days. In the case of the brine preservation method, the vine leaves were separated from the brine and further stored in food-grade plastic bags at 4°C. The brine solutions were stored in 50 ml falcon tubes at 5°C for further extraction of pesticide residues and pH measurement using a digital pH meter.

2.3.2. Leaves Washing Procedure at the laboratory.

Upon arrival at the laboratory, the vine leaf samples (1350 g/plot) were further sub-sampled to 3 portions of 150 g using a scale (Sartorius Entris II, Germany). The sample portions were washed using tap water, 10% vinegar and boiled water. Washing with tap water: The leaves were immersed for 1 minute in 2 L of tap water, the water temperature was 25 °C and the pH was 7.3. Washing with 10% vinegar: The leaves were immersed for 1 minute in 2 L of tap water containing 200 ml of white vinegar, purchased from a national producer (Production of Al-Assil, Lebanon). The temperature of 25 °C and the pH was 3.3. Washing with boiling water: The leaves were immersed for 1 minute in 2 L of boiling tap water. The boiling water temperature was 100 °C, and the pH was 6.5. The washing of vine leaves was done in a clean stainless-steel container by simulating the artisanal practices applied by consumers or the industrial processes (Figure IV- 2). After washing, the vine leaves were drained with a sieve, placed on aluminum foil, and dried for 30 min in the open air at room temperature. After drying, the washed vine leaves were placed in freezer bags and set at -20 °C until analytical extractions. A total of 36 samples of vine leaves (27 washed and 9 controls) were obtained from this process.

2.4. Analytical sample preparation

The original unbuffered QuEChERS method was used for residue extraction from vine leaf and brine samples (Anastassiades, Lehotay, Štajnbaher & Schenck, 2003). 10 grams of a homogenized vine leave sample and 10 mL of acetonitrile (ACN) and added in a Falcon tube. The mixture was manually shaken for one minute, and then 4 g of MgSO₄ and 1 g of NaCl were added. The tube was manually shaken for one minute, then the tube was centrifuged

for 10 minutes at 2066 g. One milliliter of the supernatant was removed and placed in a dispersive solid-phase extraction (d-SPE) tube with 150 mg MgSO₄, 25 mg primary secondary amine, and 50 mg graphitized carbon black. The tube was shaken for one minute, centrifuged at 3000 rpm for ten minutes. The supernatant was placed in a 15 mL polypropylene tube and stored in a refrigerator overnight. A 0.20 µm PTFE filter was used to filter the extract into LC vials. The extracts were diluted with acetonitrile (100 times and 200 times dilution) to a level within the validated analytical range (0.01 – 0.1 µg/g) and analyzed by liquid chromatography mass spectrometry (LC-MS/MS).

2.5. LC-MS/MS Analytical Conditions and Instrumentation

Liquid Chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Thermo Scientific) was used to analyze the sample extracts (LC-MS). The injection volume was 5 µL with a flow rate of 0.3 ml min⁻¹; the eluant was composed of two mobile phases A and B. Mobile phase A consisted of methanol buffered with 0.1% formic acid and 5mM ammonium formate. Mobile phase B was water buffered with 0.1% formic acid and 5mM ammonium formate. The column temperature was maintained at 25°C. The gradient elution program started with 100% of A, shifted to 30% A and 70% B at 0.5 min, held for 7 min, then switched to 100% B at 9–12 min, and finally returned to initial conditions at 12–15 min. The LC-MS/MS was run in positive mode, and data was acquired in multiple reaction monitoring (MRM) mode (Annexe III-2 - Figure SIV - 1). The ion source temperature and the ion spray voltages were 500 °C and 5 kV, respectively. The ions underwent fragmentation by collisions with nitrogen (inert gas), which also served as nebulizer curtain gas. The retention time of Azoxystrobin, Fenazaquin and Indoxacarb were 10.07, 12.50 and 11.24 min, respectively. The qualification and quantification of Azoxystrobin, Fenazaquin and Indoxacarb molecules according to the EU SANTE/12682/2019 guidelines (EC 2019) The analytical conditions and the method were validated according to Hayar et al. (2021). The supporting information for this paper includes the validation information (Annexe III-2 - Table SIV- 3).

2.6. Half-life estimation and Statistical Analysis

Data were subjected to statistical analysis using R software version 3.6.3 (suitable packages were used) and Microsoft Excel's XLMiner Analysis Toolpak. As per Lu, Yang, Shen, Liu, Zhou and Diao (2013), to evaluate dissipation kinetics during the conservation period, data were fit to first-order decay model according to eq (1):

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t designates the residual concentration at sampling time t , C_0 indicates the initial concentration, and k represents the molecule's rate of dissipation as well as the slope of the exponential regression curve used to calculate the half-life. The slope of the regression (k) was on a logarithmic scale.

The equation used to estimate the half-life was as follows:

$$DT_{50} = \frac{\ln 2}{k} \quad (2)$$

The confidence interval of the slope was also assessed using its standard error and an inverse t value based on 8 degrees of freedom.

The distribution of the residues between the brine and the leaves during the conservation process was assessed using the Ratio B/L , according to Lu et al. (2013). It was calculated using equation (3), as the ratio between the concentration of residues in the brine (C_{brine}) and , the concentration of residues in the vine leaves (C_{leaves}).

$$Ratio\ B/L = \frac{C_{brine}}{C_{leaves}} \quad (3)$$

The effect of conservation or washing, in general the processing, on residue levels is determined by calculating the processing factor (PF). It is the ratio of the concentration of pesticide residues ($mg.kg^{-1}$) in the processed product and the concentration of pesticide residues ($mg.kg^{-1}$) in the unprocessed product (Yigit & Velioglu, 2020). A PF of less than 1 indicates a reduction in pesticide content.

$$PF = \frac{C_{Processed\ leaves} (mg.kg^{-1})}{C_{Unprocessed\ leaves} (mg.kg^{-1})} \quad (4)$$

The Student's T-test was used to determine the statistical significance of the conservation methods, the means of residues for each sampling date and the dissipation rates. Analysis of variance (ANOVA) in combination with the Tukey post hoc test, with a significance level was set at 0.05, were used to analyze the statistical significance of the washing treatment.

2.7. Dietary risk assessment

The Estimated Daily Intake (EDI) is the total intake amounts of pesticides measured in $mg\ Kg^{-1}$ body weight per day [$mg.kg^{-1}\ bw\ day^{-1}$] calculated according to equation 5. The Hazard quotient (HQ) was calculated according to equation 6, where the ADI was taken from Lewis, Tzilivakis, Warner and Green (2016) and a body weight of 60 kg (FAO/WHO 1997) .

$$EDI = \frac{Average\ Food\ Consumption\ (kg.day^{-1}) \times Residue\ Amount\ (mg.kg^{-1})}{Body\ weight\ (kg)} \quad (5)$$

$$HQ = \frac{EDI \text{ in } (\text{mg.kg}^{-1} \text{ bw.day}^{-1})}{ADI \text{ in } (\text{mg.kg}^{-1} \text{ bw.day}^{-1})} \quad (6)$$

3. Results and Discussion

3.1 Effect of brine and dry industrial processing on the residues

3.1.1. Dissipation Kinetics of the residues in dry and brined vine leaves

The dissipation of Azoxystrobin, Fenazaquin and Indoxacarb residues over 60 days is presented in Figure IV- 3. The dissipation rates (k), their uncertainties (estimated for the slopes of the regressions), intercepts, half-lives and confidence intervals (CI) are given in Table IV- 1.

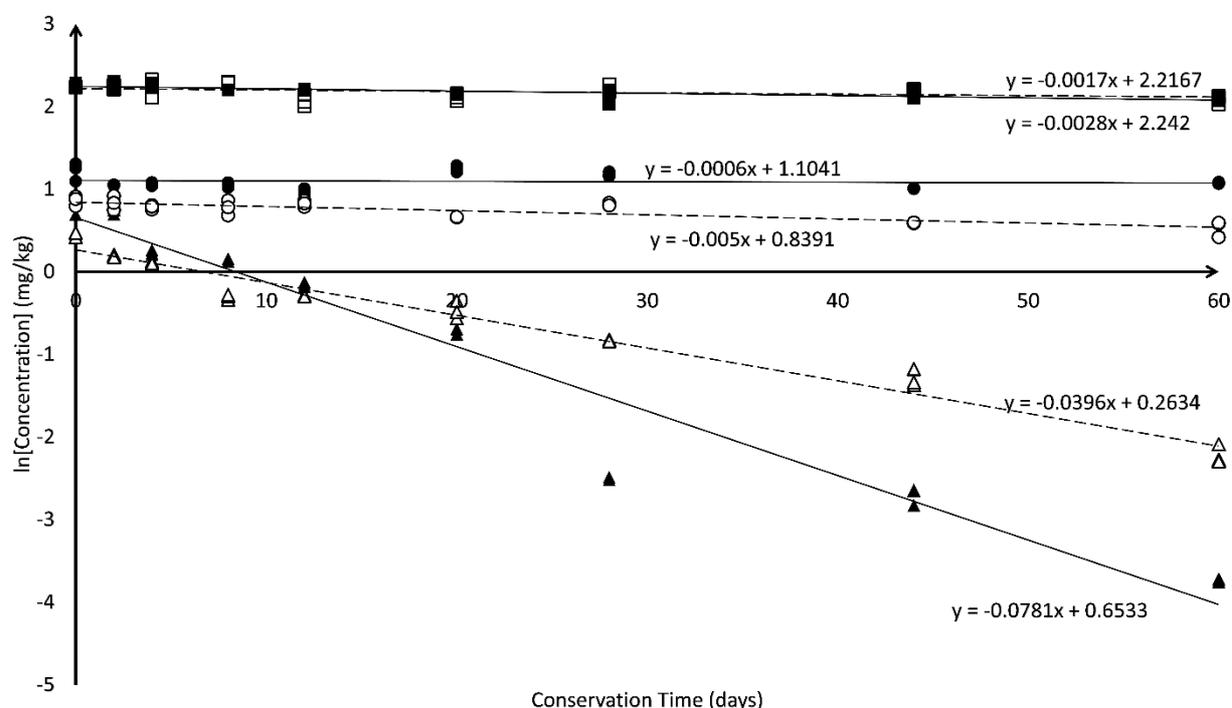


Figure IV- 3: Dissipation of Azoxystrobin (\square), Fenazaquin (Δ) and Indoxacarb (\circ) over time in brine(—) and dry (----) conservation

The results from the industrial study showed that for Azoxystrobin, other than the residues detected in T8, the difference between in residue levels between the two processing techniques was not statistically significant for all the sampling dates (Annexe III-2 - Table SIV - 4). For the acaricide Fenazaquin, however, the difference in residue levels between the two conservation methods was significant, for all the sampling dates, except for T20. For the insecticide Indoxacarb, residue concentrations were significantly different for almost all the sampling dates, except for T0 and T8 (the p-values from Student's t test for each sampling date are given in the supporting information Annexe III-2 - Table SIV - 4). For the dissipation

rates, Fenazaquin had higher dissipation rates than those of Indoxacarb and Azoxystrobin under both conservation methods. Consequently, the estimated half-life values for Fenazaquin were much lower than Indoxacarb and Azoxystrobin. The p-value of the dissipation rates showed that for Fenazaquin, important dissipations were obtained during the storage period with $p \ll 0.001$, and the estimated DT50s were 9 days for the brine conservation method versus 18 days for the dry conservation method. Additionally, residue reduction was observed only in brine conservation for Azoxystrobin and only in dry conservation for Indoxacarb ($p \ll 0.001$), with estimated DT50s of 261 and 142 days, respectively. No significant differences for Azoxystrobin residues in dry conservation ($p = 0.056$) and for Indoxacarb residues in brine conservation ($p = 0.58$) were observed, indicating no residue dissipation during the 60 days of conservation in these two cases, with DT50s exceeding one year for both molecules.

Table IV- 1: Details of the slopes of regressions, intercepts, their standard errors, p-values of the slope, the pesticide half-life their confidence interval and R squared of the regression.

| Pesticide | Treatment | Slope (K) | p-value of slope | Intercept | Half-life (days) | R ² |
|--------------|-----------|-----------------|------------------|--------------|------------------|----------------|
| Azoxystrobin | Brined | -0.003 ±0.0005* | <<0.001 | 2.238 ±0.015 | 261 [185, 443]** | 0.51 |
| | Dry | -0.002 ±0.0008 | 0.056 | 2.215 ±0.023 | > 1 year | 0.14 |
| Fenazaquin | Brined | -0.078 ±0.004 | << 0.001 | 0.650 ±0.11 | 9 [8,10] | 0.95 |
| | Dry | -0.04 ±0.001 | <<0.001 | 0.247 ±0.041 | 18 [16,19] | 0.97 |
| Indoxacarb | Brined | -0.001 ±0.001 | 0.58 | 1.105 ±0.029 | >> 1 year | 0.013 |
| | Dry | -0.005 ±0.0007 | << 0.001 | 0.834 ±0.021 | 142 [108,207] | 0.64 |

*Standard Error; ** 95 % Confidence Interval ; NA: Not Applicable

The processing factor calculations (Annexe III-2 - Table SIV- 4), demonstrated that after 60 days of conservation, brine conservation resulted in reductions of 36%, 99%, and 14% for Azoxystrobin (PF = 0.64), Fenazaquin (PF = 0.005), and Indoxacarb (PF = 0.86), respectively. Similarly, dry conservation showed reductions of 37%, 97%, and 50% for Azoxystrobin (PF = 0.63), Fenazaquin (PF = 0.03), and Indoxacarb (PF = 0.50), respectively. These findings indicate that dry conservation, as a processing technique, achieves higher rates of residue removal (an average of 60 %) compared to brine processing (an average of 48 %), irrespective of the type

of molecules. Thus, dry conservation is recommended based on its efficacy in removing different types of residues.

3.1.2. Residues concentration in brine waters

The mean concentrations of residues measured in the brine waters for the 3 pesticides, as well as the Ratio B/L used to study the distribution pattern of the residues between vine leaves and the brine waters, are supplied in the supporting information Annexe III-2 - Table SIV - 5. For Fenazaquin, the residues in brine waters remained constant during all the conservation period with a mean concentration of 0.002 mg.kg^{-1} . The Ratio B/L increased with time from 0.001 to 0.064 mg.kg^{-1} . This is because Fenazaquin adsorbs fairly to the leaves matrix and does not dissolve in water (Kumar, Tewary, Ravindranath & Shanker, 2006). The increase of the Ratio B/L was mainly due to the degradation of Fenazaquin in leaves which can be related to the physiochemical properties of Fenazaquin favoring its rapid degradation. For Azoxystrobin and Indoxacarb, the residues in brine waters showed the same dissipation behavior as in leaves which is reflected in an increase-decrease-increase fluctuating pattern in the residue's concentration in the brine waters. This behavior suggests an exchange of residues between the leaves and the brine waters (2 % on average for the three molecules) and could reflect the alteration of the cuticula of the vine leaves that releases fractions of the residue embedded in the waxes during the storage period.

Indeed, it is possible to observe that also in the leaves the decrease of Azoxystrobin residues was not constant, and a sudden spike of residue was measured at T44 (8.6 mg.kg^{-1}) for the brine processing technique, and at T20 (8.24 mg.kg^{-1}) and T28 (9.14 mg.kg^{-1}) for the dry processing technique. A similar result was obtained for Indoxacarb. A sudden increase of residues of indoxacarb in leaves were measured at T20 (3.46 mg.kg^{-1}) for the brine processing technique, and at T28 (2.25 mg.kg^{-1}) for the dry processing technique (Figure IV - 3 & Annexe III-2 - Table SIV- 4). Lu et al. (2013) made a similar observation. They compared the residues of ten pesticides in the brine and in pickled cabbages observing a similar fluctuation in residues in brine waters. They reported a sudden increase of Chlorpyrifos and Triazophos residue concentration, after consistent decay, in cabbage leaves preserved in 20% NaCl brine. Their explanation was the effect of residue concentration resulting from the loss of water from the leaf matrix during storage (Lu et al., 2013). They also discussed the effect of the brine composition on the transfer of pesticides to the brine (Lu et al., 2013)

3.1.3. The effect of the mode of action and physico-chemical properties of pesticides

The results from the industrial study strongly displayed the effect of the mode of action and the molecules' physicochemical properties on the dissipation, where lower dissipation rates were obtained for Azoxystrobin and Indoxacarb compared to Fenazaquin, although they both have higher solubility factors in water ($S_{\text{Azoxystrobin}} = 6.7 \text{ mg.L}^{-1}$; $S_{\text{Indoxacarb}} = 0.2 \text{ mg.L}^{-1}$) and lower octanol water partition coefficients ($\text{LogP}_{\text{Azoxystrobin}} = 2.5$; $\text{LogP}_{\text{Indoxacarb}} = 4.6$) than Fenazaquin's ($S_{\text{Fenazaquin}} = 0.102 \text{ mg.L}^{-1}$; $\text{LogP}_{\text{Fenazaquin}} = 5.51$). Indeed, Azoxystrobin is a systemic pesticide that allows it to move deeper in the plant tissues than Fenazaquin and Indoxacarb having both a non-systemic mode of action (contact) making Azoxystrobin less prone to dissipation despite having a higher solubility. In fact, the elimination of residue strongly depends on the localization of the pesticide in the plant material, a high-water solubility of some systemic pesticides does not always guarantee their dissipation (Lozowicka, Jankowska, Hrynko & Kaczynski, 2016). Acoglu and Omeroglu (2021) argued that processing factors are not only influenced by the physico-chemical properties of the pesticide but also by the mode of action of the pesticide that dictates the pesticide partitioning and its presence in the treated matrix. The combined effect of the mode of action (systemic versus non-systemic) and the washing of crops is discussed in the work of Hwang, Seok and Kim (2019). They studied the permeation of the systemic Azoxystrobin and the contact (non-systemic) Chlorothalonil pesticides into apples' pulp by analyzing the residues from pesticides applied as wettable powder (WP) formulations in unwashed, washed, and wax removed apple samples (after washing). They found that Azoxystrobin was irremovable in washings and strongly bound to the matrix. They attributed this behavior due to the adjuvants in the commercial formulation, which confer Azoxystrobin's systemic nature, embedding it in the waxes and cuticular tissues of the matrix (Hwang et al., 2019). As to Chlorothalonil, the washing eliminated all residues with a removal efficiency close to 98.5 % (Hwang et al., 2019). Indeed, surfactants, penetrant enhancers, and co-solvents are added to pesticide commercial formulations to increase effectiveness and stability of the active ingredient of the commercial product (Kalyabina, Esimbekova, Kopylova & Kratasyuk, 2021). However, contradictory results regarding the removal of Azoxystrobin in a variety of matrices by different processing techniques can be found in the literature and explained by Aguilera, Valverde, Camacho, Boulaid, and García-Fuentes (2012) as due to the variability of the cuticular composition in the studied matrices, which influences the residue

removal to a greater or lower extent. As well they demonstrated that among non-systemic pesticides, Pyridaben, having the highest octanol/water partition coefficient compared to Acrinathrin, Fipronil and Kresoxim-methyl, also had the greatest residue reduction, and this was explained by its non-systemic mode of action (Aguilera et al., 2012). Angioni, Schirra, Garau, Melis, Tuberoso and Cabras (2004) along with Lozowicka et al. (2016) demonstrated and discussed the lack of correlation between the pesticide's solubility and the residues dissipation in washing water. Vemuri, Sreenivasa Rao, Rao, Darsi, Reddy and Swarupa (2014) demonstrated that Dimethoate, a systemic pesticide, had the lowest rate of reduction, compared to non-systemic molecules Methyl-parathion, Quinalphos, Endosulfan and Profenophos, when washing tomatoes with salt water (2%). Guardia-Rubio, Ayora-Cañada and Ruiz-Medina (2007) showed that despite the water solubility characteristic of some pesticides, washing olives with water was more effective in removing Simazine residues, a non-systemic pesticide, than in removing the residues of Diuron and Terbutylazine, two systemic pesticides, despite they had greater solubility in water than Simazine. The authors also related the removal to the different octanol-water partition (K_{ow}) coefficients that influence the partition of the pesticides between the cuticula and the washing water (Guardia-Rubio et al., 2007). The results obtained by Yang, Doherty, Zhao, Kinchla, Clark and He (2017) are in line with those previous findings. They investigated the effectiveness of commercial and homemade washing agents in the removal of contact and systemic pesticides in apples. Contact pesticide residues, present on the surface of the peel, were most effectively removed by sodium bicarbonate solution when compared to either tap water or chlorine-based bleach. Thiabendazole, a systemic pesticide, penetrated four times more into the apple peel than Phosmet, a non-systemic pesticide, which led to more Thiabendazole residues in the apple pulp, and could not be washed away using the sodium bicarbonate washing solution. They concluded that peeling may be the best method to reduce the number of systemic pesticides, such as Thiabendazole (Yang et al., 2017) .

While the mode of action played a primary role in the dissipation of Azoxystrobin, the physicochemical properties of Indoxacarb emerged as a significant factor influencing its slow dissipation. Despite both Indoxacarb and Fenazaquin being non-systemic pesticides, the higher solubility of Indoxacarb, in comparison to Fenazaquin, resulted in lower dissipation rates. Undoubtedly, Indoxacarb exhibits greater physicochemical stability than Fenazaquin, as evidenced by its lower vapor pressure value, which likely contributed to its recalcitrance.

Gao et al. (2020) showed that two studied organophosphate pesticides (OP), Trichlorfon and Acetamiprid, had higher vapor pressure than the three studied neonicotinoids pesticides, but presented larger penetration ratios in tea leaves than neonicotinoids. However, they showed that the OP pesticides dissipated more quickly and left behind smaller levels of residues on and in the entire leaves than the neonicotinoid pesticides (Gao et al., 2020). A similar behavior for Indoxacarb, as in this study, was shown by Xu, Lu, Xu, Lu, Qiu and Zha (2022). They demonstrated that only 10.7% of Indoxacarb residues passed from tea leaves to the brew (Xu et al. 2022). Noh et al. (2022) stated that Indoxacarb's long half-life in the field was due to its resistance to rainfall mainly because of its low solubility, which reflects, in general, the stability of the pesticide. Fenazaquin dissociates in an acidic environment (pKa 2.44) and undergoes degradation by hydrolysis in an aqueous medium with a DT50= 9.6 at pH 5 (see supporting information Annexe III-2 - Table SIV- 2). On the other hand, Indoxacarb is a relatively stable pesticide. This was shown by the significant difference in the half-lives obtained for the mode of preservation in brine being 9days for Fenazaquin and exceeding one year for Indoxacarb (see Table IV- 1). With Fenazaquin showing a significant residue reduction (dissipation) in both the dry and the brine conservation mode after 60 days (>97%) (Table IV- 1 and Figure IV - 3). Kumar et al. (2006) yielded alike findings in tea crops. They explained the fast dissipation of Fenazaquin under field conditions and during the processing of tea leaves by the pesticide physico-chemical properties, as Fenazaquin exhibits a short half-life under agroclimatic conditions, and degrades under boiling temperatures such as those encountered with hot brine poured on the leaves, or when acidic conditions, such as the one used in the brine processing techniques (pH= 3.5) from this study, are applied (Kumar et al., 2006).

3.2. Effect of the washing method at the laboratory on residues removal in vine leaves

The concentration of the residues of the three molecules in vine leaves after washing with tap water, 10% vinegar solution and boiling water are presented in Figure IV- 4. The studied pesticides exhibited different behaviors for each of washing method, namely, tap water, boiled water and 10% vinegar solution (refer to Annexe III-2 - Table SIV- 6 in the supporting information).

For Fenazaquin, the three washing methods had significantly different effects on the residues, washing with tap water was found to be the most effective method for reducing the Fenazaquin residues (PF = 0.57), followed by washing with 10% vinegar solution (PF = 0.66)

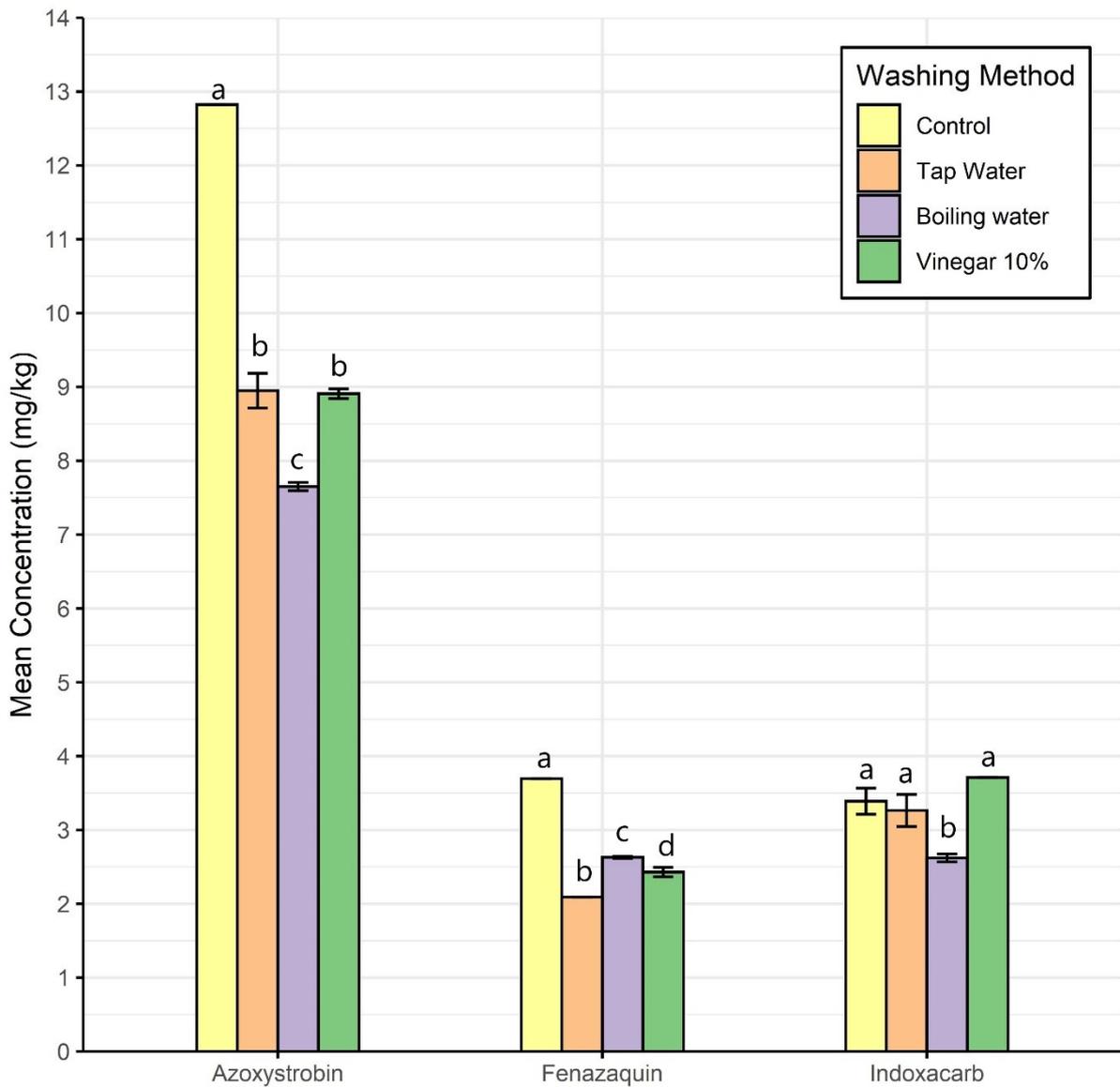


Figure IV- 4: Residues concentration of azoxystrobin, fenazaquin and indoxacarb in vine leaves (mg/kg) after washing with three different methods, as compared to unwashed samples

and boiling water (PF = 0.71). For Azoxystrobin washing with boiling water was significantly more effective in reducing residues (PF = 0.60) compared to the two other methods, which had similar effects on the residues with PFs of 0.69 and 0.70 after 10% vinegar solution and tap water washing, respectively. For Indoxacarb, washing with boiling water was the only significantly effective method to reduce Indoxacarb residues (PF = 0.78). Tukey's post-hoc test showed no significant difference between the residue amount in the control (unwashed) group and the amount detected after washing with tap water (PF = 0.96) or with the 10% vinegar solution (PF = 1.10). In this latter case, an increase in residue amount was observed (+9.44%). The increase of the Indoxacarb residues after washing with the 10% vinegar solution in addition to the residues in the control samples not being significantly different from the residues after washing with tap water and 10% vinegar solution, may be explained by the

analytical uncertainty value. Some authors also suggest that the high matrix effects in the analytical determination may have an enhancement effect on the reported residue values. Andrade, Monteiro, Francisco, Figueiredo, Rocha and Tornisielo (2015) also reported higher residues of the insecticide Fipronil in tomatoes after washing with vinegar. This behavior was potentially attributed to the analytical sample preparation, and the ionization suppression effect due to the acidic matrix as observed in the mass spectrometric detection (Andrade et al., 2015). Matrix effects in the analysis of fresh fruits and vegetables are common, and inevitable (Silvestro, Tarcomnicu & Savu, 2013) .

The laboratory washing study results align with the industrial processing outcomes. Fenazaquin showed the highest residue reduction (28.82-43.38%), compared to Azoxystrobin (30.21-40.34%) and Indoxacarb (0-22.39%). As mentioned earlier, the lower PFs of Azoxystrobin and Indoxacarb, compared to Fenazaquin, may primarily be due to Azoxystrobin's systemic formulation and the relatively stable physiochemical characteristics of Indoxacarb residues. The decrease of Azoxystrobin residues left after washing with water and vinegar solution (~30%) can be explained by the physical removal of the dust present on the leaves; a similar effect was noticed at T0 for the dry and brine processing. In the industrial study, except for Indoxacarb in brine conservation, at T0, the pesticides residues had already dissipated by 25-57% (see supporting information Annexe III-2 - Table SIV- 4). These residue reductions may be due to the degradation (enzymatic, environmental degradation, etc.) that took place after harvest, and between the arrival of the samples at the factory and their processing, with an estimated time of about 24 hours for the entire process. Another explanation for the quick decrease in the residue concentration 24hr after harvest is the removal of dust from the vine leaves' surface, and consequently, the elimination of potentially adsorbed pesticides. A similar observation was made by Angioni et al. (2004). The agricultural practices implemented in Lebanon for the "Grenache" variety of vines may have played a role in increasing the amount of dust on leaves, thus an important fraction of the pesticide residues detected in the samples might have come from the dust. Therefore, its elimination is important towards ensuring minimal residues in the final product (the vine leaves).

Furthermore, it has been demonstrated that temperature and humidity are two important regulators of the cuticula's biomechanical properties. The combination of elevated temperature and high humidity is detrimental to the cuticula (Lara, Heredia & Domínguez,

2019); this explains the enhanced release of pesticides, especially Azoxystrobin and Indoxacarb, from the leaf matrix after washing with boiled water. Maestroni et al. (2018) reported the effectiveness of washing vine leaves with hot water in removing Dimethoate residues, achieving a 92 % removal success; however, no discernable effect was observed for Chlorpyrifos, the most apolar compound in their study. Lee and Jung (2009) described similar findings on pepper leaves. The blanching step, using boiling water, increased the removal ratio of Dichlofluanid, Flusilazole, Folpet, Iprodione, λ -cyhalothrin, and Lufenuron. This effect was explained by hydrolysis, volatilization and leaching of the molecules during blanching and cooking processes (Lee et al., 2009). Cangı et al. (2019) showed that hot brining conservation produced the highest reduction of Azoxystrobin residues in grapevine leaves (96.7-99.2%); whereas cold water application produced lower reduction of residues. They also mentioned the importance of combining washing, boiling, and then brining vine leaves to ensure increased residues removal (Cangı et al., 2019). Duhan, Kumari and Gulati (2010) studied the effects of processing on Fenazaquin residues and found that the maximum reduction rate was observed by a combination of washing and boiling with a 61% reduction rate, compared to the individual steps of either boiling, cooking, or washing only (Duhan et al., 2010). The authors also suggest that a combination of processing steps is useful to further reduce the level of residues on the commodity (Duhan et al., 2010). In terms of achieving the highest residue reduction, Ta, Madan, Chauhan and Kumari (2018) demonstrated for tomato fruits, that reduction rates of Indoxacarb ranged between 12 - 54 % after washing only, and between 71-85 % after a combined washing and boiling, whereas peeling was the most effective processing technique with a decrease of residues up to 89%. Andrade et al. (2015) concluded that different washing methods (water, 10% bicarbonate solution and 10 % vinegar solution) shall be adopted for each of the studied pesticides. Lozowicka et al. (2016) recommended boiling along with ultrasonic cleaning as one of the most effective methods in removing the residues of the 16 studied molecules in strawberries having different formulation types (EC/SC/WG).

From the evidence presented, washing with boiled water resulted in an average residue reduction of 30% for the three pesticides, regardless of the molecule's mode of action or its physicochemical properties. Thus, washing with boiled water could be recommended for vine leaves processing.

3.3. Dietary risk assessment

The dietary risk assessment was based on the estimation of the hazard quotient (HQ). The HQ reflects the long-term health risk for consumers when consuming a certain quantity of vine leaves, contaminated with pesticide residues, over a certain amount of time. When the HQ is lower than 1, the lifetime consumption of a commodity containing the measured level of pesticide residues does not pose health risks to the consumers; on the contrary, a HQ higher than 1, indicates a possible risk for the consumers.

As shown in Table IV- 2, the estimated HQs for all pesticides and all processing methods were below 1. The HQ was also lower than 1 for all control samples. This indicates that the consumption of the vine leaves would not pose a health risk to the consumers under such a scenario. The EDI used in the estimation of the HQ for the control samples represented the worst-case scenario, where the vine leaves were harvested just after 12 hours from the pesticide applications and where no conservation or processing method was applied. For Azoxystrobin, washing with boiling water showed the lowest HQ value of 0.0191. For Fenazaquin, the 60 days brine conservation had the lowest HQ value of 0.002. Finally, for Indoxacarb, the 60 days dry conservation showed the lowest HQ with a value of 0.17. The food consumption value, which was used in equation 5, was estimated based on the assumption that an adult would consume, under certain traditional eating habits, on average 100 stuffed vine leaves per week. Since a single vine leaf weight about 2 g, an adult consumes a total of 200 g of vine leaves per week (0.03 kg/day). This represents a potentially overestimated value. Still, risk assessment by HQ calculations only reflects the risk of the chronic dietary cumulative exposure to the individual pesticide in the vine leaves and do not consider the risk stemming from the exposure to the same molecule from other food sources or the risk associated by the exposure to pesticide cocktails that may be present in vine leaves. From this comes the importance of setting realistic MRLs for vine leaves to define pre-harvest intervals to minimize pesticide exposure from this commodity and limit its contribution to the overall pesticide exposure of the consumers.

Table IV- 2: The risk assessment was based on the estimation of the EDI and HQ for the pesticides according to the different conservation / washing methods. The EDI is reported as $\text{mg.kg}^{-1}.\text{day}^{-1}$.

| Molecule | ADI* (mg. kg ⁻¹ .day ⁻¹) | Processing Method | Mean Concentration (mg.kg ⁻¹) (±SD) | EDI** (mg.kg ⁻¹ .day ⁻¹) | HQ*** |
|--------------|---|----------------------|---|--|--------|
| Azoxystrobin | 0.2 | Control | 12.82 (±0.002) | 0.0064 | 0.0321 |
| | | In Brine | 8.24 (±0.252) | 0.0041 | 0.0206 |
| | | Dry | 8.05 (±0.326) | 0.0040 | 0.0201 |
| | | Tap water | 8.95 (±0.235) | 0.0045 | 0.0224 |
| | | Boiling water | 7.65 (±0.056) | 0.0038 | 0.0191 |
| | | Vinegar 10% | 8.91 (±0.066) | 0.0045 | 0.0223 |
| Fenazaquin | 0.005 | Control | 3.69 (±0.002) | 0.0018 | 0.3690 |
| | | In Brine | 0.02 (±0.000) | 0.0000 | 0.0020 |
| | | Dry | 0.11 (±0.011) | 0.0001 | 0.0110 |
| | | Tap water | 2.09 (±0.001) | 0.0010 | 0.2090 |
| | | Boiling water | 2.63 (±0.013) | 0.0013 | 0.2630 |
| | | Vinegar 10% | 2.43 (±0.064) | 0.0012 | 0.2430 |
| Indoxacarb | 0.005 | Control | 3.39 (±0.177) | 0.0017 | 0.3390 |
| | | In Brine | 2.92 (±0.025) | 0.0015 | 0.2920 |
| | | Dry | 1.70 (±0.133) | 0.0009 | 0.1700 |
| | | Tap water | 3.26 (±0.217) | 0.0016 | 0.3260 |
| | | Boiling water | 2.62 (±0.053) | 0.0013 | 0.2620 |
| | | Vinegar 10% | 3.71 (±0.003) | 0.0019 | 0.3710 |

*According to Lewis et al. (2016)

**Average Adult Body weight = 60 Kg; Average vine leaves consumption = 0.03 kg.day⁻¹; EDI= (Average Food Consumption (kg.day⁻¹)×Residue Amount(mg.kg⁻¹))/(Body weight (kg))

N.B. The mean concentrations for in brine and dry processing methods represent the remaining concentrations after 60 days of conservation time.

4. Conclusion

This study confirms that different preservation and washing methods have varying effects on pesticide residues in vine leaves. The mode of action and physicochemical properties of the molecules contributed to dissipation rate variations, influencing residue levels. The dissipation rates of Azoxystrobin (DT50 = 261 days in brine conservation and exceeding one year in dry conservation) and Indoxacarb (DT50 exceeding one year in brine conservation and of 142 days in dry conservation) were lower compared to Fenazaquin (DT50 = 9 days in brine

conservation and of 18 days in dry conservation). Regardless of the molecule's nature, dry conservation demonstrated a more pronounced residue reduction for all three molecules (60% average reduction) compared to brined conservation (48% average reduction). If these results are confirmed by studying other molecules, dry conservation could be consequently recommended. Furthermore, residue levels in brine waters indicate pesticide diffusion from leaves to brine, emphasizing the need for industrialists and consumers to treat brine solutions as contaminated. Washing with boiling water showed 30% residues reductions on average, regardless of the molecule nature, suggesting the preference to favor hot water washing or blanching for leaf processing. Health risk assessment shows no risk in extreme scenarios, with Hazard Quotients consistently below 1 for all pesticides and treatment methods; however, the potential contribution of these molecules to the overall pesticide exposure must be taken into consideration. Therefore, it is crucial for the industry to consider the specific characteristics and behavior of each pesticide when selecting processing techniques. Thus, the findings of this study support the hypothesis of the varying effects of different preservation and washing methods on pesticide residues in vine leaves. Since conservation procedures do not ensure residue elimination during the storage, vine leaf producers are encouraged to minimize phytosanitary product use during the leaf harvest period and employ targeted treatments only when necessary. Additionally, they are also encouraged to use phytosanitary treatment records to enable industrialists to accurately estimate the level of residues to avoid the risk of export rejections. Further studies are crucial to understand the processing behavior of all viticulture authorized pesticides. Developing regulatory measures, implementing good agricultural practices, and setting realistic MRLs-PHI for vine leaves will enhance international trade and protect consumer health.

Credit authorship contribution statement

Liliane MAJED: Conceptualization, methodology, software, formal analysis, investigation, data curation, writing—original draft preparation and visualization. **Salem HAYAR:** Conceptualization, methodology, validation, formal analysis, resources, writing—review and editing, supervision, project administration and funding acquisition. **Sylvie DOUSSET:** writing—review and editing and supervision. **Britt Marianna MAESTRONI:** Validation and writing—review and editing. **Khaled EL OMARI:** Residues LCMSMS Analysis. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting Information

The following Supporting Information is available: **Table S1.** Phytosanitary products, percentage of active substance, application rate and pre-harvest intervals (PHI) as mentioned on the product label ; **Table S2.** Physico-chemical and toxicological properties of azoxystrobin, fenazaquin and indoxacarb; **Table S3.** Method validation results for the molecules at the three fortification levels (n= 5 at each level) in vine leaves; **Table S4.** Mean residue concentrations, half-lives (DT50), processing factors (PF) and Student's t-test p-values of the 3 pesticides for the two industrial processing techniques, brining and drying; **Table S5.** Analysis of brine waters for azoxystrobin, fenazaquin and indoxacarb (n= 3); **Table S6.** Mean residue concentrations in mg.kg⁻¹ before and after the washing procedures of vine leaves samples (n=3), processing factors and Tukey's test comparing these different procedures; **Figure S1.** the extracted ion chromatogram for azoxystrobin, fenazaquin and indoxacarb in vine leaves at 50µg/L

References

- Acoglu, B., & Omeroglu, P. Y. (2021). Effectiveness of different type of washing agents on reduction of pesticide residues in orange (*Citrus sinensis*). *LWT*, *147*, 111690. <https://doi.org/10.1016/j.lwt.2021.111690>
- Aguilera, A., Valverde, A., Camacho, F., Boulaid, M., & García-Fuentes, L. (2012). Effect of household processing and unit to unit variability of azoxystrobin, acrinathrin and kresoxim methyl residues in zucchini. *Food Control*, *25*(2), 594–600. <https://doi.org/10.1016/j.foodcont.2011.11.038>
- Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce. *Journal of AOAC International*, *86*(2), 412–431. <https://doi.org/10.1093/jaoac/86.2.412>
- Andrade, G., Monteiro, S., Francisco, J., Figueiredo, L., Rocha, A., & Tornisielo, V. (2015). Effects of Types of Washing and Peeling in Relation to Pesticide Residues in Tomatoes. *Journal of the Brazilian Chemical Society*, *26*. <https://doi.org/10.5935/0103-5053.20150179>
- Angioni, A., Schirra, M., Garau, V. L., Melis, M., Tuberoso, C. I. G., & Cabras, P. (2004). Residues of azoxystrobin, fenhexamid and pyrimethanil in strawberry following field treatments and the effect of domestic washing. *Food Additives and Contaminants*, *21*(11), 1065–1070. <https://doi.org/10.1080/02652030400010066>
- Bajwa, U., & Sandhu, K. S. (2014). Effect of handling and processing on pesticide residues in food- a review. *Journal of Food Science and Technology*, *51*(2), 201–220. <https://doi.org/10.1007/s13197-011-0499-5>
- Cangi, R., Yanar, Y., & Dülgeroğlu, Y. (2019). Effects of Brining and Picking Time on The Degradation of Pesticide Residue in Grapevine Leaves. *Türk Tarım - Gıda Bilim ve Teknoloji Dergisi*, *7*(11), 1773–1779. <https://doi.org/10.24925/turjaf.v7i11.1773-1779.2505>
- Cantwell, M. I., Hong, G., Albornoz, K., & Berlanga, M. (2022). Fresh grapevine (*Vitis vinifera* L.) leaves: Postharvest biology and handling recommendations. *Scientia Horticulturae*, *292*, 110627. <https://doi.org/10.1016/j.scienta.2021.110627>
- Codex online databases | CODEXALIMENTARIUS* FAO-WHO. (2021). Retrieved from <https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/en/> . Accessed April 4, 2021
- Duhan, A., Kumari, B., & Gulati, R. (2010). Effect of Household Processing on Fenazaquin Residues in Okra Fruits. *Bulletin of Environmental Contamination and Toxicology*, *84*(2), 217–220. <https://doi.org/10.1007/s00128-009-9863-9>
- EU Pesticides Database (v.2.2) Pesticide residue(s) and maximum residue levels (mg/kg)*. (2021). Retrieved from https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=details&pest_res_ids=22,132,2692&product_ids=&v=1&e=search.pr . Accessed April 4, 2021
- Gao, W., Guo, J., Xie, L., Peng, C., He, L., Wan, X., & Hou, R. (2020). Washing fresh tea leaves before picking decreases pesticide residues in tea. *Journal of the Science of Food and Agriculture*, *100*(13), 4921–4929. <https://doi.org/10.1002/jsfa.10553>
- Guardia-Rubio, M., Ayora-Cañada, M. J., & Ruiz-Medina, A. (2007). Effect of washing on pesticide residues in olives. *Journal of Food Science*, *72*(2), C139-143. <https://doi.org/10.1111/j.1750-3841.2006.00252.x>
- EC. (2019). Guidance SANTE/12682/2019 – Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. In

Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Retrieved from <https://www.accredia.it/en/documento/guidance-sante-12682-2019-guidance-document-on-analytical-quality-control-and-method-validation-procedures-for-pesticides-residues-analysis-in-food-and-feed/>. Accessed October 19, 2020

FAO/WHO. (1997). *Guidelines for predicting dietary intake of pesticide residues / prepared by the Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme (GEMS/Food); in collaboration with the Codex Committee on Pesticide Residues*. Retrieved from <https://apps.who.int/iris/handle/10665/63787>. Accessed August 8, 2021

Hayar, S., Zeitoun, R., & Maestroni, B. M. (2021). Validation of a Rapid Multiresidue Method for the Determination of Pesticide Residues in Vine Leaves. Comparison of the Results According to the Different Conservation Methods. *Molecules*, 26(4), 1176. <https://doi.org/10.3390/molecules26041176>

Hwang, J.-I., Seok, D.-R., & Kim, J.-E. (2019). Effects of cuticular waxes on permeation of fungicides azoxystrobin and chlorothalonil into apples. *Applied Biological Chemistry*, 62(1), 34. <https://doi.org/10.1186/s13765-019-0441-5>

Kalyabina, V. P., Esimbekova, E. N., Kopylova, K. V., & Kratasyuk, V. A. (2021). Pesticides: formulants, distribution pathways and effects on human health – a review. *Toxicology Reports*, 8, 1179. <https://doi.org/10.1016/J.TOXREP.2021.06.004>

Kumar, V., Tewary, D. K., Ravindranath, S. D., & Shanker, A. (2006). Investigation in tea on fate of fenazaquin residue and its transfer in brew. *Food and Chemical Toxicology*, 44(4), 596–600. <https://doi.org/10.1016/j.fct.2005.10.010>

Lara, I., Heredia, A., & Domínguez, E. (2019). Shelf Life Potential and the Fruit Cuticle: The Unexpected Player. *Frontiers in Plant Science*, 10. <https://www.frontiersin.org/articles/10.3389/fpls.2019.00770>

Lee, M.-G., & Jung, D.-I. (2009). Processing Factors and Removal Ratios of Select Pesticides in Hot Pepper Leaves by a Successive Process of Washing, Blanching, and Drying. *Food Science and Biotechnology*, 18(5), 1076–1082. <https://koreascience.kr/article/JAKO200935736659085.kr&sa=U>

Lewis, K. A., Tzilivakis, J., Warner, D. J., & Green, A. (2016). An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal*, 22(4), 1050–1064. <https://doi.org/10.1080/10807039.2015.1133242>

Lozowicka, B., Jankowska, M., Hrynko, I., & Kaczynski, P. (2016). Removal of 16 pesticide residues from strawberries by washing with tap and ozone water, ultrasonic cleaning and boiling. *Environmental Monitoring and Assessment*, 188(1), 51. <https://doi.org/10.1007/s10661-015-4850-6>

Lu, Y., Yang, Z., Shen, L., Liu, Z., Zhou, Z., & Diao, J. (2013). Dissipation behavior of organophosphorus pesticides during the cabbage pickling process: Residue changes with salt and vinegar content of pickling solution. *Journal of Agricultural and Food Chemistry*, 61(9). <https://doi.org/10.1021/jf304500f>

Maestroni, B. M., Ghanem, I., Correll, R., Alnaser, A. A., Islam, M., Cesio, V., Heinzen, H., & Cannavan, A. (2018). Required Withholding Period for Vine Leaves Following Spraying with Pesticide. *Journal of Health and Environmental Research*, 4(4), 140. <https://doi.org/10.11648/j.jher.20180404.14>

Noh, H.-H., Jo, S.-H., Shin, H.-W., Kim, D.-J., Ham, Y.-J., Kim, J.-Y., Kim, D.-B., Kwon, H.-Y., & Kyung, K.-S. (2022). Dissipation and Residue Pattern of Dinotefuran, Fluazinam, Indoxacarb, and Thiacloprid in Fresh and Processed Persimmon Using LC-MS/MS. *Foods*, 11(3), 416.

<https://doi.org/10.3390/foods11030416>

OECD. (2016). *OECD Guidance Document on Crop Field Trials*. Retrieved from [https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2011\)50/rev1&doclanguage=en](https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2011)50/rev1&doclanguage=en) . Accessed March 19, 2021

Joint FAO/WHO Food Standards. (1999). *Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs (CAC/GL 33-1999)*. Retrieved from https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXG%2B33-1999%252FCXG_033e.pdf . Accessed March 19, 2021

RASFF. (2021). European Commission RASFF Window. Retrieved from <https://webgate.ec.europa.eu/rasff-window/screen/search> . Accessed March 19, 2021

Silvestro, L., Tarcomnicu, I., & Savu, S. R. (2013). Matrix Effects in Mass Spectrometry Combined with Separation Methods — Comparison HPLC, GC and Discussion on Methods to Control these Effects. In *Tandem Mass Spectrometry - Molecular Characterization*. Intech. <https://doi.org/10.5772/55982>

Ta, A., Madan, V. K., Chauhan, R., & Kumari, B. (2018). Dissipation Behaviour and Effect of Different Decontamination Processes on Reduction of Residues of Novaluron in Tomato (*Lycopersicon esculentum* Mill.). *International Journal of Current Microbiology and Applied Sciences*, 7(05), 648–656. <https://doi.org/10.20546/IJCMAS.2018.705.079>

Thorsen, S. (2021). Timeanddate.com. Retrieved from <https://www.timeanddate.com/>. Accessed June 15, 2021

Vemuri, S., Sreenivasa Rao, C., Rao, R., Darsi, H., Reddy, A., & Swarupa, S. (2014). Methods for Removal of Pesticide Residues in Tomato. *Food Science and Technology*, 2(5), 64-68. <https://doi.org/10.13189/fst.2014.020502>

Xu, F., Lu, Z., Xu, D., Lu, H., Qiu, J., & Zha, X. (2022). Dissipation behavior, residue transfer, and safety evaluation of chlorantraniliprole and indoxacarb during tea growing and brewing by ultrahigh-performance liquid chromatography-tandem mass spectrometry. *Environmental Science and Pollution Research*, 29(42), 63735–63752. <https://doi.org/10.1007/s11356-022-20321-y>

Yang, T., Doherty, J., Zhao, B., Kinchla, A. J., Clark, J. M., & He, L. (2017). Effectiveness of Commercial and Homemade Washing Agents in Removing Pesticide Residues on and in Apples. *Journal of Agricultural and Food Chemistry*, 65(44), 9744–9752. <https://doi.org/10.1021/acs.jafc.7b03118>

Yigit, N., & Velioglu, Y. S. (2020). Effects of processing and storage on pesticide residues in foods. *Critical Reviews in Food Science and Nutrition*, 60(21), 3622–3641. <https://doi.org/10.1080/10408398.2019.1702501>

Conclusion générale

Rappel de la problématique

L'absence de Limites Maximales de Résidus (LMR) dans les feuilles de vigne limite le commerce international de cette denrée alimentaire. Elle impacte les producteurs et industriels libanais qui cherchent à exploiter le potentiel économique des feuilles de vigne, souvent considérées comme un sous-produit de la production de raisins. Bien que les feuilles de vigne farcies soient un des plats classiques du mezzé libanais et soient consommées dans de nombreux pays méditerranéens (Chypre, Turquie, Grèce, Espagne, Italie et Maroc) (Rizzuti et al. 2013). Cette denrée est classée comme culture orpheline au niveau international et a été très peu étudiée par la communauté scientifique. Ceci a entraîné une absence de LMR dans le Codex Alimentarius, et une spécification de LMR européenne correspondant à la limite inférieure de détermination analytique pour 98 % des matières (European Commission 2023; FAO-WHO 2023). Ainsi, fournir des données scientifiques sur les teneurs et la dissipation des résidus dans les feuilles de vigne pourrait contribuer à l'établissement de LMR afin de protéger la santé des consommateurs.

Des études ont porté sur l'estimation des teneurs en résidus de pesticides dans diverses cultures au Liban, telles que le néflier (Abou Zeid et al. 2021), les pommes (El Hawari et al. 2019), et les fraises (Kfoury, Hilan, et El-Amil 2002). Toutefois, une revue exhaustive de la littérature a révélé une absence de données sur les teneurs en résidus de pesticides dans les raisins et les feuilles de vigne dans le contexte libanais (**Chapitre 1**). Au Liban, seules deux études, centrées sur l'exposition alimentaire aux résidus de pesticides provenant d'aliments d'origine végétale à Beyrouth, fournissent des informations sur les résidus de pesticides dans les raisins et les feuilles de vigne (Nasreddine et al. 2016; Khazaal et al. 2022). De plus, une étude menée par Hayar, Zeitoun, et Maestroni (2021) a examiné les résidus dans les feuilles de vigne conservées commercialisées au Liban. Cependant, les résultats présentés dans ces études présentent plusieurs limitations qui entravent leur représentativité. Ces limitations comprennent la restriction des études à une seule région (Beyrouth), un petit nombre d'échantillons (5 - feuilles de vigne et 12 - raisins, (Khazaal et al. 2022), et le regroupement des résultats avec d'autres aliments de la même catégorie, tels que les raisins et les fraises ensemble, ainsi que les légumes-feuilles verts cuits pour les feuilles de vigne avec les épinards (Nasreddine et al. 2016)). L'absence de traçabilité et l'impossibilité d'identifier la provenance des feuilles de vigne commercialisées (Hayar, Zeitoun, et Maestroni 2021) ne permettent pas

de connaître les teneurs en résidus de pesticides des feuilles de vigne produites au Liban. À l'échelle mondiale, les résultats des études incluant les feuilles de vigne en parallèle aux raisins (Sama'neh 2004; Arora et al. 2009; Salama 2009; Jyot et al. 2010; Mohapatra et al. 2011; Patil et al. 2018) soulignent l'importance d'une analyse approfondie des résidus de pesticides dans les feuilles de vigne, ainsi que des facteurs influençant ces résidus, vu que ces études ont démontré que les feuilles de vigne accumulent des niveaux élevés de résidus de pesticides. De plus, peu d'études ont exploré les facteurs affectant les teneurs en résidus de pesticides dans les feuilles de vigne (Maestroni et al. 2018; Türköz Bakirci, Çınar, et Karakaya 2019; Cangı, Yanar, et Yılmaz 2019), tels que l'efficacité du lavage à l'eau chaude (Maestroni et al. 2018 ; Cangı, Yanar, et Yılmaz 2019) et la conservation en saumure des feuilles de vigne (Türköz Bakirci, Çınar, et Karakaya 2019; Cangı, Yanar, et Yılmaz 2019). Par ailleurs, aucune étude n'a examiné l'effet du type de formulation des pesticides sur la dissipation des résidus, ni comparé les effets de différents types de conservation et de lavage sur les résidus de cette denrée. De plus, une seule étude menée en Égypte a intégré l'évaluation des risques pour la santé humaine liés aux résidus de pesticides dans les feuilles de vigne (Hamzawy 2022). Cet auteur a souligné la nécessité d'une vigilance constante pour assurer la sécurité des produits à base de feuilles de vigne et réduire au minimum les risques potentiels pour la santé des consommateurs.

Questionnement et démarche scientifique

Pour évaluer l'utilisation des produits phytosanitaires en viticulture au Liban, des données de surveillance sur les teneurs en résidus de pesticides dans les raisins de table entre 2012 et 2014 ont été obtenus auprès du ministère de l'agriculture. Les résultats ont montré la présence de 48 pesticides dans 1 594 échantillons de raisin de table dans les principales régions agricoles : la Bekaa, le Nord, le Mont-Liban et le Sud. Les données ont été analysées quantitativement et qualitativement au moyen de méthodes statistiques et d'évaluation des risques, en tenant compte des propriétés des pesticides et du mode d'action des fongicides et des insecticides. Les résultats obtenus ont contribué à identifier les molécules les plus utilisées en viticulture au Liban, ainsi que celles présentant des teneurs dépassant les LMRs du Codex Alimentarius. Ces résultats ont été comparés aux données de la base de données du réseau d'alerte rapide européen pour l'alimentation humaine et animale (RASFF)

répertoriant les teneurs en résidus de pesticides ayant entraîné une interdiction d'exportation des feuilles de vigne du Liban. Ainsi, quatre molécules ont été retenues : Azoxystrobine, Fenezaquin, Imidaclopride et Indoxacarb.

Pour estimer les vitesses de dégradation des pesticides sur différentes matrices (raisins et feuilles de vigne) et pour différentes formulations, une parcelle de vigne (situé à Tamnine-El-Tahta, Gouvernorat de Baalbeck Hermel, Liban) a été traitée avec deux formulations d'imidaclopride. La vitesse de dégradation de l'imidaclopride dans les raisins et les feuilles de vigne a été suivie sur le terrain, avec un échantillonnage aléatoire des feuilles de vigne et des raisins tous les 2 jours jusqu'à 18 jours après le traitement.

Afin d'approfondir notre compréhension des facteurs influençant les résidus de pesticides dans les feuilles de vigne, nous avons étudié les pratiques post-récolte, notamment le mode de conservation (à sec et en saumure) et le lavage (eau du robinet, solution de vinaigre à 10% ou eau bouillie), en adoptant une approche semblable à la réalité. Trois pesticides ont été appliqués en juin 2021 sur une parcelle viticole de la variété "Grenache", situé à Kefraya, Bekaa, au Liban : azoxystrobine (0,25 kg/ha), fenazaquin (0,08 kg/ha) et indoxacarbe (0,036 kg/ha). Les teneurs en résidus de pesticides ont été suivies sur une période de 60 jours pour les 2 méthodes de conservation. De plus, les teneurs en résidus de pesticides dans l'eau de saumure ont été mesurées. Pour l'étude de l'effet des méthodes de lavage, les teneurs en résidus avant et après le lavage ont été déterminées. Le risque pour la santé humaine a également été évalué par le calcul des quotients de risque (HQ).

Synthèse des résultats

➤ Quel est l'état des pratiques phytosanitaires dans la viticulture libanaise ?

Les données de 2012 à 2014 sur les raisins montrent des teneurs élevées en résidus de pesticides (56 % à 97 % d'échantillons positifs), dont 15 % à 33 % de ces échantillons présentant des dépassements de LMRs pour un ou plusieurs pesticides. Les variations observées d'une année à l'autre suggèrent une efficacité accrue des mesures de surveillance du ministère de l'agriculture, à savoir l'accompagnement des agriculteurs, l'élaboration du guide pour la production du raisin de table et la surveillance des teneurs en résidus durant la saison de production. En effet, une diminution significative des teneurs en résidus a été observée, avec les médiane de concentrations étant de 0,18 mg/kg en 2012, comparée à 0,06

mg/kg en 2013 et 0,07 mg/kg en 2014.

L'analyse des 48 pesticides quantifiés indique une prédominance de pesticides systémiques, représentant 78% des pesticides appliqués en 2012, 89% en 2013 et 86% en 2014. Les fongicides sont largement utilisés, constituant 73% en 2012, 84% en 2013 et 76% en 2014, ciblant principalement l'oïdium, le mildiou et la pourriture grise.

Bien que les risques pour la santé humaine soient évalués comme négligeables avec des HQ inférieurs à 1, ces HQs ne tiennent compte que du risque des molécules individuellement; par conséquent, il est essentiel de prendre en considération l'exposition globale aux résidus. Surtout, la présence de cocktails de résidus de pesticides dans les échantillons soulève des inquiétudes quant à leurs effets cumulatifs sur la santé humaine (WHO 2018). En effet, 60 % des échantillons contenaient entre 2 et 9 pesticides. De manière préoccupante, plus de 89% des résidus sont classés comme toxines du développement et de la reproduction, plus de 43% comme cancérigènes et plus de 36% comme génotoxiques. Ces résultats soulignent la nécessité d'une gestion plus stricte des pratiques phytosanitaires dans la viticulture libanaise par le ministère pour garantir la conformité aux normes internationales et la protection de la santé humaine et de l'environnement.

➤ *La nature de la matrice (feuilles, baies de vigne) influence-t-elle les teneurs et la dissipation des résidus de pesticides ?*

Les résultats obtenus ont montré une différence significative sur les teneurs et la persistance des résidus de pesticides pour les deux matrices (feuilles, baies de vigne). Les teneurs en résidus de pesticides sont de 20 à 70 fois supérieures dans les feuilles par rapport aux baies (feuilles : 16 mg/kg (SL) et 7 mg/kg (WDG) ; baies : 0,7 mg/kg (SL) et 0,1 mg/kg (WDG)). Nos résultats s'expliqueraient par le fait que les feuilles recouvrent les baies, engendrant une surface de contact plus exposée expliquant ainsi les teneurs en résidus de pesticides plus élevées sur les feuilles.

➤ *La formulation des pesticides affecte-t-elle leurs dépôts initiaux et leurs cinétiques de dissipation dans les feuilles et baies de vigne ?*

La formulation de l'imidaclopride (SL ou WDG) influence les dépôts initiaux des pesticides mais pas la dissipation des résidus dans les feuilles et baies de vigne. Les résidus initiaux dans les feuilles et les baies traitées avec la formulation SL étaient significativement plus élevés (15,6 mg kg⁻¹ et 0,7 mg kg⁻¹, respectivement) que ceux de la formulation WDG (6,7 mg kg⁻¹ et

0,1 mg kg⁻¹). Effectivement, bien que la nature et le ratio des coformulants soient gardés confidentiels par les fabricants et uniquement mentionnés comme 'inertes' sur l'étiquette du produit (Kalyabina et al. 2021), les formulations solubles sont connues pour avoir une taille de particules plus petite que les formulations granulées, et en général, elles contiennent plus de coformulants que les formulations WDG, à savoir : des agents dispersants, des agents mouillants, des antigels, des conservateurs, des agents antifoam, et de l'eau (Ohkouchi et Tsuji 2022). Cela pourrait expliquer les dépôts initiaux plus importants avec la formulation SL, lesquels ont engendré une teneur en résidus plus élevée. En revanche, les cinétiques de dissipation de l'imidaclopride présentent des similitudes entre les deux formulations, avec des demi-vies de 0,5 jours pour les feuilles et les baies, ce qui est lié aux caractéristiques physico-chimiques de la matière active.

➤ Les méthodes de lavage sont-elles efficaces pour éliminer les résidus de pesticides des feuilles de vigne avant leur consommation ou leur conservation ?

Les méthodes de lavage courantes, telles que l'eau du robinet, la solution de vinaigre à 10 % et l'eau bouillante, sont efficaces pour réduire les résidus de pesticides des feuilles de vigne. L'efficacité est variable en fonction des propriétés du pesticide. Par exemple, le lavage à l'eau du robinet s'est avéré être le plus efficace pour réduire les résidus de fenazaquin avec un FT de 0,57, tandis que le lavage à l'eau bouillante était plus efficace pour l'azoxystrobine (FT = 0,60). Seul le lavage à l'eau bouillante a montré une efficacité significative pour réduire les résidus d'indoxacarbe (FT = 0,78). À partir de ces résultats, le lavage à l'eau bouillante est recommandé, puisque des réductions de résidus (30%) ont été obtenues, comparé à 26% pour l'eau du robinet et 18% pour l'eau vinaigrée) indépendamment de la nature des molécules.

➤ Les méthodes de conservation des feuilles de vigne influencent-elles leur teneur en résidus de pesticides ?

Les résultats révèlent des teneurs en résidus d'azoxystrobine, de fenazaquin et d'indoxacarbe différentes pour les deux méthodes de conservation la conservation à sec et en saumure. Pour la fenazaquin, par exemple, des demi-vies plus courtes sont observées sous les deux méthodes de conservation (à sec – 16 jours, en saumure – 8 jours) par rapport à l'indoxacarbe (à sec – 100 jours, en saumure – 644 jours) et l'azoxystrobine (à sec – 210 jours, en saumure – 164 jours). Ainsi, la dissipation des résidus de pesticides dans les feuilles de vigne conservées dépend grandement des caractéristiques physico-chimiques des molécules. Il est

donc important pour les industriels de comprendre que les techniques employées de conservation ne garantissent pas une élimination totale des résidus de pesticides dans les feuilles de vigne. Cependant, les résultats de cette étude démontrent que la conservation à sec est recommandée, puisqu'après 60 jours de conservation, des réductions de résidus de 60 % ont été obtenues, comparé à 48% pour la conservation en saumure, indépendamment de la nature de la molécule. De plus, les teneurs en résidus des trois pesticides dans les eaux de saumure montrent une diffusion des pesticides des feuilles vers l'eau de saumure, un phénomène potentiellement lié à des altérations de la structure cuticulaire des feuilles de vigne pendant l'intervalle de stockage. De plus, étant donnée que une fraction des résidus passent dans les eaux de saumure, et que les feuilles de vigne sont parfois stockées dans de l'eau de saumure en attendant leur conservation en industrie tout au long de l'année, les industriels doivent manipuler ces eaux de saumure avec précaution, et garantir une élimination sûre de ces eaux, évitant ainsi le rejet de ces contaminants dans l'environnement. Il est également recommandé aux consommateurs de ne pas boire ces eaux, et de laver les feuilles saumurées avant de les consommer.

Apport du travail mené et perspectives

Le travail présenté dans cette thèse révèle un manque de compréhension concernant l'utilisation des molécules phytosanitaires, poussant les viticulteurs à recourir à l'utilisation d'un mélange de molécules pour lutter contre les maladies. Plus l'utilisation des molécules non recommandée sur la vigne (> 18%), il est ainsi recommandé que le Ministère de l'Agriculture renforce la mise en œuvre des Bonnes Pratiques Agricoles (BPA) et propose une formation aux producteurs de raisins/feuilles de vigne. L'obligation de l'utilisation de registres agricoles est cruciale pour établir une traçabilité, permettant des traitements uniquement par des professionnels formés afin de protéger la santé humaine et l'environnement.

L'étude sur l'effet de la formulation a indiqué que, bien que le type de formulation n'affecte pas la dissipation des résidus dans les raisins et les feuilles de vigne, les formulations SL entraînent des dépôts initiaux plus élevés que les formulations WDG. Cela doit être pris en compte sur le choix des produits commerciaux à appliquer sur la vigne, puisque nos résultats ont montré que les feuilles accumulent 70 fois plus de résidus que les raisins. Dans les cas où deux formulations d'une même substance active ont des BPA comparables, le choix de

formulations granulées plutôt que de formes liquides est préférable, étant donné la fréquence quotidienne ou bi-hebdomadaire de la récolte des feuilles sans tenir compte du nombre de jours après le traitement. Le lavage à l'eau bouillante élimine efficacement les résidus pour les 3 différentes molécules étudiées (~30%), ainsi nous recommandons un lavage à l'eau bouillante pour maximiser l'élimination des résidus. De même, la conservation à sec est recommandée puisque 60% de réductions comparée à 48% pour la conservation en saumure ont été obtenus indépendamment de la nature de la molécule. En revanche, étant donné que la conservation des feuilles de vigne ne garantit pas l'élimination totale des résidus durant la période de stockage, et une fraction des résidus passe dans les eaux de saumure, l'élimination sûre des eaux de saumure utilisées pour stocker les feuilles de vigne en attendant leur conservation en industrie est essentielle pour éviter tout rejet dans l'environnement des contaminants. Par conséquent, il pourrait être recommandé d'éviter les traitements phytosanitaires pendant la période de récolte des feuilles de vigne, qui s'étend de mai à juin. En cas de pression fongique pendant la saison de croissance des feuilles, il est conseillé d'utiliser des molécules ayant des demi-vies courtes et d'appliquer des traitements curatifs au lieu de traitements préventifs.

L'établissement de pratiques et de directives spécifiques pour la production de feuilles de vigne, avec une liste de pesticides recommandés, serait bénéfique. En ce qui concerne l'établissement des LMRs, nos résultats soulignent l'importance de prendre en compte la fréquence de la récolte des feuilles de vigne (quotidienne ou bi-hebdomadaire) lors de la détermination des durées avant récolte, assurant une production de feuilles de vigne sûre pour la santé humaine et économiquement viable.

Mais du travail reste à faire...

En raison des défis financiers liés à la dépréciation monétaire au Liban, qui ont impacté le financement de cette thèse et la disponibilité des matériaux nécessaires à l'analyse des échantillons, ainsi que des confinements liés au Covid-19 qui ont conduit à la fermeture du laboratoire, il a été impossible de mener des campagnes d'échantillonnage auprès des producteurs de feuilles de vigne pour estimer les quantités de pesticides appliquées en vigne au Liban. Dans le futur, il serait intéressant de réaliser de telles campagnes pour obtenir des données sur les résidus présents dans les feuilles de vigne produites localement et fournies aux industries ou vendues sur le marché libanais.

Il serait également pertinent d'étudier les dissipations et les éliminations des autres

molécules identifiées durant cette thèse en comparant les résultats de 2012-2014 avec ceux de la plateforme RASFF, comprenant le Boscalid, le Chlorpyrifos, le Difénoconazole, le Diflubenzuron, le Lambda-cyhalothrine, le Myclobutanil et le Thiophanate Méthyl. Étant donné que les circonstances de cette thèse ont restreint le travail à quatre molécules (l'Azoxystrobine, l'Imidaclopride, l'Indoxacarbe et le Fenazaquin), il serait important d'étudier le reste des molécules pour garantir la disponibilité de données scientifiques suffisantes sur la dissipation de ces substances fréquemment quantifiées dans les feuilles de vigne. Il serait également intéressant d'explorer l'effet des technologies modernes de lavage, telles que l'ozonation et l'utilisation de solutions détergentes, destinées à l'élimination des résidus. Ces travaux sont actuellement en cours et feront l'objet de publications futures.

Bibliographie générale

A

- Abdallah, O. Dissipation Behavior of Chlorfenapyr and Difenconazole Residues in/on Grapes (*Vitis Vinifera* L.). *nature and science* 2014, 12, 49–54.
- Abdel-Hamid, M, Walaa El-Sayed, et Nevein Ahmed. 2013. « The Relationship Between Different Formulation Types And The Residue Levels Of Pesticides On Tomato Fruits ». *Research Journal of Agriculture and Biological Sciences*, 9(1), 8-16.
- Abou Zeid, Mohamad I, Mireille Kallassy Awad, Khalil C Melki, Yusuf Abou Jawdah, et Adla M Jammoul. 2021. « Pesticides residues on Loquat: A minor crop in Lebanon ». *Food Control* 130 (décembre): 108297. <https://doi.org/10.1016/j.foodcont.2021.108297>.
- Abou Zeid, Mohamad I, Adla M Jammoul, Khalil C Melki, Yusuf Abou Jawdah, et Mireille Kallassy Awad. 2020. « Suggested policy and legislation reforms to reduce deleterious effect of pesticides in Lebanon ». *Heliyon* 6 (12): e05524. <https://doi.org/10.1016/j.heliyon.2020.e05524>.
- Abou Zeid, Mohamad, Adla Jammoul, Khalil Melki, Yusuf Abou-Jawda, et Mireille Kallassy. 2020. « Dissipation of 3 commercial formulations of penconazole 10% EC on greenhouse tomatoes in Lebanon ». *RESEARCH JOURNAL OF CHEMISTRY AND ENVIRONMENT* 13 (septembre): 2230.
- Acoglu, B, et P Yolci Omeroglu. 2021. « Effectiveness of Different Type of Washing Agents on Reduction of Pesticide Residues in Orange (*Citrus Sinensis*) ». *LWT* 147 (juillet): 111690. <https://doi.org/10.1016/j.lwt.2021.111690>.
- Ad Meijs, W.H.M. New Type of Formulations and New Application Techniques; Consequences for the Authorisation of Pesticides. *Environmentalist* 2008, 28, 5–8, doi:10.1007/s10669-007-9038-3.
- Aguilera, Ana, Antonio Valverde, Francisco Camacho, Mourad Boulaid, et Luis García-Fuentes. 2012. « Effect of Household Processing and Unit to Unit Variability of Azoxystrobin, Acrinathrin and Kresoxim Methyl Residues in Zucchini ». *Food Control* 25 (2): 594-600. <https://doi.org/10.1016/j.foodcont.2011.11.038>.
- Akkouch, Nisreen, et Jalal Halwani. 2023. « Pesticide Occurrence in Food, Water, and Human Biomonitoring in Lebanon-A Health Repercussion ». *Annals of Epidemiology and Public Health* 6 (1): 1101.
- Aktar, Md. Wasim, Dwaipayan Sengupta, et Ashim Chowdhury. 2009. « Impact of pesticides use in agriculture: their benefits and hazards ». *Interdiscip Toxicol* 2 (1): 1-12. <https://doi.org/10.2478/v10102-009-0001-7>.
- Aktop, Simge, Pınar Şanlıbaba, et Yalçın Güçer. 2023. « Grape-based traditional foods produced in Turkey ». *Italian Journal of Food Science* 35 (3): 55-74. <https://doi.org/10.15586/IJFS.V35I3.2339>.
- Arkam, M., Alves, A., Lopes, A. et al. Diversity of Botryosphaeriaceae causing grapevine trunk diseases and their spatial distribution under different climatic conditions in Algeria. *Eur J Plant Pathol* 161, 933–952 (2021). <https://doi.org/10.1007/s10658-021-02377-7>
- Alister, Claudio, Manuel Araya, Kevin Becerra, Jorge Saavedra, et Marcelo Kogan. 2017. « Preharvest

Interval Periods and Their Relation to Fruit Growth Stages and Pesticide Formulations ». *Food Chem* 221 (avril): 548-54. <https://doi.org/10.1016/j.foodchem.2016.11.140>.

Alonso González, Pablo, Eva Parga Dans, Andrea Carolina Acosta Dacal, Manuel Zumbado Peña, et Octavio Pérez Luzardo. 2022. « Differences in the levels of sulphites and pesticide residues in soils and wines and under organic and conventional production methods ». *Journal of Food Composition and Analysis* 112 (septembre): 104714. <https://doi.org/10.1016/J.JFCA.2022.104714>.

Alonso González, Pablo, Eva Parga-Dans, et Octavio Pérez Luzardo. 2021. « Big sales, no carrots: Assessment of pesticide policy in Spain ». *Crop Protection* 141 (mars): 105428. <https://doi.org/10.1016/j.cropro.2020.105428>.

Ambrus, Árpád, Júlia Szenczi-Cseh, Vy Vy N Doan, et Adrienn Vásárhelyi. 2023. « Evaluation of Monitoring Data in Foods ». *Agrochemicals* 2 (1): 69-95. <https://doi.org/10.3390/agrochemicals2010006>.

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce. *Journal of AOAC International*, 86(2), 412–431. <https://doi.org/10.1093/jaoac/86.2.412>

Andrade, Graziela, Sérgio Monteiro, Jeane Francisco, Leila Figueiredo, Aderbal Rocha, et Valdemar Tornisielo. 2015. « Effects of Types of Washing and Peeling in Relation to Pesticide Residues in Tomatoes ». *Journal of the Brazilian Chemical Society* 26 (octobre). <https://doi.org/10.5935/0103-5053.20150179>.

Angioni, A, M Schirra, V L Garau, M Melis, C I G Tuberoso, et P Cabras. 2004. « Residues of Azoxystrobin, Fenhexamid and Pyrimethanil in Strawberry Following Field Treatments and the Effect of Domestic Washing ». *Food Additives and Contaminants* 21 (11): 1065-70. <https://doi.org/10.1080/02652030400010066>.

Ansari, Iqbal, Maha M El-Kady, Charu Arora, Muniyan Sundararajan, Deblina Maiti, et Aarif Khan. 2021. « A review on the fatal impact of pesticide toxicity on environment and human health ». In *Global Climate Change*, édité par Suruchi Singh, Pardeep Singh, S Rangabhashiyam, et K K Srivastava, 361-91. Elsevier. <https://doi.org/10.1016/B978-0-12-822928-6.00017-4>.

Arora, Parshotam Kumar, Gagan Jyot, Baljeet Singh, Raminderjit Singh Battu, Balwinder Singh, et Pushpinder Singh Aulakh. 2009. « Persistence of Imidacloprid on Grape Leaves, Grape Berries and Soil ». *Bull Environ Contam Toxicol* 82 (2): 239-42. <https://doi.org/10.1007/s00128-008-9554-y>.

B

Bajwa, Usha, et Kulwant Singh Sandhu. 2014. « Effect of Handling and Processing on Pesticide Residues in Food- a Review ». *J Food Sci Technol* 51 (2): 201-20. <https://doi.org/10.1007/s13197-011-0499-5>.

Balkan, Tarik, et Kenan Kara. 2023. « Dissipation kinetics of some pesticides applied singly or in mixtures in/on grape leaf ». *Pest Management Science* 79 (3): 1234-42.

<https://doi.org/10.1002/ps.7299>.

Baçoğlu, Fikri, İsmet Şahin, Mihriban Korukluoğlu, Vildan Uylaser, et Arzu Akpınar. 1996. « A Research on the Effects of Fermentation Type and Additives on Quality and Preservation and Development of Adequate Technique in Brined Vine-Leaves Production ». *Turk J Agric For* 20 (6): 535-45. <https://journals.tubitak.gov.tr/agriculture/abstract.htm?id=820>.

Baur, P.; Marzouk, H.; Schönherr, J.; Bauer, H. Mobilities of Organic Compounds in Plant Cuticles as Affected by Structure and Molar Volumes of Chemicals and Plant Species. *Planta* 1996, 199, 404–412, doi:10.1007/BF00195732.

Bletsou, A.A.; Hanafi, A.H.; Dasenaki, M.E.; Thomaidis, N.S. Development of Specific LC-ESI-MS/MS Methods to Determine Bifenthrin, Lufenuron, and Iprodione Residue Levels in Green Beans, Peas, and Chili Peppers Under Egyptian Field Con-ditions. *Food Anal. Methods* 2013, 6, 1099–1112, doi:10.1007/s12161-012-9515-2.

Bo, Li Ying, Ying Hua Zhang, et Xin Huai Zhao. 2011. « Degradation kinetics of seven organophosphorus pesticides in milk during yoghurt processing ». *Journal of the Serbian Chemical Society* 76 (3): 353-62. <https://doi.org/10.2298/JSC100615035B>.

Bois, Benjamin, S. Zito, et A. Calonnec. 2017. « Climate vs grapevine pests and diseases worldwide: the first results of a global survey ». *OENO One* 51 (2): 133-39. <https://doi.org/10.20870/OENO-ONE.2017.51.2.1780>.

Bouagga, A., H. Chaabane, K. Toumi, A. Mougou Hamdane, B. Nasraoui, et L. Joly. 2019. « Pesticide residues in Tunisian table grapes and associated risk for consumer's health ». *Food Additives & Contaminants: Part B* 12 (2): 135-44. <https://doi.org/10.1080/19393210.2019.1571532>.

Bou-Mitri, Christelle, Alexandria Nivelte Mekanna, Sabine Dagher, Sara Moukarzel, et Antoine Farhat. 2022. « Occurrence and exposure to glyphosate present in bread and flour products in Lebanon ». *Food Control* 136 (juin): 108894. <https://doi.org/10.1016/j.foodcont.2022.108894>.

Buzzetti, Karina. 2017. « Role of the Formulation in the Efficacy and Dissipation of Agricultural Insecticides ». In *Insecticides - Agriculture and Toxicology*. IntechOpen. <https://doi.org/10.5772/intechopen.72340>.

C

« CAC/RCP 1-1969, Rev. 4-20031 - RECOMMENDED INTERNATIONAL CODE OF PRACTICE GENERAL PRINCIPLES OF FOOD HYGIENE ». s. d. <https://www.mhlw.go.jp/english/topics/importedfoods/guideline/dl/04.pdf>.

Cabras, P.; Gennari, M.; Meloni, M.; Cabitza, F.; Cubeddu, M. Pesticide Residues in Lettuce. 2. Influence of Formulations. *J. Agric. Food Chem.* 1989, 37, 1405–1407, doi:10.1021/jf00089a043.

Cangi, R., Y. Yanar, A. Yağcı, N. Topçu, S. Sucu, et Y. Dülgeroğlu. 2014. « The Effect of Picking Period and Brining Applications on Fungicide Residue Levels in Brined Vine Leaves Production ». *JAFAG* 31 (2): 23-30.

Cangi, Rustem, Yusuf Yanar, et Yağmur Dülgeroğlu. 2019. « Effects of Brining and Picking Time on The

- Degradation of Pesticide Residue in Grapevine Leaves ». *Türk Tarım - Gıda Bilim ve Teknoloji Dergisi* 7 (11): 1773-79. <https://doi.org/10.24925/turjaf.v7i11.1773-1779.2505>.
- Cangi, Rüstem, Yusuf Yanar, et Yağmur Dülgeroğlu Yılmaz. 2019. « Effects of Brining and Picking Time on The Degradation of Pesticide Residue in Grapevine Leaves ». *Turkish Journal of Agriculture - Food Science and Technology* 7 (11): 1773-79. <https://doi.org/10.24925/turjaf.v7i11.1773-1779.2505>.
- Cantwell, M I, G Hong, K Albornoz, et M Berlanga. 2022. « Fresh Grapevine (*Vitis Vinifera* L.) Leaves: Postharvest Biology and Handling Recommendations ». *Scientia Horticulturae* 292 (janvier): 110627. <https://doi.org/10.1016/j.scienta.2021.110627>.
- CBI. 2018. « Value chain analysis Lebanon fresh fruit and vegetables ». <https://www.cbi.eu/market-information/fresh-fruit-vegetables/vca-lebanon-fresh-fruit-vegetables-2018>.
- Chatzigeorgiou, Ioanna, Georgios Liantas, Petros Spanos, Vasiliki Gkriniari, Eleni Maloupa, et Georgios K. Ntinas. 2022. « Hydroponic Cultivation of Vine Leaves with Reduced Carbon Footprint in a Mediterranean Greenhouse ». *Sustainability* 2022, Vol. 14, Page 8011 14 (13): 8011. <https://doi.org/10.3390/SU14138011>.
- Chowdhury, A.; Jepson, P.; Ford, M.; Frampton, G. The Role of Cuticular Waxes and Surface Roughness in Determining the Insecticidal Efficacy of Deltamethrin and Dimethoate Applied as Emulsifiable Concentrates to Leaf Surfaces. *International Journal of Pest Management - INT J PEST MANAGE* 2005, 51, 253–263, doi:10.1080/09670870500404674.
- Codex online databases | CODEXALIMENTARIUS FAO-WHO. (2021). Retrieved from <https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/en/> . Accessed April 4, 2021
- Corrias, Francesco, Alessandro Atzei, Carla Lai, Fabrizio Dedola, Enrico Ibba, Gianluca Zedda, Francesca Canu, et Alberto Angioni. 2020. « Effects of Industrial Processing on Pesticide Multiresidues Transfer from Raw Tomatoes to Processed Products ». *Foods* 9 (10): 1497. <https://doi.org/10.3390/foods9101497>.
- Costa, J. Miguel, Ricardo Egipto, Francisca C. Aguiar, Paulo Marques, Amaia Nogales, et Manuel Madeira. 2023. « The role of soil temperature in mediterranean vineyards in a climate change context ». *Frontiers in Plant Science* 14 (mai): 1145137. <https://doi.org/10.3389/FPLS.2023.1145137/BIBTEX>.
- Cox, Caroline, et Michael Sorgan. 2006. « Unidentified Inert Ingredients in Pesticides: Implications for Human and Environmental Health ». *Environ Health Perspect* 114 (12): 1803-6. <https://doi.org/10.1289/ehp.9374>.
- Creasy, Glen L., et Leroy L. Creasy. 2018. *Grapes Crop Production Science in Horticulture Series*. 2nd éd. Cambridge, MA, USA: CABI. https://books.google.com.lb/books/about/Grapes_2nd_Edition.html?id=1hMntAEACAAJ&redir_esc=y.
- Cress, D. Factors Affecting Pesticide Behavior and Breakdown Available online: <https://bookstore.ksre.ksu.edu/pubs/MF958.pdf> (accessed on 17 April 2021).
- Commission Implementing Regulation (EU) 2020/1643 of 5 November 2020 Amending Implementing

Regulation (EU) No 540/2011 as Regards the Approval Periods of the Active Substances Calcium Phosphide, Denathonium Benzoate, Halox-yfop-P, Imidacloprid, Pencycuron and Zeta-Cypermethrin (Text with EEA Relevance); 2020; Vol. 370

Cumming, Hemi, et Christoph R cker. 2017. « Octanol–Water Partition Coefficient Measurement by a Simple ¹H NMR Method ». *ACS Omega* 2 (9): 6244-49. <https://doi.org/10.1021/acsomega.7b01102>.

D

Daniele, Penna., Paolo, Benettin. (2023). Ecohydrological dynamics and temporal water origin in European Mediterranean vineyards: a case study in Tuscany, Central Italy. doi: 10.5194/egusphere-egu23-16318

Das, S K. 2013. « Mode of action of pesticides and the novel trends – A critical review ». *Int. Res. J. Agric. Sci. Soil Sci.* 3 (11): 393-401. <https://doi.org/http://dx.doi.org/10.14303/irjas.2013.118>.

Dikshit, A.K.; Pachauri, D.C.; Jindal, T. Maximum Residue Limit and Risk Assessment of Beta-Cyfluthrin and Imidacloprid on Tomato (*Lycopersicon Esculentum* Mill). *Bull. Environ. Contam. Toxicol.* 2003, 70, 1143–1150, doi:10.1007/s00128-003-0101-6.

Din, Ali, Mohamed Azab, et Mohamed Shalaby. 2018. « Pesticide Residues Monitoring of Organophosphorus and Carbamates in Grapes in Three Egyptian Governorates ». *Archives of Current Research International* 12 (1): 1-12. <https://doi.org/10.9734/ACRI/2018/38986>.

Duhan, Anil, et Beena Kumari. 2011. « Degradation Studies of Fenazaquin in Soil under Field and Laboratory Conditions ». *Bulletin of Environmental Contamination and Toxicology* 87 (2): 180-83. <https://doi.org/10.1007/s00128-011-0318-8>.

Dumitriu (Gabur), G.-D., Teodosiu, C., & V. Cotea, V. (2022). Management of Pesticides from Vineyard to Wines: Focus on Wine Safety and Pesticides Removal by Emerging Technologies. IntechOpen. doi: 10.5772/intechopen.98991

E

EastFruit. 2019. « Grape Leaf as a Business or How Much Money Dolma Brings • EastFruit ». *EastFruit*. <https://east-fruit.com/en/news/grape-leaf-as-a-business-or-how-much-money-dolma-brings/>.

EC. (2019). Guidance SANTE/12682/2019 – Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. In Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Retrieved from <https://www.accredia.it/en/documento/guidance-sante-12682-2019-guidance-document-on-analytical-quality-control-and-method-validation-procedures-for-pesticides-residues-analysis-in-food-and-feed/> . Accessed October 19, 2020

EC. 2023. « SANCO/12495/2011 EURL Residues of Pesticides Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed ». <https://www.eurl-pesticides.eu/docs/public/tmpl/article.asp?CntID=727&LabID=100&Lang=EN>.

- Ecobichon, Donald J. 1997. « The basis of toxicity testing », 220.
- Edwards, C A. 1975. « Factors that affect the persistence of pesticides in plants and soils ». *Pure and Applied Chemistry* 42 (1-2): 39-56. <https://doi.org/10.1351/pac197542010039>.
- EFSA. 2013. « Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters ». *EFSA Journal* 11 (7): 3290.
- . 2022. « Pesticides in Food: Latest Report Published EFSA ». <https://www.efsa.europa.eu/en/news/pesticides-food-latest-report-published>.
- . 2023a. « Glossary ». 2023. <https://www.efsa.europa.eu/en/glossary-taxonomy-terms>.
- . 2023b. « Pesticide evaluation: Tools ». 2023. <https://www.efsa.europa.eu/en/applications/pesticides/tools>.
- Elmarsafy, Ashraf M., et Taher M. Kadah. 2018. « MEASURING THE ECONOMIC IMPACT AND HEALTH RISKS OF PESTICIDE RESIDUES IN POTATOES AND GRAPES CROPS ». *Egyptian Journal of Agricultural Research* 96 (3): 1203-28. <https://doi.org/10.21608/EJAR.2018.141160>.
- EPA. 2023. « IRIS Glossary | US ». 2023. <https://www.epa.gov/iris/iris-glossary>.
- Ertürk, A. 2009. « Determination of Fungicide Residues in Grape Leaves (Cv Yapıncak) Grown in Tekirdag Province, Before and After Pickling ». Master, Science Institute - Namık Kemal Üniv.
- EU Pesticides Database (v.2.2) Pesticide residue(s) and maximum residue levels (mg/kg). (2021). Retrieved from https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=details&pest_res_ids=22,132,2692&product_ids=&v=1&e=search.pr. Accessed April 4, 2021
- Europe, CropLife. 2022. « PESTICIDE USE AND FOOD SAFETY ». <https://croplifeurope.eu/wp-content/uploads/2022/05/35429-Pesticides-Use-and-Food-Safety.pdf>.
- European Commission. 2023. « EU Pesticides Database - Active substances ». <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances>.

F

- Fader, R G, et M J Bukovac. 1997. « Triton X-45: A Unique Effect on Growth Regulator Sorption by and Penetration of Isolated Plant Cuticles ». In *Pesticide Formulations and Application Systems: 17th Volume*, 310-26. ASTM International. <https://doi.org/10.1520/STP13851S>.
- Fahed, Christina, et Salem Hayar. 2021. « Conventional vs. Organic Cucumber Production in Lebanon: Risk Assessment of the Recommended Agrochemicals on Consumer Health and the Environment ». *Chemistry Africa* 4 (2): 463-76. <https://doi.org/10.1007/S42250-021-00238-8/METRICS>.
- FAO. 2021. « Definitions and Classifications of Pesticides ». https://www.fao.org/fileadmin/user_upload/faoweb/statistics/questionnaires/FAO_Definitions_and_classifications_of_pesticides_2021.xlsx#:~:text=Pesticides%20refer%20to%20insecticides%2C%20mineral,animal%20disease%2C%20unwanted%20species%20of.

FAO CAC/GL 33-1999 RECOMMENDED METHODS OF SAMPLING FOR THE DETERMINATION OF PESTICIDE RESIDUES FOR COMPLIANCE WITH MRLS Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXG%2B33-1999%252FCXG_033e.pdf (accessed on 19 March 2021).

FAOSTAT. 2021. « FAOSTAT ». 2021. <https://www.fao.org/faostat/en/#home>.

FAO/WHO. (1997). *Guidelines for predicting dietary intake of pesticide residues / prepared by the Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme (GEMS/Food); in collaboration with the Codex Committee on Pesticide Residues*. Retrieved from <https://apps.who.int/iris/handle/10665/63787> . Accessed August 8, 2021

FAO-WHO. 2023. « Guidelines | CODEXALIMENTARIUS FAO-WHO ». 2023. <https://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/>.

Federal Food, Drug, and Cosmetic Act , 2018. FDA. <https://www.fda.gov/regulatory-information/laws-enforced-fda/federal-food-drug-and-cosmetic-act-fdc-act>.

Fernández, V, et T Eichert. 2009. « Uptake of Hydrophilic Solutes Through Plant Leaves: Current State of Knowledge and Perspectives of Foliar Fertilization ». *Critical Reviews in Plant Sciences* 28 (1-2): 36-68. <https://doi.org/10.1080/07352680902743069>.

Food and Agriculture Organization of the United Nations Guidelines on Good Practice for Ground Application of Pesticides Available online: <http://www.fao.org/3/y2767e/y2767e00.htm> (accessed on 19 March 2021).

Fouillet, E., Delière, L., Chartier, N., Munier-Jolain, N., Cortel, S., Rapidel, B., & Merot, A. (2022). Reducing pesticide use in vineyards: Evidence from the analysis of the French DEPHY network. *European Journal of Agronomy*, 136, 126503. <https://doi.org/10.1016/j.eja.2022.126503>

FRAC. 2023. « Database | Home ». 2023. <https://www.frac.info/>.

G

Gabel, B. 2019. « New concept of vine grape protection – knowledge-based approach & high tech ». *BIO Web of Conferences* 15: 01020. <https://doi.org/10.1051/BIOCONF/20191501020>.

Gabur, Georgiana-Diana Dumitriu, Carmen Teodosiu, Valeriu V. Cotea, Georgiana-Diana Dumitriu Gabur, Carmen Teodosiu, et Valeriu V. Cotea. 2021. « Management of Pesticides from Vineyard to Wines: Focus on Wine Safety and Pesticides Removal by Emerging Technologies ». *Grapes and Wine*, juillet. <https://doi.org/10.5772/INTECHOPEN.98991>.

Gajbhiye, Vijay Tularam, Suman Gupta, Irani Mukherjee, Shashi Bala Singh, Neera Singh, Prem Dureja, et Yogesh Kumar. 2011. « Persistence of Azoxystrobin in/on Grapes and Soil in Different Grapes Growing Areas of India ». *Bull Environ Contam Toxicol* 86 (1): 90-94. <https://doi.org/10.1007/s00128-010-0170-2>.

Gao, Wanjun, Jiagang Guo, Liang Xie, Chuanyi Peng, Lili He, Xiaochun Wan, et Ruyan Hou. 2020.

« Washing Fresh Tea Leaves before Picking Decreases Pesticide Residues in Tea ». *J Sci Food Agric* 100 (13): 4921-29. <https://doi.org/10.1002/jsfa.10553>.

Golge, Ozgur, et Bulent Kabak. 2018. « Pesticide Residues in Table Grapes and Exposure Assessment ». *Journal of Agricultural and Food Chemistry* 66 (7): 1701-13. https://doi.org/10.1021/ACS.JAFC.7B05707/ASSET/IMAGES/MEDIUM/JF-2017-05707A_0004.GIF.

Goumenou, Marina, et Aristidis Tsatsakis. 2019. « Proposing new approaches for the risk characterisation of single chemicals and chemical mixtures: The source related Hazard Quotient (HQS) and Hazard Index (HIS) and the adversity specific Hazard Index (HIA) ». *Toxicology Reports* 6: 632-36. <https://doi.org/10.1016/j.toxrep.2019.06.010>.

Gu, Meng-Ying, Peng-Si Wang, Shang-Mei Shi, et Jian Xue. 2021. « Dietary Risk Assessment and Ranking of Multipesticides in Dendrobium Officinale ». *Journal of Food Quality* 2021 (juin): e9916758. <https://doi.org/10.1155/2021/9916758>.

Guardia-Rubio, M, M J Ayora-Cañada, et A Ruiz-Medina. 2007. « Effect of Washing on Pesticide Residues in Olives ». *Journal of Food Science* 72 (2): C139-143. <https://doi.org/10.1111/j.1750-3841.2006.00252.x>.

Gülcü, M., et AŞ Demirci. 2014. « Pesticide Residue Problem in Brined Leaves Production and Marketing ». In *4th Traditional Foods Symposium*, 331. Adana, Türkiye.

Guidance SANTE/12682/2019 – Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed Available online: <https://www.accredia.it/en/documento/guidance-sante-12682-2019-guidance-document-on-analytical-quality-control-and-method-validation-procedures-for-pesticides-residues-analysis-in-food-and-feed/> (accessed on 19 October 2021).

H

Halleen, F., et G. Holz. 2017. « An Overview of the Biology, Epidemiology and Control of Uncinula necator (Powdery Mildew) on Grapevine, with Reference to South Africa ». *South African Journal of Enology & Viticulture* 22 (2). <https://doi.org/10.21548/22-2-2205>.

Hanafi, A.; Dasenaki, M.; Bletsou, A.; Thomaidis, N.S. Dissipation Rate Study and Pre-Harvest Intervals Calculation of Imidacloprid and Oxamyl in Exported Egyptian Green Beans and Chili Peppers after Pesticide Treatment. *Food Chemistry* 2018, 240, 1047–1054, doi:10.1016/j.foodchem.2017.08.037.

Hamzawy, Ahmed H. 2022. « Residual Pesticides in Grape Leaves (Vitis Vinifera L.) on the Egyptian Market and Human Health Risk ». *Food Addit Contam Part B Surveill* 15 (1): 62-70. <https://doi.org/10.1080/19393210.2021.2022005>.

Hassaan, Mohamed A, et Ahmed El Nemr. 2020. « Pesticides pollution: Classifications, human health impact, extraction and treatment techniques ». *The Egyptian Journal of Aquatic Research* 46 (3): 207-20. <https://doi.org/10.1016/j.ejar.2020.08.007>.

Hawari, Khaled El, Samia Mokh, Mohamad Al Iskandarani, Wafaa Halloum, et Farouk Jaber. 2019.

« Pesticide residues in Lebanese apples and health risk assessment ». *Food Additives & Contaminants: Part B* 12 (2): 81-89. <https://doi.org/10.1080/19393210.2018.1564370>.

Hayar, S.; Al Hadi, J.; Merhi, A.; Majed, L.; Saba Ayoun, L. L'effet de 3 modes de conservation sur la dissipation des résidus de pesticides dans les feuilles de vigne *Vitis vinifera*. In Proceedings of the 47ème Congrès du Groupe Français des Pesti-cides: Regards transfrontaliers sur les pesticides; Nancy, France, May 15 2017; pp. 65–75.

Hayar, Salem, Rawan Zeitoun, et Britt Marianna Maestroni. 2021. « Validation of a Rapid Multiresidue Method for the Determination of Pesticide Residues in Vine Leaves. Comparison of the Results According to the Different Conservation Methods ». *Molecules* 26 (4): 1176. <https://doi.org/10.3390/molecules26041176>.

Hobeika, Charbel, et Dani El Khouri. 2011. « CGP/LEB/021/ITA - A grower's guide to good agricultural practices for table grapes ». Ministry of Agriculture - Lebanon. 2011.

Hwang, Jeong-In, Da-Rong Seok, et Jang-Eok Kim. 2019. « Effects of Cuticular Waxes on Permeation of Fungicides Azoxystrobin and Chlorothalonil into Apples ». *Appl Biol Chem* 62 (1): 34. <https://doi.org/10.1186/s13765-019-0441-5>.

I

IRAC. 2023. « Mode of Action Classification Insecticide Resistance Management ». IRAC. <https://irac-online.org/mode-of-action/classification-online/>.

ISTAT. 2023. « Istat.it Agriculture ». 2023. <https://www.istat.it/en/agriculture?data-and-indicators>.

J

Jaume, Gil., Elena, Alter., Maria, José, La, Rota., Enric, Tello., Vittorio, Galletto., Roc, Padró., Teresa, Martínez., Soazig, Darnay., Joan, Marull. (2022). Towards an agroecological transition in the Mediterranean: A bioeconomic assessment of viticulture farming. *Journal of Cleaner Production*, 380:134999-134999. doi: 10.1016/j.jclepro.2022.134999

João, Prada., Lia-Tânia, Dinis., Elia, Soriato., Elodie, Vandelle., Oğuzhan, Soltekin., Şener, Uysal., Abdelhi, Dihazi., Conceição, Santos., João, A., Santos. (2024). Climate change impact on Mediterranean viticultural regions and site-specific climate risk-reduction strategies. *Mitigation and Adaptation Strategies for Global Change*, 29(6) doi: 10.1007/s11027-024-10146-0

Jensen, Bodil Hamborg, Annette Petersen, Pernille Bjørn Petersen, Tue Christensen, Sisse Fagt, Ellen Trolle, Mette Erecius Poulsen, et Jens Hinge Andersen. 2022. « Cumulative dietary risk assessment of pesticides in food for the Danish population for the period 2012–2017 ». *Food and Chemical Toxicology* 168 (octobre): 113359. <https://doi.org/10.1016/j.fct.2022.113359>.

Joint FAO/WHO Food Standards. (1999). Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs (CAC/GL 33-1999). Retrieved from https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B33-1999%252FCXG_033e.pdf . Accessed March 19, 2021

Jürgens, Monika D, John Crosse, Patrick B Hamilton, Andrew C Johnson, et Kevin C Jones. 2016. « The long shadow of our chemical past – High DDT concentrations in fish near a former agrochemicals factory in England ». *Chemosphere* 162 (novembre): 333-44. <https://doi.org/10.1016/j.chemosphere.2016.07.078>.

Jyot, Gagan, Parshotam Kumar Arora, Sanjay Kumar Sahoo, Balwinder Singh, et Raminderjit Singh Battu. 2010. « Persistence of Trifloxystrobin and Tebuconazole on Grape Leaves, Grape Berries and Soil ». *Bull Environ Contam Toxicol* 84 (3): 305-10. <https://doi.org/10.1007/s00128-009-9925-z>.

K

Kalyabina, Valeriya P., Elena N. Esimbekova, Kseniya V. Kopylova, et Valentina A. Kratasyuk. 2021. « Pesticides: formulants, distribution pathways and effects on human health – a review ». *Toxicol. Rep.* 8 (janvier): 1179. <https://doi.org/10.1016/J.TOXREP.2021.06.004>.

Katagi, Toshiyuki, et Hitoshi Tanaka. 2016. « Metabolism, bioaccumulation, and toxicity of pesticides in aquatic insect larvae ». *J Pestic Sci* 41 (2): 25-37. <https://doi.org/10.1584/jpestics.D15-064>.

Katalinic, V.; Mozina, S.S.; Generalic, I.; Skroza, D.; Ljubenkovic, I.; Klancnik, A. Phenolic Profile, Antioxidant Capacity, and Antimicrobial Activity of Leaf Extracts from Six Vitis Vinifera L. Varieties. *International Journal of Food Properties* 2013, 16, 45–60, doi:10.1080/10942912.2010.526274

Kfoury, Linda, Christo Hilan, et Rola El-Amil. 2002. « Residue Analysis Of Pesticides Commonly Used By Lebanese Farmers On Strawberries ». *Lebanese Science Journal* 3 (1): 37-46. <http://lsj.cnrs.edu.lb/wp-content/uploads/2016/01/kfoury.pdf>.

Kharel, Menila, Bed Mani Dahal, et Nani Raut. 2022. « Good agriculture practices for safe food and sustainable agriculture in Nepal: A review ». *Journal of Agriculture and Food Research* 10 (décembre): 100447. <https://doi.org/10.1016/J.JAFR.2022.100447>.

Khazaal, Salma, Nada El Darra, Amal Kobeissi, Rola Jammoul, et Adla Jammoul. 2022. « Risk assessment of pesticide residues from foods of plant origin in Lebanon ». *Food Chemistry* 374 (avril): 131676. <https://doi.org/10.1016/j.foodchem.2021.131676>.

Koubeissy, Amal. 2014. « The Challenge of Facilitating Exports of Table Grapes In Agriculture in Lebanon ». Dekweneh, Lebanon: Lebanese University.

Karthik, P.; Venugopal, S.; Datchina Murthy, K.; Lokesh, S.; Karthik, G.; Sharmila, U.; Paramasivam, M.; Senguttuvan, K.; Gunasekaran, K.; Kuttalam, S. Bioefficacy, Phytotoxicity, Safety to Natural Enemies and Residue Dynamics of Imidacloprid 70 WG in Okra (*Abelmoschus Esculenta* (L) Moench) under Open Field Conditions. *Crop Protection* 2015, 71, 88–94, doi:10.1016/j.cropro.2015.01.025.

Kumar, V., Tewary, D. K., Ravindranath, S. D., & Shanker, A. (2006). Investigation in tea on fate of fenazaquin residue and its transfer in brew. *Food and Chemical Toxicology*, 44(4), 596–600. <https://doi.org/10.1016/j.fct.2005.10.010>

Kutlu Kuşaksız, Esen, et Hüseyin Çimer. 2019. « Asma(Vitis vinifera var. Sultani çekirdeksiz) Yapraklarında Farklı Salamura Ortamlarının Pestisit Kalıntı Düzeylerine Etkisi ». *Ege Üniversitesi Ziraat Fakültesi Dergisi* 56 (3): 267-72. <https://doi.org/10.20289/zfdergi.446022>.

L

Lara, I., Heredia, A., & Domínguez, E. (2019). Shelf Life Potential and the Fruit Cuticle: The Unexpected Player. *Frontiers in Plant Science*, 10. <https://www.frontiersin.org/articles/10.3389/fpls.2019.00770>

Lacerda, D.S.; Costa, P.C.; Funchal, C.; Gomez, C.D. and R. Benefits of Vine Leaf on Different Biological Systems. *Grape and Wine Biotechnology* 2016, doi:10.5772/64930.

Lau, Ke Qi, Mohd Redzwan Sabran, et Siti Raihanah Shafie. 2021. « Utilization of Vegetable and Fruit By-products as Functional Ingredient and Food ». *Front Nutr* 8 (juin): 661693. <https://doi.org/10.3389/fnut.2021.661693>.

Lee, Mi-Gyung, et Da-I. Jung. 2009. « Processing Factors and Removal Ratios of Select Pesticides in Hot Pepper Leaves by a Successive Process of Washing, Blanching, and Drying ». *Food Science and Biotechnology* 18 (5): 1076-82. <https://koreascience.kr/article/JAKO200935736659085.kr&sa=U>.

Leifer, K B. 1997. « New Developments in the Regulation of Pesticide Inert Ingredients in the United States ». In *Pesticide Formulations and Application Systems: 17th Volume*, 5-10. ASTM International. <https://doi.org/10.1520/STP13828S>.

Lelay, G., Roger, T., Delhomme, B., and Roby, J.-P. (2003). La filière viticole au Liban : Analyse et propositions pour une évolution. ENITA de Bordeaux.

Lewis, Kathleen A, John Tzilivakis, Douglas J Warner, et Andrew Green. 2016. « An International Database for Pesticide Risk Assessments and Management ». *Human and Ecological Risk Assessment: An International Journal* 22 (4): 1050-64. <https://doi.org/10.1080/10807039.2015.1133242>.

Li, Changjian, Huimin Zhu, Changyan Li, He Qian, Weirong Yao, et Yahui Guo. 2021. « The Present Situation of Pesticide Residues in China and Their Removal and Transformation during Food Processing ». *Food Chemistry* 354: 129552. <https://doi.org/10.1016/j.foodchem.2021.129552>.

Li, Zijian. 2022. « Improved Pesticide Product Labeling Information for Household Lawn Management: Recommended Safe Durations in Support of Minimizing Children's Exposure to Pesticides ». *ACS Chemical Health and Safety* 29 (2): 230-39. https://doi.org/10.1021/ACS.CHAS.1C00092/SUPPL_FILE/HS1C00092_SI_002.XLSX.

———. 2023. « Modeling pesticide dissipation half-lives in plants with effects of fertilizer application: A flexible simulation tool ». *Science of The Total Environment* 893 (octobre): 164739. <https://doi.org/10.1016/J.SCITOTENV.2023.164739>.

Lia-Tânia, Dinis., Sara, Bernardo., Chenyao, Yang., Helder, Fraga., Aureliano, C., Malheiro., José, Moutinho-Pereira., João, A., C., Santos. (2022). Mediterranean viticulture in the context of climate change. *Ciencia E Tecnica Vitivinicola*, 37(2):139-158. doi: 10.1051/ctv/ctv20223702139

- Lichiheb, Nebila, Carole Bedos, Erwan Personne, Pierre Benoit, Valerie Bergheaud, Olivier Fanucci, Jihene Bouhleb, et Enrique Barriuso. 2015. « Measuring Leaf Penetration and Volatilization of Chlorothalonil and Epoxiconazole Applied on Wheat Leaves in a Laboratory-Scale Experiment ». *Journal of Environmental Quality* 44 (6): 1782-90. <https://doi.org/10.2134/jeq2015.03.0165>.
- Limon-Miro, Ana Teresa, Maria Lourdes Aldana-Madrid, Gerardo Alvarez-Hernandez, Lesley Evelyn Antunez-Roman, Guillermo Rodriguez-Olibarria, et Mauro E Valencia Juillerat. 2017. « Breast milk intake and mother to infant pesticide transfer measured by deuterium oxide dilution in agricultural and urban areas of Mexico ». *Chemosphere* 181 (août): 682-89. <https://doi.org/10.1016/j.chemosphere.2017.04.110>.
- Lozowicka, Bozena, Magdalena Jankowska, Izabela Hrynko, et Piotr Kaczynski. 2016. « Removal of 16 Pesticide Residues from Strawberries by Washing with Tap and Ozone Water, Ultrasonic Cleaning and Boiling ». *Environ Monit Assess* 188 (1): 51. <https://doi.org/10.1007/s10661-015-4850-6>.
- Lu, Yuele, Zhonghua Yang, Luyao Shen, Zhenmin Liu, Zhiqiang Zhou, et Jinling Diao. 2013. « Dissipation behavior of organophosphorus pesticides during the cabbage pickling process: Residue changes with salt and vinegar content of pickling solution ». *Journal of Agricultural and Food Chemistry* 61 (9). <https://doi.org/10.1021/jf304500f>.

M

- Maclachlan, D.J.; Hamilton, D. A New Tool for the Evaluation of Crop Residue Trial Data (Day-Zero-plus Decline). *Food Additives & Contaminants: Part A* 2010, 27, 347–364, doi:10.1080/19440040903403024.
- Maestroni, Britt, Amer Abu Alnaser, Iyad Ghanem, Marivil Islam, Veronica Cesio, Horacio Heinzen, Simon Kelly, et Andrew Cannavan. 2018. « Validation of an Analytical Method for the Determination of Pesticide Residues in Vine Leaves by GC-MS/MS ». *J. Agric. Food Chem.* 66 (25): 6421-30. <https://doi.org/10.1021/acs.jafc.8b00453>.
- Maestroni, Britt Marianna, Iyad Ghanem, Raymond Correll, Amer Abu Alnaser, Marivil Islam, Veronica Cesio, Horacio Heinzen, et Andrew Cannavan. 2018. « Required Withholding Period for Vine Leaves Following Spraying with Pesticide ». *JHER* 4 (4): 140. <https://doi.org/10.11648/j.jher.20180404.14>.
- Maia, Marisa, Ana Rita Cavaco, Gonçalo Laureano, Jorge Cunha, José Eiras-Dias, Ana Rita Matos, Bernardo Duarte, et Andreia Figueiredo. 2021. « More than Just Wine: The Nutritional Benefits of Grapevine Leaves ». *Foods* 10 (10): 2251. <https://doi.org/10.3390/foods10102251>.
- Mdh, P.; Mw, A.; Sn, A. Determination of Pre-Harvest Interval for Quinalphos, Malathion, Diazinon and Cypermethrin in Major Vegetables. 2018, doi:10.4172/2161-0525.1000553.
- Mehta, Rukshan V, M A Sreenivasa, Mathen Mathew, Amy Webb Girard, Sunita Taneja, Samriddhi Ranjan, Usha Ramakrishnan, Reynaldo Martorell, P Barry Ryan, et Melissa F Young. 2020. « A mixed-methods study of pesticide exposures in Breastmilk and Community & Lactating Women's perspectives from Haryana, India ». *BMC Public Health* 20 (1): 1877. <https://doi.org/10.1186/s12889-020-09966-x>.
- Mekonen, Seblework, Argaw Ambelu, Mekitie Wondafrash, Patrick Kolsteren, et Pieter Spanoghe.

2021. « Exposure of infants to organochlorine pesticides from breast milk consumption in southwestern Ethiopia ». *Sci Rep* 11 (novembre): 22053. <https://doi.org/10.1038/s41598-021-01656-x>.
- MoA. 2012. « Decision No. 380, dated March 5, 2012, Subjects table grapes and apples to pesticide residue testing. » *The Lebanese University - Center for Legal Informatics*. <http://77.42.251.205/LawView.aspx?opt=view&LawID=231764>.
- Mohapatra, Soudamini, Ashok Ahuja, Debi Sharma, Deepa Manthirachalam, Gondakar Prakash, et Sampath Kumar. 2011. « Residue study of imidacloprid in grapes (*Vitis vinifera* L.) and soil ». *Quality Assurance and Safety of Crops & Foods* 3 (mars). <https://doi.org/10.1111/j.1757-837X.2010.00084.x>.
- Mohapatra, Soudamini, M Deepa, G K Jagdish, N Rashmi, Sampath Kumar, et G S Prakash. 2010. « Fate of Fipronil and Its Metabolites in/on Grape Leaves, Berries and Soil under Semi Arid Tropical Climatic Conditions ». *Bull Environ Contam Toxicol* 84 (5): 587-91. <https://doi.org/10.1007/s00128-010-9965-4>.
- Mohapatra, Soudamini, Lekha Siddamallaiiah, Nagapooja Yogendraiah Matadha, Veena Rao Udupi, Danish Poothotathil Raj, et Shambulinga Gadigeppa. 2019. « Dissipation of neonicotinoid insecticides imidacloprid, indoxacarb and thiamethoxam on pomegranate (*Punica granatum* L.) ». *Ecotoxicology and Environmental Safety* 171 (avril): 130-37. <https://doi.org/10.1016/j.ECOENV.2018.12.070>.
- Mohasseb, Rita, Youssef Sassine, et Zeina el Sebaaly. 2019. « STATE OF VINICULTURE IN LEBANON ». <https://doi.org/10.13140/RG.2.2.33847.57766>.
- Montemurro, Nicola, Francesco Grieco, Giovanni Lacertosa, et Angelo Visconti. 2002. « Chlorpyrifos Decline Curves and Residue Levels from Different Commercial Formulations Applied to Oranges ». *J Agric Food Chem* 50 (21): 5975-80. <https://doi.org/10.1021/jf0256687>.
- Mostafalou, Sara, et Mohammad Abdollahi. 2016. « Pesticides: an update of human exposure and toxicity ». *Archives of Toxicology* 2016 91:2 91 (2): 549-99. <https://doi.org/10.1007/S00204-016-1849-X>.

N

- Nagy, Károly, Radu Corneliu Duca, Szabolcs Lovas, Matteo Creta, Paul T J Scheepers, Lode Godderis, et Balázs Ádám. 2020. « Systematic review of comparative studies assessing the toxicity of pesticide active ingredients and their product formulations ». *Environmental Research* 181 (février): 108926. <https://doi.org/10.1016/j.envres.2019.108926>.
- Nasreddine, Lara, Maria Rehaime, Zeina Kassaify, Roula Rechmany, et Farouk Jaber. 2016. « Dietary exposure to pesticide residues from foods of plant origin and drinks in Lebanon ». *Environmental Monitoring and Assessment* 188 (8): 1-21. <https://doi.org/10.1007/S10661-016-5505-Y/TABLES/7>.
- Noh, H.-H., Jo, S.-H., Shin, H.-W., Kim, D.-J., Ham, Y.-J., Kim, J.-Y., Kim, D.-B., Kwon, H.-Y., & Kyung, K.-S. (2022). Dissipation and Residue Pattern of Dinotefuran, Fluazinam, Indoxacarb, and Thiacloprid in Fresh and Processed Persimmon Using LC-MS/MS. *Foods*, 11(3), 416.

<https://doi.org/10.3390/foods11030416>

Nougadère, Alexandre, Véronique Sirot, Jean-Pierre Cravedi, Paule Vasseur, Cyril Feidt, Richard J Fussell, Renwei Hu, et al. 2020. « Dietary exposure to pesticide residues and associated health risks in infants and young children – Results of the French infant total diet study ». *Environment International* 137 (avril): 105529. <https://doi.org/10.1016/j.envint.2020.105529>.

Nowowi*, M F M, M a. M Ishak, K Ismail, et S R Zakaria. 2016. « Study on the Effectiveness of Five Cleaning Solutions in Removing Chlorpyrifos Residues in Cauliflower (Brassica Oleracea) ». *JECE* 8 (7): 69-72. <https://doi.org/10.5897/JECE2015.0370>.

O

Odewale, Gideon Oluwasogo, Mosudi Babatunde Sosan, John Adekunle Oyedele Oyekunle, et Adeoluwa Oluwaseyi Adeleye. 2022. « Assessment of systemic and carcinogenic health risks of persistent organochlorine pesticide residues in four fruit vegetables in south-western Nigeria ». *British Food Journal* 124 (5): 1755-74. <https://doi.org/10.1108/BFJ-05-2021-0573/FULL/XML>.

OECD. (2016). OECD Guidance Document on Crop Field Trials. Retrieved from [https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2011\)50/rev1&doclanguage=en](https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2011)50/rev1&doclanguage=en) . Accessed March 19, 2021

Ohkouchi, Takeo, et Kozo Tsuji. 2022. « Basic Technology and Recent Trends in Agricultural Formulation and Application Technology ». *J. Pestic. Sci* 47 (4): 155-71. <https://doi.org/10.1584/jpestics.D22-055>.

Oliva, J.; Cermeño, S.; Cámara, M.A.; Martínez, G.; Barba, A. Disappearance of Six Pesticides in Fresh and Processed Zucchini, Bioavailability and Health Risk Assessment. *Food Chem* 2017, 229, 172–177, doi:10.1016/j.foodchem.2017.02.076.

OMS. 2019. « The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification, 2019 Edition ». <https://www.who.int/publications-detail-redirect/9789240005662>.

Oruç, Esen, Rüstem Cangi, et Aysel Ergün. 2020. « Consumers Preferences on Pickled Vine Leaves the Case of Tokat Province ». *Turkish Journal of Agriculture - Food Science and Technology* 8 (3): 668-77. <https://doi.org/10.24925/turjaf.v8i3.668-677.3172>.

P

Pathak, Vinay Mohan, Vijay K. Verma, Balwant Singh Rawat, Baljinder Kaur, Neelesh Babu, Akansha Sharma, Seeta Dewali, et al. 2022. « Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review ». *Frontiers in Microbiology* 13 (août): 962619. <https://doi.org/10.3389/FMICB.2022.962619/BIBTEX>.

Patil, Reshma, Zareen Khan, Anjali Pudale, Raviraj Shinde, T P Ahammed Shabeer, Ashlesha Patil, et Kaushik Banerjee. 2018. « Comprehensive multiresidue determination of pesticides and plant growth regulators in grapevine leaves using liquid- and gas chromatography with tandem mass

spectrometry ». *Journal of Chromatography A* 1579 (décembre): 73-82. <https://doi.org/10.1016/j.chroma.2018.10.025>.

Patil, Shreya R., et Rahul B Patil. 2022. « Global Scenario of Pesticides and Benefits from Pesticide Usage: A Review ». *International journal of zoological investigations* 08 (02): 805-13. <https://doi.org/10.33745/IJZI.2022.V08I02.097>.

Popp, József, Károly Pető, et János Nagy. 2013. « Pesticide Productivity and Food Security. A Review ». *Agron. Sustain. Dev.* 33 (1): 243-55. <https://doi.org/10.1007/s13593-012-0105-x>.

Possingham, Jv, Tc Chambers, F Radler, et M Grncarevic. 1967. « Cuticular Transpiration and Wax Structure and Composition of Leaves and Fruit of *Vitis Vinifera* ». *Aust. Jnl. Of Bio. Sci.* 20 (6): 1149. <https://doi.org/10.1071/BI9671149>.

Poulsen, M. E., H. K. Hansen, J. J. Sloth, H. B. Christensen, et J. H. Andersen. 2007. « Survey of pesticide residues in table grapes: Determination of processing factors, intake and risk assessment ». *Food Additives and Contaminants* 24 (8): 886-95. <https://doi.org/10.1080/02652030701245320>.

Q

Qi, Shi-Yu, Xue-Ling Xu, Wen-Zhi Ma, Shou-Long Deng, Zheng-Xing Lian, et Kun Yu. 2022. « Effects of Organochlorine Pesticide Residues in Maternal Body on Infants ». *Frontiers in Endocrinology* 13. <https://www.frontiersin.org/articles/10.3389/fendo.2022.890307>.

R

Rahimi, Shahrzad, Khalil Talebi, Ehssan Torabi, et Vahid Hoseini Naveh. 2015. « The dissipation kinetics of malathion in aqueous extracts of different fruits and vegetables ». *Environmental Monitoring and Assessment* 187 (11): 693. <https://doi.org/10.1007/s10661-015-4865-z>.

Ramadan, Mohamed F A, Mohamed M A Abdel-Hamid, Montasser M F Altorgoman, Hamed A AlGaramah, Mohammed A Alawi, Ali A Shati, Hoda A Shweeta, et Nasser S Awwad. 2020. « Evaluation of Pesticide Residues in Vegetables from the Asir Region, Saudi Arabia ». *Molecules* 25 (1): 205. <https://doi.org/10.3390/molecules25010205>.

R Core Team R: A Language and Environment for Statistical Computing.; R Foundation for Statistical Computing: Vienna, Austria, 2020;

RASFF. (2021). European Commission RASFF Window. Retrieved from <https://webgate.ec.europa.eu/rasff-window/screen/search> . Accessed March 19, 2021

RASFF. 2023. « RASFF Window - Version 2.6.0 ». 2023. <https://webgate.ec.europa.eu/rasff-window/screen/search>.

Reddy, Banka Kanda Kishore, Kaithamalai Bhuvaneshwari, Padmanaban Geetha, Natarajan Thamilarasi, Angappan Suganthi, et Mariappan Paramasivam. 2022. « Effect of Decontamination and Processing on Insecticide Residues in Grape (Muscat Hamburg) ». *Environ Sci Pollut Res*, juin.

<https://doi.org/10.1007/s11356-022-21165-2>.

« Règlement (CE) n o 1107/2009 du Parlement européen et du Conseil du 21 octobre 2009 concernant la mise sur le marché des produits phytopharmaceutiques et abrogeant les directives 79/117/CEE et 91/414/CEE du Conseil ». 2009. <http://data.europa.eu/eli/reg/2009/1107/oj/fra>.

« RÈGLEMENT (CE) No 178/2002 DU PARLEMENT EUROPÉEN ET DU CONSEIL DU 28 janvier 2002 ». s. d. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002R0178:20080325:FR:PDF>.

« Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC Text with EEA Relevance. » 2005. <http://data.europa.eu/eli/reg/2005/396/oj/eng>.

Rizzuti, Antonino, Rocco Caliendo, Vito Gallo, Piero Mastroilli, Giuseppe Chita, et Mario Latronico. 2013. « A Combined Approach for Characterisation of Fresh and Brined Vine Leaves by X-Ray Powder Diffraction, NMR Spectroscopy and Direct Infusion High Resolution Mass Spectrometry ». *Food Chem* 141 (3): 1908-15. <https://doi.org/10.1016/j.foodchem.2013.05.044>.

Roby, Jean-Philippe. 2003. « LA FILIERE VITICOLE AU LIBAN : ANALYSE ET PROPOSITIONS POUR UNE EVOLUTION ». Lebanon. <http://www.agriculture.gov.lb/getattachment/Statistics-and-Studies/Studies-and-Publications/agri-production-chains/%D8%A7%D9%84%D8%A7%D9%86%D8%AA%D8%A7%D8%AC-%D8%A7%D9%84%D8%B2%D8%B1%D8%A7%D8%B9%D9%8A/VIGNE.pdf?lang=ar-LB>.

Rogiers, S.Y.; Hatfield, J.M.; Jaudzems, V.G.; White, R.G.; Keller, M. Grape Berry Cv. Shiraz Epicuticular Wax and Transpiration during Ripening and Preharvest Weight Loss. 2004, 7.

S

Salama, Ahmed. 2017. « Lactational Exposure to Pesticides: A Review ». *Toxicology: Open Access* 3 (janvier): 1-9. <https://doi.org/10.4172/2476-2067.1000122>.

Salama, E Y. 2009. « PERSISTENCE OF SOME PESTICIDES ON AND IN GRAPE AND GRAPE LEAVES ». *J. Agric. Sci. Mansoura Univ.* 34 (5): 5349 – 5355. https://jppp.journals.ekb.eg/article_188388_1b89a13bcec45da09d3073292dca6550.pdf.

Salameh, Pascale R., Isabelle Baldi, Patrick Brochard, et Bernadette Abi Saleh. 2004. « Pesticides in Lebanon: a knowledge, attitude, and practice study ». *Environmental Research* 94 (1): 1-6. [https://doi.org/10.1016/S0013-9351\(03\)00092-6](https://doi.org/10.1016/S0013-9351(03)00092-6).

Sandikly, N.; Hayar, S.; Millet, M. Monitoring Survey of Pesticide Residues in Table Grapes and Risk Assessment to Human in Lebanon. In Proceedings of the 49ème Congrès du Groupe Français des Pesticides; Montpellier, France, May 21 2019.

Sama'neh, Safa'. 2004. « Detection of Chlorpyrifos and Penconazole Residues in Grape Leaves and Fruit by Gas Chromatography/ Mass Spectrometry ». Nablus, Palestine: An-Najah National University - Faculty of Graduate Studies. https://scholar.najah.edu/sites/default/files/all-thesis/detection_of_chlorpyrifos_and_penconazole_residues_in_grape_leaves_and_fruit_by_g

[as_chromatography_mass_spectrometry.pdf](#).

- Sameut Bouhaik, Azzeddine. 2009. « Modélisation de dépôt des pesticides sur des plans d'eau ». 5, boulevard Descartes Champs-sur-Marne 77454 Marne-la-Vallée Cedex 2: Université Paris-Est Marne-la-Vallée - Laboratoire de Géo matériaux et Environnement. https://www.generations-futures.fr/wp-content/uploads/2019/09/rapportfinalazzed_repaired.pdf.
- Sánchez-Bayo, Francisco, Koichi Goka, et Daisuke Hayasaka. 2016. « Contamination of the Aquatic Environment with Neonicotinoids and its Implication for Ecosystems ». *Frontiers in Environmental Science* 4. <https://www.frontiersin.org/articles/10.3389/fenvs.2016.00071>.
- Sannakki, S.; Rajpurohit, V.; Nargund, V.; Kulkarni, P. "Diagnosis and Classification of Grape Leaf Diseases Using Neural Networks"; 2013; p. 5; ISBN 978-1-4799-3926-8.
- Schusterova, Dana, Jana Hajslova, Vladimir Kocourek, et Jana Pulkrabova. 2021. « Pesticide Residues and Their Metabolites in Grapes and Wines from Conventional and Organic Farming System ». *Foods* 2021, Vol. 10, Page 307 10 (2): 307. <https://doi.org/10.3390/FOODS10020307>.
- Scholz, K.; Reinhard, F. Photolysis of Imidacloprid (NTN 33893) on the Leaf Surface of Tomato Plants. *Pesticide Science* 1999, 55, 652–654, doi:10.1002/(SICI)1096-9063(199906)55:6<652::AID-PS997>3.0.CO;2-I.
- Şensoy, RİG., L. Ersayar, et A. Doğan. 2017. « Determination of Pesticide Residue Amounts in Fresh Grapes, Raisins and Pickled Grape Leaves Sold in Van Province ». *Yuzuncu Yil Univ. J. Of Agr. Sci.* 27 (3): 436-46.
- Shao, Wenyong, Youfu Zhao, et Zhonghua Ma. 2021. « Advances in understanding fungicide resistance in botrytis cinerea in China ». *Phytopathology* 111 (3): 455-63. <https://doi.org/10.1094/PHYTO-07-20-0313-IA/ASSET/IMAGES/LARGE/PHYTO-07-20-0313-IAT1.JPG>.
- Shokoohi, R., M. T. Samadi, M. Karami, A. Heshmati, M. Leili, et S. Khamutian. 2021. « Residue Content of Organophosphorus Pesticides and their Toxic Metabolites in Greenhouse-Grown Tomatoes during Pre-Harvest Interval and Post-Harvest Processing: A Kinetic Study ». *Iranian Red Crescent Medical Journal* 23 (1): 1. <https://doi.org/10.32592/ircmj.2021.23.1.33>.
- SHOKOOHI, Reza, Mohammad Taghi SAMADI, Manoochehr KARAMI, et Razieh KHAMUTIAN. 2019. « The Necessity of Monitoring Pesticide Residues in Vegetables and Fruits Using Hazard Index among Consumers ». *Iran J Public Health* 48 (6): 1189-90. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6635347/>.
- Shokr, S., I. Nasr, et Hend A. Mahmoud. 2015. « RESIDUAL BEHAVIOUR OF FENARIMOL AND FLUSILAZOLE FUNGICIDES IN GRAPES ».
- Silvestro, L., Tarcomnicu, I., & Savu, S. R. (2013). Matrix Effects in Mass Spectrometry Combined with Separation Methods — Comparison HPLC, GC and Discussion on Methods to Control these Effects. In *Tandem Mass Spectrometry - Molecular Characterization*. Intech. <https://doi.org/10.5772/55982>
- Singh, R Paul. 1996. *Computer applications in food technology : use of spreadsheets in graphical, statistical, and process analyses*. Academic Press. <http://p5070-www.sciencedirect.com.bases-doc.univ-lorraine.fr/book/9780126463828/computer-applications-in-food-technology>.

Smadi, Nadia, Adla Jammoul, et Nada El Darra. 2019. « Assessment of Antibiotic and Pesticides Residues in Breast Milk of Syrian Refugee Lactating Mothers ». *Toxics* 7 (3): 39. <https://doi.org/10.3390/toxics7030039>.

Socorro, Joanna, Amandine Durand, Brice Temime-Roussel, Sasho Gligorovski, Henri Wortham, et Etienne Quivet. 2016. « The persistence of pesticides in atmospheric particulate phase: An emerging air quality issue ». *Scientific Reports* 2016 6:1 6 (1): 1-7. <https://doi.org/10.1038/srep33456>.

Stefl, Philip. 2017. « What Is Log Pow? The O/W Partition Coefficient in SDSs ». *GeSi3*. <https://www.gesi.de/en/blog/2017/02/06/what-is-log-pow-the-o-w-partition-coefficient-in-sdss/>.

T

Ta, A., Madan, V. K., Chauhan, R., & Kumari, B. (2018). Dissipation Behaviour and Effect of Different Decontamination Processes on Reduction of Residues of Novaluron in Tomato (*Lycopersicon esculentum* Mill.). *International Journal of Current Microbiology and Applied Sciences*, 7(05), 648–656. <https://doi.org/10.20546/IJCMAS.2018.705.079>

Tabaja, N., M. Sibliini, et L. Chalak. 2020. « Current status of registered grapevine saplings in Lebanon ». *Acta Horticulturae* 1276 (avril): 265-70. <https://doi.org/10.17660/ACTAHORTIC.2020.1276.38>.

Tadele, Zerihun. 2019. « Orphan Crops: Their Importance and the Urgency of Improvement ». *Planta* 250 (3): 677-94. <https://doi.org/10.1007/s00425-019-03210-6>.

Talabi, Abidemi Olutayo, Prashant Vikram, Sumitha Thushar, Hifzur Rahman, Hayatullah Ahmadzai, Nhamo Nhamo, Mohammed Shahid, et Rakesh Kumar Singh. 2022. « Orphan Crops: A Best Fit for Dietary Enrichment and Diversification in Highly Deteriorated Marginal Environments ». *Frontiers in Plant Science* 13. <https://www.frontiersin.org/articles/10.3389/fpls.2022.839704>.

Tang, Fiona H M, Manfred Lenzen, Alexander McBratney, et Federico Maggi. 2021. « Risk of Pesticide Pollution at the Global Scale ». *Nat. Geosci.* 14 (4): 206-10. <https://doi.org/10.1038/s41561-021-00712-5>.

Tann, R S. 1997. *A Review of Surfactants Used in Novel Agricultural Applications*. ASTM International.

Thorsen, S. (2021). Timeanddate.com. Retrieved from <https://www.timeanddate.com/>. Accessed June 15, 2021

TRADE, MINISTRY O F ECONOMY &TRADE. 2022. « LEBTRADE-Table Grapes ». *LEBTRADE*. <https://lebtrade.gov.lb/>.

Tucker, Samuel, Georgiana Diana Dumitriu, et Carmen Teodosiu. 2022. « Pesticides Identification and Sustainable Viticulture Practices to Reduce Their Use: An Overview ». *Molecules* 2022, Vol. 27, Page 8205 27 (23): 8205. <https://doi.org/10.3390/MOLECULES27238205>.

Tudi, Muyesaier, Huada Daniel Ruan, Li Wang, Jia Lyu, Ross Sadler, Des Connell, Cordia Chu, et Dung Tri Phung. 2021. « Agriculture Development, Pesticide Application and Its Impact on the

Environment ». *Int J Environ Res Public Health* 18 (3): 1112.
<https://doi.org/10.3390/ijerph18031112>.

Turkoz Bakirci, Gozde. 2018. « Evaluation of pesticide residues in brined vine leaves by liquid chromatography coupled to tandem mass spectrometry ». *Fresenius Environmental Bulletin* 27 (janvier): 4543-58.

TÜRKÖZ BAKIRCI, Gözde, Ezgi Çınar, et Senem KARAKAYA. 2019. « Manisa İlinden Toplanan Asma Yapraklarında Pestisit Kalıntıları ». *Akademik Gıda* 17 (1): 55-60.
<https://doi.org/10.24323/akademik-gida.544073>.

U

University of Kentucky. 2016. « Pesticide Formulations – Chapter 3: Using Pesticide Formulations ». University of Kentucky Department of Entomology.
<https://www.uky.edu/Ag/Entomology/PSEP/pdfs/3formulations.pdf>.

USAID/LEBANON. 2021. « LIVCD POST-ACTIVITY ASSESSMENT – TABLE GRAPE VC - Final Report ».

V

Vemuri, S., Sreenivasa Rao, C., Rao, R., Darsi, H., Reddy, A., & Swarupa, S. (2014). Methods for Removal of Pesticide Residues in Tomato. *Food Science and Technology*, 2(5), 64-68.
<https://doi.org/10.13189/fst.2014.020502>

Volza. 2023. « Grape Leaves Exports from World - Export Data with Price, Buyer, Supplier, HSN Code ». <https://www.volza.com/p/grape-leaves/export/>.

W

WHO. 2018. « Generic Risk Assessment Model for Insecticides Used for Larviciding and Mollusciciding – Second Edition ». <https://www.who.int/publications-detail-redirect/9789241515047>.

———. 2019. « The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification, 2019 Edition ». <https://www.who.int/publications-detail-redirect/9789240005662>.

World Weather. 2023. « Local Weather Forecast ». 2023. <https://en.tutiempo.net/>.

Wu, Yangliu, Quanshun An, Dong Li, Jun Wu, et Canping Pan. 2019. « Comparison of Different Home/Commercial Washing Strategies for Ten Typical Pesticide Residue Removal Effects in Kumquat, Spinach and Cucumber ». *Int J Environ Res Public Health* 16 (3): E472.
<https://doi.org/10.3390/ijerph16030472>.

X

Xu, F., Lu, Z., Xu, D., Lu, H., Qiu, J., & Zha, X. (2022). Dissipation behavior, residue transfer, and safety evaluation of chlorantraniliprole and indoxacarb during tea growing and brewing by ultrahigh-performance liquid chromatography-tandem mass spectrometry. *Environmental Science and Pollution Research*, 29(42), 63735–63752. <https://doi.org/10.1007/s11356-022-20321-y>

Y

Yanar, Y., R. Cangı, et K. Ozata. 2015. « The Determination of Pesticide Residue Levels in Brined Vine Leaves Produced in Tokat Province ». *Selcuk Agr. and Food sci.* 27: 267-75.

Yang, Gui-ling, Wen Wang, Sen-miao Liang, Yi-jun Yu, Hui-yu Zhao, Qiang Wang, et Yong-zhong Qian. 2017. « Pesticide residues in bayberry (*Myrica rubra*) and probabilistic risk assessment for consumers in Zhejiang, China ». *Journal of Integrative Agriculture* 16 (9): 2101-9. [https://doi.org/10.1016/S2095-3119\(16\)61600-3](https://doi.org/10.1016/S2095-3119(16)61600-3).

Yang, Tianxi, Bin Zhao, Amanda J Kinchla, John M Clark, et Lili He. 2017. « Investigation of Pesticide Penetration and Persistence on Harvested and Live Basil Leaves Using Surface-Enhanced Raman Scattering Mapping ». *J. Agric. Food Chem.* 65 (17): 3541-50. <https://doi.org/10.1021/acs.jafc.7b00548>.

Yang, T., Doherty, J., Zhao, B., Kinchla, A. J., Clark, J. M., & He, L. (2017). Effectiveness of Commercial and Homemade Washing Agents in Removing Pesticide Residues on and in Apples. *Journal of Agricultural and Food Chemistry*, 65(44), 9744–9752. <https://doi.org/10.1021/acs.jafc.7b03118>

Yigit, N., & Velioglu, Y. S. (2020). Effects of processing and storage on pesticide residues in foods. *Critical Reviews in Food Science and Nutrition*, 60(21), 3622–3641. <https://doi.org/10.1080/10408398.2019.1702501>

Yildirim Kumral, Aysegul, Nabi Alper Kumral, Aysenur Kolcu, Busra Maden, et Buse Artik. 2020. « Simulation Study for the Degradation of Some Insecticides during Different Black Table Olive Processes ». *ACS Omega* 5 (23): 14164-72. <https://doi.org/10.1021/acsomega.0c01907>.

Youssef, Lobna, Ghassan Younes, Abir Kouzayha, et Farouk Jaber. 2015. « Occurrence and levels of pesticides in South Lebanon water ». *Chemical Speciation & Bioavailability* 27 (2): 62-70. <https://doi.org/10.1080/09542299.2015.1023092>.

Z

Zacharia, James Tano, Zacharia, et James Tano. 2011. « Identity, Physical and Chemical Properties of Pesticides ». In *Pesticides in the Modern World - Trends in Pesticides Analysis*. IntechOpen. <https://doi.org/10.5772/17513>.

Zarn, Jürg A, et Caitlyn D O'Brien. 2018. « Current Pesticide Dietary Risk Assessment in Light of Comparable Animal Study NOAELs after Chronic and Short-Termed Exposure Durations ». *Arch Toxicol* 92 (1): 157-67. <https://doi.org/10.1007/s00204-017-2052-4>.

Zhang, Jiguang, Xiaojing Sheng, Jianmin Cao, Song Fang, Xue Liu, Xiuhua Liu, Liangrui Peng, Huiqing Sun, et Rui Weng. 2023. « Occurrence and risk exposure assessment of multiple pesticide residues in edible mint in China ». *Journal of Food Composition and Analysis* 116 (mars): 105071. <https://doi.org/10.1016/j.jfca.2022.105071>.

Zhou, You, Kun Wang, Chao Yan, Wei-Sheng Li, Hong Li, Ning Zhang, et Zhi-Xiang Zhang. 2014. « Effect of Two Formulations on the Decline Curves and Residue Levels of Rotenone in Cabbage and Soil under Field Conditions ». *Ecotoxicol Environ Saf* 104 (juin): 23-27. <https://doi.org/10.1016/j.ecoenv.2014.02.014>.

ZORLU ÜNLÜ, Tuba, Semra TOPUZ, Mustafa BAYRAM, Tarik BALKAN, et Cemal KAYA. 2023. « DETERMINATION OF PESTICIDE RESIDUES IN PICKLED VINE (*Vitis vinifera* L.) LEAVES BY A VALIDATED LC-MS/MS METHOD ». *Gıda* 48 (6): 1335-50. <https://doi.org/10.15237/gida.GD23096>.

Zubrod, J. P., Bundschuh, M., Arts, G., Brühl, C. A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J. J., Rohr, J., Scharmüller, A., Smalling, K., Stehle, S., Schulz, R., & Schäfer, R. B. (2019). Fungicides: An Overlooked Pesticide Class?. *Environmental science & technology*, 53(7), 3347–3365. <https://doi.org/10.1021/acs.est.8b04392>

40 CFR Part 180 – Tolerances and Exemptions for Pesticide Chemical Residues in Food. <https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-180?toc=1>.

Annexes

Annexe I : Matériel supplémentaire de l'article : "Majed L., Hayar S. & Dousset S. 2024. Monitoring of pesticide residue levels in Lebanese table grapes from 2012-2014 and human health risk assessment. Food Additives & Contaminants: Part B, X–X. <https://doi.org/10.1080/XXXXX> "

Table SII- 1: List of the 80 molecules analyzed in the grape samples

| NB | Compounds | Instrument | NB | Compounds | Instrument |
|-----------|----------------------|-------------------|-----------|----------------------|-------------------|
| 1 | Acetamiprid | LCMSMS | 41 | Kresoxim methyl | LCMSMS/GCMSMS |
| 2 | Azoxystrobin | LCMSMS/GCMSMS | 42 | Lambda cyhalothrin | GCMSMS |
| 3 | Bifenthrin | LCMSMS/GCMSMS | 43 | Linuron | LCMSMS |
| 4 | Boscalid | LCMSMS/GCMSMS | 44 | Lufenuron | LCMSMS |
| 5 | Carbendazim | LCMSMS | 45 | Malaoxon | LCMSMS |
| 6 | Carbofuran | LCMSMS | 46 | Malathion | LCMSMS/GCMSMS |
| 7 | Carbosulfan | LCMSMS | 47 | Metalaxyl | LCMSMS/GCMSMS |
| 8 | Chlorpyriphos | LCMSMS/GCMSMS | 48 | Metalaxyl M | LCMSMS/GCMSMS |
| 9 | Chlorpyriphos methyl | LCMSMS/GCMSMS | 49 | Methamidophos | LCMSMS |
| 10 | Clodinafop propargyl | LCMSMS/GCMSMS | 50 | Methiocarb | LCMSMS |
| 11 | Cyflufenamid | LCMSMS/GCMSMS | 51 | Methiocarb Sulfoxide | LCMSMS |
| 12 | Cymoxanil | LCMSMS | 52 | Methomyl | LCMSMS |
| 13 | Cypermethrin | GCMSMS | 53 | Metribuzin | LCMSMS/GCMSMS |
| 14 | Cyproconazole | LCMSMS/GCMSMS | 54 | Monocrotophos | LCMSMS |
| 15 | Cyprodinil | LCMSMS/GCMSMS | 55 | Myclobutanil | LCMSMS/GCMSMS |
| 16 | Deltamethrin | GCMSMS | 56 | Omethoate | LCMSMS |
| 17 | Diafenthiuron | LCMSMS | 57 | Oxamyl | LCMSMS |
| 18 | Diazinon | LCMSMS/GCMSMS | 58 | Parathion | LCMSMS |
| 19 | Difenoconazole | LCMSMS/GCMSMS | 59 | Penconazole | LCMSMS/GCMSMS |
| 20 | Diflubenzuron | LCMSMS | 60 | Pendimethalin | LCMSMS/GCMSMS |

| | | | | | |
|----|--------------------|---------------|----|----------------------|---------------|
| 21 | Dimethoate | LCMSMS/GCMSMS | 61 | Piperonyl Butoxide | GCMSMS |
| 22 | Dimethomorph | LCMSMS | 62 | Pirimiphos-methyl | GCMSMS |
| 23 | Etoazole | LCMSMS/GCMSMS | 63 | Pirimicarb | LCMSMS/GCMSMS |
| 24 | Famoxadone | LCMSMS/GCMSMS | 64 | Procymidone | GCMSMS |
| 25 | Fenazaquin | LCMSMS/GCMSMS | 65 | Propamocarb | LCMSMS |
| 26 | Fenhexamid | LCMSMS/GCMSMS | 66 | Propargite | GCMSMS |
| 27 | Fenpropathrin | LCMSMS/GCMSMS | 67 | Propiconazole | LCMSMS/GCMSMS |
| 28 | Fenpyroximate | LCMSMS | 68 | Pyraclostrobin | LCMSMS |
| 29 | Fluazinam | LCMSMS | 69 | Pyridaben | LCMSMS/GCMSMS |
| 30 | Fludioxonil | LCMSMS/GCMSMS | 70 | Pyrimethanil | LCMSMS/GCMSMS |
| 31 | Flufenoxuron | LCMSMS | 71 | Spinosad | LCMSMS |
| 32 | Flusilazole | LCMSMS | 72 | Spirodiclofen | LCMSMS/GCMSMS |
| 33 | Flutriafol | LCMSMS/GCMSMS | 73 | Sulfotep | GCMSMS |
| 34 | Fluazifop-butyl | LCMSMS | 74 | Tebuconazole | LCMSMS/GCMSMS |
| 35 | Haloxypop-P-methyl | LCMSMS/GCMSMS | 75 | Tetraconazole | LCMSMS/GCMSMS |
| 36 | Hexaconazole | LCMSMS/GCMSMS | 76 | Thiamethoxam | LCMSMS |
| 37 | Hexythiazoxe | LCMSMS | 77 | Thiophanate - methyl | LCMSMS |
| 38 | Imazalil | LCMSMS | 78 | Tolclofos-methyl | GCMSMS |
| 39 | Imidacloprid | LCMSMS | 79 | Triadimenol | GCMSMS |
| 40 | Indoxacarb | LCMSMS/GCMSMS | 80 | Trifloxystrobin | LCMSMS/GCMSMS |

Table SII- 2: Number of grape samples containing pesticide residues or not in Lebanese regions in 2012, 2013 and 2014

| | | Year of sampling | | | | | |
|--|---------------|------------------|------------|-----------|------------|-----------|------------|
| | | 2012 | | 2013 | | 2014 | |
| | | <i>RD</i> | <i>NRD</i> | <i>RD</i> | <i>NRD</i> | <i>RD</i> | <i>NRD</i> |
| Region | Bekaa | 469 | 0 | 165 | 97 | 235 | 185 |
| | North | 102 | 0 | 92 | 54 | 68 | 54 |
| | South | 2 | 15 | 9 | 5 | 14 | 11 |
| | Mount-Lebanon | - | - | 2 | 1 | 8 | 6 |
| | Totals | 573 | 15 | 268 | 157 | 325 | 256 |
| | | 588 | | 425 | | 581 | |
| | | 1594 | | | | | |
| *RD : residues detected; NRD: no residues detected | | | | | | | |

Table SII- 3: Occurrences, means, medians and MRL exceedances of detected pesticides in grapes in 2012, 2013, and 2014 years

| Molecule | All Regions | | | | | | | | | | | | | | | | | |
|---------------------|-------------|-------------------|--------|------|-------|--------|------|-------------------|--------|------|-------|--------|------|-------------------|--------|------|-------|--------|
| | 2012 | | | | | | 2013 | | | | | | 2014 | | | | | |
| | N | Mean (±SD) | Median | MRL | N>MRL | %N>MRL | N | Mean (±SD) | Median | MRL | N>MRL | %N>MRL | N | Mean (±SD) | Median | MRL | N>MRL | %N>MRL |
| Acetamiprid | 34 | 0.276 (±0.237) | 0.096 | 0.5 | 15 | 44.1 | 26 | 0.244 (±0.277) | 0.080 | 0.5 | 9 | 34.6 | 73 | 0.134 (±0.121) | 0.113 | 0.5 | 0 | 0.0 |
| Azoxystrobin | 95 | 0.456 (±0.332) | 0.358 | 2 | 0 | 0.0 | 59 | 0.193 (±0.376) | 0.058 | 2 | 1 | 1.7 | 108 | 0.118 (±0.124) | 0.071 | 2 | 0 | 0.0 |
| Boscalid | 58 | 1.271 (±1.084) | 0.978 | 5 | 0 | 0.0 | 30 | 0.1 (±0.058) | 0.090 | 5 | 0 | 0.0 | 123 | 0.328 (±0.416) | 0.200 | 5 | 0 | 0.0 |
| Carbendazim | 73 | 0.950 (±0.663) | 0.874 | 3 | 1 | 1.4 | 59 | 0.31 (±0.344) | 0.200 | 3 | 0 | 0.0 | 58 | 0.3 (±0.325) | 0.201 | 3 | 0 | 0.0 |
| Carbofuran | 2 | 0.008 (±0.001) | 0.008 | 0.01 | 0 | 0.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Chlorpyrifos | 56 | 0.243 (±0.202) | 0.202 | 0.5 | 5 | 8.9 | 5 | 0.332 (±0.491) | 0.116 | 0.5 | 1 | 20.0 | 44 | 0.426 (±0.396) | 0.327 | 0.5 | 14 | 31.8 |
| Chlorpyrifos-methyl | 1 | 0.142 (±0) | 0.142 | 1 | 0 | 0.0 | ND | ND | ND | 1 | ND | ND | ND | ND | ND | 1 | ND | ND |
| Cymoxanil | 1 | 0.009 (±0) | 0.009 | 0.01 | 0 | 0.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Cypermethrin | 75 | 0.443 (±0.399) | 0.365 | 0.2 | 48 | 64.0 | 36 | 0.281 (±0.187) | 0.254 | 0.2 | 29 | 80.6 | 36 | 0.37 (±0.225) | 0.291 | 0.2 | 35 | 97.2 |
| Cyproconazole | 8 | 0.084 (±0.110) | 0.009 | 0.01 | 3 | 37.5 | 4 | 0.008 (±0.001) | 0.008 | 0.01 | 0 | 0.0 | ND | ND | ND | 0.01 | ND | ND |
| Cyprodinil | 101 | 0.386 (±0.277) | 0.345 | 3 | 0 | 0.0 | 46 | 0.167 (±0.163) | 0.123 | 3 | 0 | 0.0 | 69 | 0.302 (±0.36) | 0.163 | 3 | 0 | 0.0 |
| Deltamethrin | 4 | 0.164 (±0.062) | 0.155 | 0.2 | 1 | 25.0 | ND | ND | ND | 0.2 | ND | ND | ND | ND | ND | 0.2 | ND | ND |
| Difenoconazole | 85 | 0.082 (±0.090) | 0.060 | 0.1 | 15 | 17.6 | 48 | 0.117 (±0.144) | 0.075 | 0.1 | 16 | 33.3 | 50 | 1.466 (±1.901) | 0.057 | 3 | 19 | 38.0 |
| Diflubenzuron | 1 | 0.008 (±0) | 0.008 | 0.01 | 0 | 0.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Dimethoate | 4 | 0.077 (±0.082) | 0.068 | 0.01 | 2 | 50.0 | 2 | 0.085 (±0.114) | 0.085 | 0.01 | 1 | 50.0 | 1 | 0.134 (±0) | 0.134 | 0.01 | 1 | 100.0 |

| | | | | | | | | | | | | | | | | | | |
|--------------------|----|-------------------|-------|------|----|-------|----|-------------------|-------|------|----|-------|----|-------------------|-------|------|----|------|
| Dimethomorph | 1 | 0.040 (±0) | 0.040 | 2 | 0 | 0.0 | 3 | 0.025 (±0.013) | 0.020 | 2 | 0 | 0.0 | ND | ND | ND | 2 | ND | ND |
| Etozazole | 1 | 0.090 (±0) | 0.090 | 0.5 | 0 | 0.0 | ND | ND | ND | 0.5 | ND | ND | ND | ND | ND | 0.5 | ND | ND |
| Fenhexamid | 61 | 0.641 (±0.541) | 0.505 | 15 | 0 | 0.0 | 35 | 0.483 (±0.657) | 0.200 | 15 | 0 | 0.0 | 12 | 0.462 (±0.583) | 0.158 | 15 | 0 | 0.0 |
| Fluazifop-butyl | 1 | 0.009 (±0) | 0.009 | 0.01 | 0 | 0.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Flufenoxuron | 4 | 0.269 (±0.185) | 0.196 | 0.01 | 4 | 100.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Flusilazole | 13 | 0.189 (±0.342) | 0.079 | 0.2 | 3 | 23.1 | 4 | 0.105 (±0.125) | 0.056 | 0.2 | 1 | 25.0 | 2 | 0.054 (±0.023) | 0.054 | 0.2 | 0 | 0.0 |
| Hexaconazole | 37 | 0.017 (±0.038) | 0.007 | 0.01 | 3 | 8.1 | 5 | 0.007 (±0.002) | 0.007 | 0.01 | 0 | 0.0 | 3 | 0.098 (±0.079) | 0.130 | 0.01 | 2 | 66.7 |
| Imidacloprid | 24 | 0.422 (±0.359) | 0.310 | 1 | 1 | 4.2 | 3 | 0.283 (±0.142) | 0.276 | 1 | 0 | 0.0 | 6 | 0.244 (±0.109) | 0.233 | 1 | 0 | 0.0 |
| Indoxacarb | 43 | 0.257 (±0.279) | 0.100 | 2 | 0 | 0.0 | 5 | 0.127 (±0.026) | 0.127 | 2 | 0 | 0.0 | 2 | 0.334 (±0.233) | 0.334 | 2 | 0 | 0.0 |
| Kresoxim-methyl | 1 | 0.652 (±0) | 0.652 | 1 | 0 | 0.0 | 6 | 0.537 (±0.414) | 0.380 | 1 | 1 | 16.7 | 6 | 0.283 (±0.248) | 0.227 | 1 | 0 | 0.0 |
| Lambda-Cyhalothrin | 2 | 0.380 (±0.028) | 0.380 | 0.01 | 2 | 100.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Lufenuron | 19 | 0.138 (±0.165) | 0.008 | 0.01 | 8 | 42.1 | 2 | 0.39 (±0.015) | 0.390 | 0.01 | 2 | 100.0 | 23 | 0.292 (±0.379) | 0.125 | 0.01 | 12 | 52.2 |
| Metalaxyl | 7 | 0.178 (±0.198) | 0.141 | 1 | 0 | 0.0 | ND | ND | ND | 1 | ND | ND | 4 | 0.128 (±0.182) | 0.038 | 1 | 0 | 0.0 |
| Metalaxyl-M | 3 | 0.198 (±0.068) | 0.237 | 0.01 | 3 | 100.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Methamidophos | 1 | 0.150 (±0) | 0.150 | 0.01 | 1 | 100.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Methiocarb | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND | 1 | 0.004 (±0) | 0.004 | 0.01 | 0 | 0.0 |
| Methomyl | 2 | 0.179 (±0.021) | 0.179 | 0.3 | 0 | 0.0 | ND | ND | ND | 0.3 | ND | ND | ND | ND | ND | 0.3 | ND | ND |
| Myclobutanil | 45 | 0.304 (±0.289) | 0.167 | 1 | 0 | 0.0 | 58 | 0.201 (±0.198) | 0.146 | 1 | 0 | 0.0 | 32 | 0.226 (±0.113) | 0.199 | 1 | 0 | 0.0 |
| Penconazole | 18 | 0.104 (±0.055) | 0.095 | 0.2 | 0 | 0.0 | 6 | 0.064 (±0.058) | 0.034 | 0.2 | 0 | 0.0 | 8 | 0.088 (±0.056) | 0.082 | 0.2 | 0 | 0.0 |
| Pirimiphos-methyl | 1 | 0.003 (±0) | 0.003 | 0.01 | 0 | 0.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |

| | | | | | | | | | | | | | | | | | | |
|--------------------|----|-------------------|-------|------|----|------|----|-------------------|-------|------|----|-------|----|-------------------|-------|------|----|------|
| Procymidone | 3 | 0.006 (±0.003) | 0.006 | 0.01 | 0 | 0.0 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Propargite | 2 | 2.400 (±0.566) | 2.400 | 7 | 0 | 0.0 | ND | ND | ND | 7 | ND | ND | ND | ND | ND | 7 | ND | ND |
| Propiconazole | ND | ND | ND | 0.01 | ND | ND | 2 | 0.002 (±0) | 0.002 | 0.01 | 0 | 0.0 | 4 | 0.005 (±0.001) | 0.005 | 0.01 | 0 | 0.0 |
| Pyraclostrobin | 18 | 0.243 (±0.315) | 0.135 | 2 | 0 | 0.0 | ND | ND | ND | 2 | ND | ND | 12 | 0.077 (±0.044) | 0.074 | 2 | 0 | 0.0 |
| Pyridaben | 16 | 0.035 (±0.053) | 0.007 | 0.01 | 4 | 25.0 | 2 | 0.006 (±0.005) | 0.006 | 0.01 | 0 | 0.0 | 3 | 0.01 (±0.001) | 0.010 | 0.01 | 0 | 0.0 |
| Pyrimethanil | 7 | 0.585 (±0.484) | 0.614 | 4 | 0 | 0.0 | 2 | 0.095 (±0.006) | 0.095 | 4 | 0 | 0.0 | 4 | 0.402 (±0.53) | 0.177 | 4 | 0 | 0.0 |
| Tebuconazole | ND | ND | ND | 6 | ND | ND | 1 | 0.086 (±0) | 0.086 | 6 | 0 | 0.0 | ND | ND | ND | 6 | ND | ND |
| Tetraconazole | 15 | 0.164 (±0.206) | 0.123 | 0.01 | 9 | 60.0 | 12 | 0.148 (±0.201) | 0.009 | 0.01 | 5 | 41.7 | 54 | 0.08 (±0.147) | 0.008 | 0.01 | 17 | 31.5 |
| Thiamethoxam | ND | ND | ND | 0.01 | ND | ND | 1 | 0.464 (±0) | 0.464 | 0.01 | 1 | 100.0 | ND | ND | ND | 0.01 | ND | ND |
| Thiophanate-methyl | 87 | 0.616 (±0.622) | 0.359 | 0.01 | 66 | 75.9 | 29 | 1.44 (±1.731) | 0.821 | 0.01 | 23 | 79.3 | 21 | 1.233 (±2.472) | 0.174 | 0.01 | 15 | 71.4 |
| Tolclofos-methyl | 7 | 0.517 (±0.656) | 0.150 | 0.01 | 5 | 71.4 | ND | ND | ND | 0.01 | ND | ND | ND | ND | ND | 0.01 | ND | ND |
| Triadimenol | 2 | 1.400 (±0.990) | 1.400 | 2 | 1 | 50.0 | ND | ND | ND | 2 | ND | ND | ND | ND | ND | 2 | ND | ND |
| Trifloxystrobin | 45 | 0.041 (±0.016) | 0.040 | 3 | 0 | 0.0 | 39 | 0.066 (±0.129) | 0.028 | 3 | 0 | 0.0 | 34 | 0.108 (±0.128) | 0.049 | 3 | 0 | 0.0 |

** the mean, median and MRLs are in mg/kg; ND: Not Detected; N: number of samples with the molecule; N>MRL: number of samples where the molecule exceeded its MRL; SD: Standard Deviation

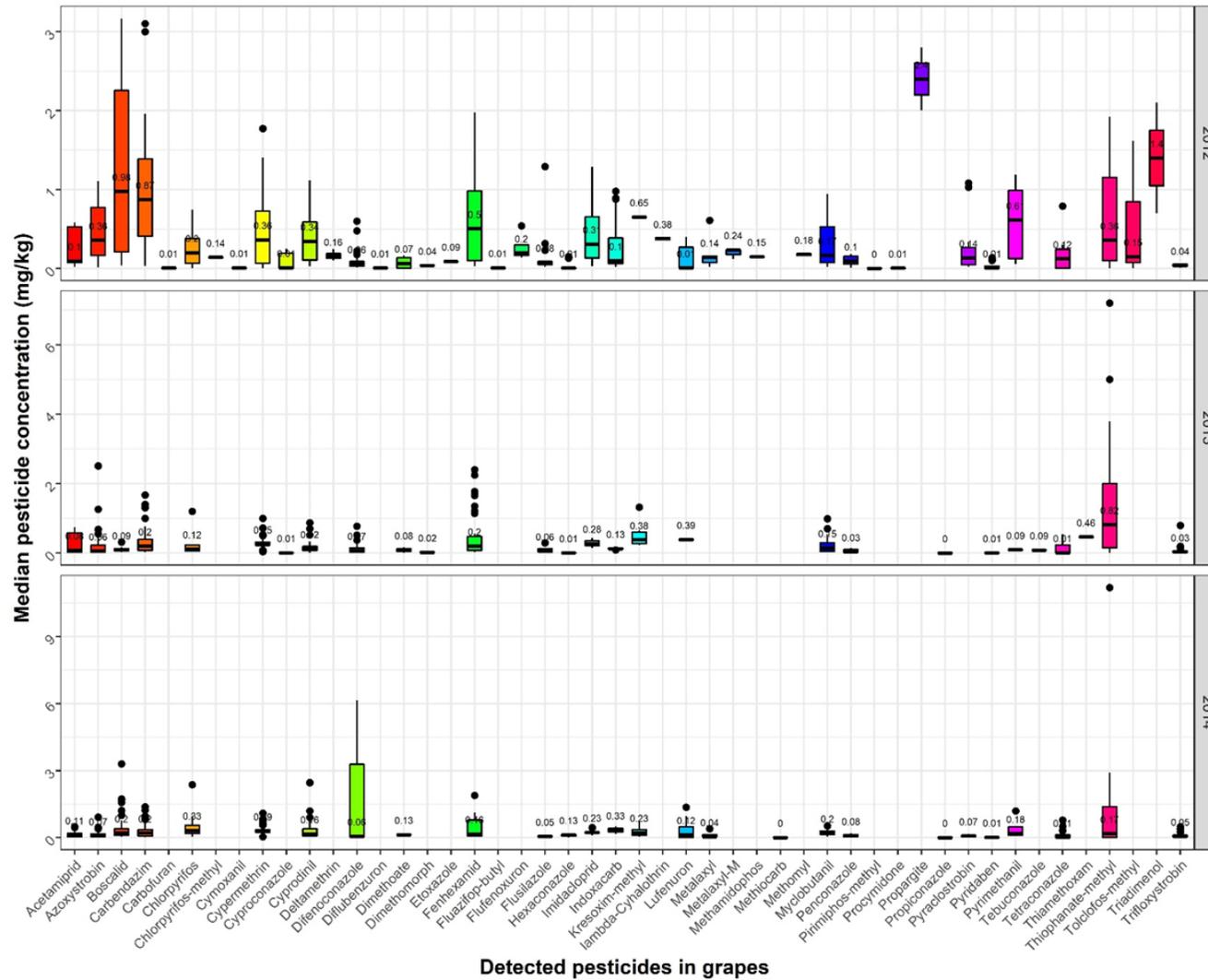
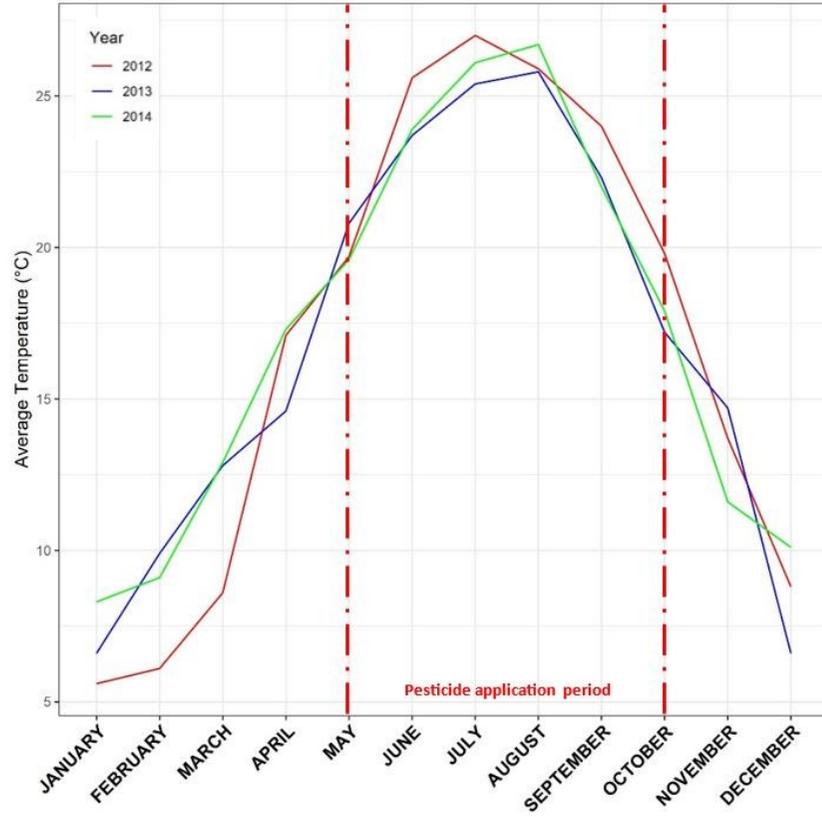


Figure SII - 1: Median concentrations of pesticides detected in grape samples in 2012, 2013, and 2014

(A) Average Temperature Over 3 Years



(B) Average Relative Humidity Over 3 Years

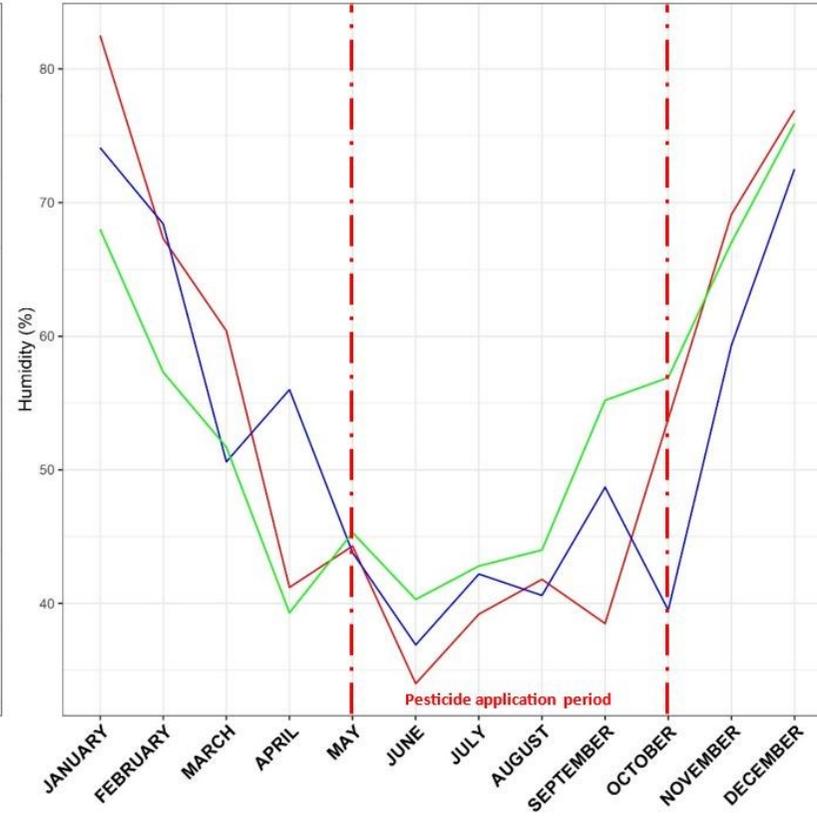


Figure SII - 2: Average monthly temperature (A) and relative humidity (B) in 2012, 2013 and 2014 in Bekaa region (according to World Weather data 2023)

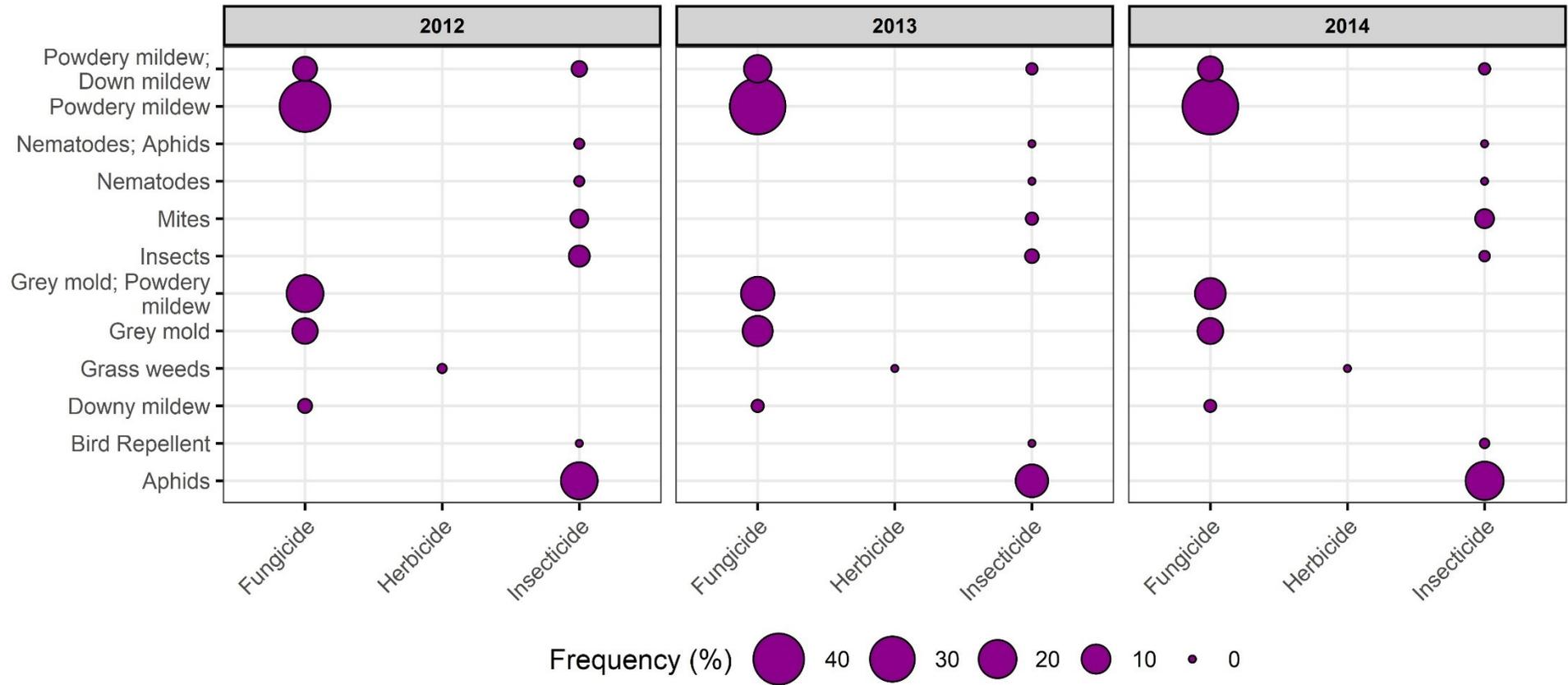


Figure SII - 3: Percentage of detected fungicides, insecticides and herbicides in grape samples by target group

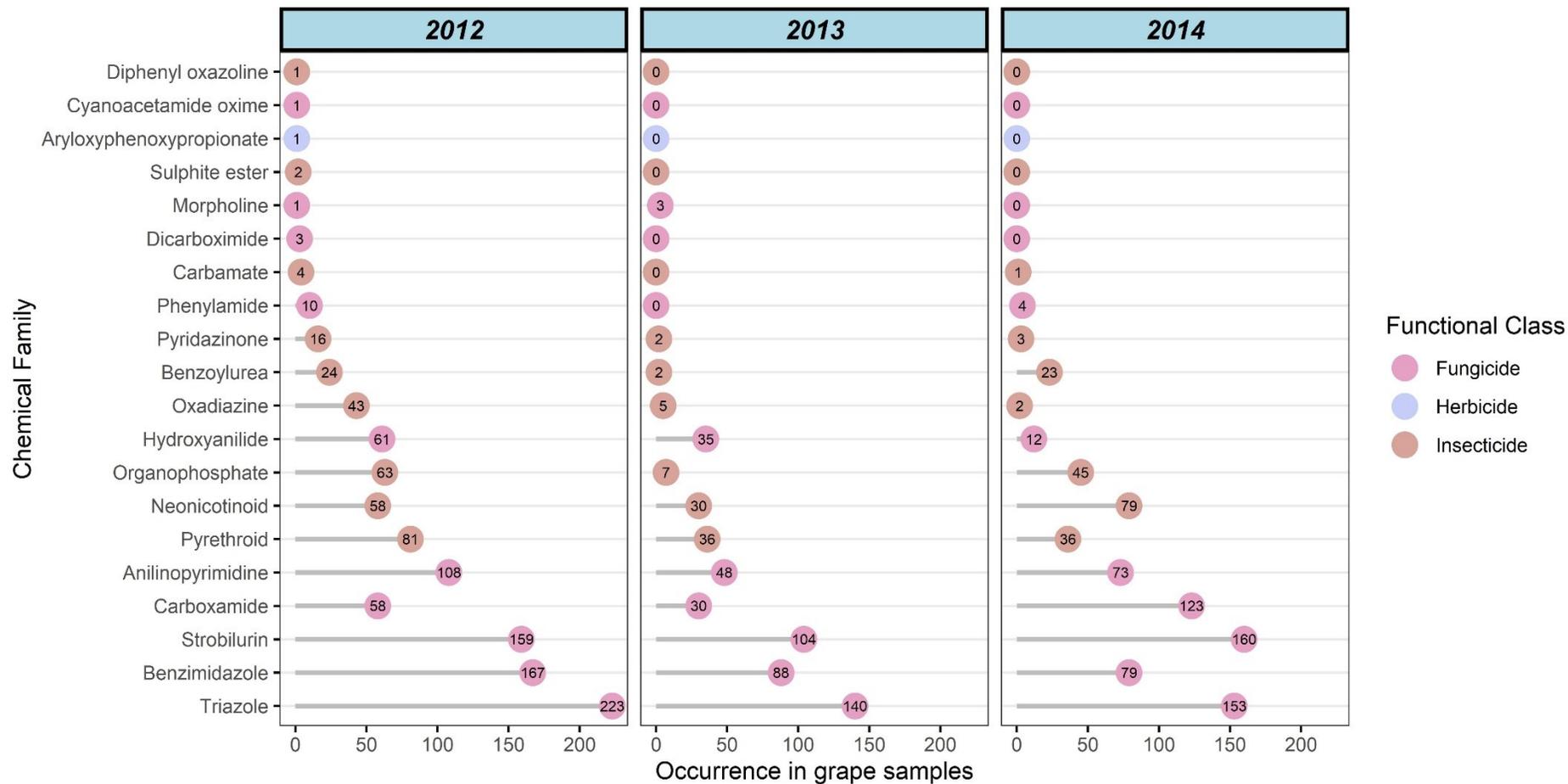


Figure SII - 4: Number of pesticide detected in grape samples in 2012, 2013 et 2014 by chemical family and functional class

Table SII- 4: Average pesticide levels (mg/kg) in grapes according to chemical groups in 2012, 2013 and 2014

| Chemical Group | Concentration in mg/kg \pm SD | | |
|--------------------------|---------------------------------|-----------------|-----------------|
| | 2012 | 2013 | 2014 |
| Anilinopyrimidine | 0.40 \pm 0.30 | 0.16 \pm 0.16 | 0.31 \pm 0.37 |
| Benzimidazole | 0.76 \pm 0.66 | 0.68 \pm 1.15 | 0.55 \pm 1.35 |
| Carboxamide | 1.27 \pm 1.08 | 0.10 \pm 0.06 | 0.33 \pm 0.42 |
| Cyanoacetamide oxime | 0.01 | - | - |
| Dicarboximide | 0.01 \pm 0.00 | - | - |
| Hydroxylanilide | 0.64 \pm 0.54 | 0.48 \pm 0.66 | 0.46 \pm 0.58 |
| Morpholine | 0.04 | 0.03 \pm 0.01 | - |
| Phenylamide | 0.18 \pm 0.17 | - | 0.13 \pm 0.18 |
| Strobilurin | 0.32 \pm 0.33 | 0.17 \pm 0.33 | 0.12 \pm 0.13 |
| Triazole | 0.14 \pm 0.24 | 0.14 \pm 0.17 | 0.56 \pm 1.26 |
| Benzoylurea | 0.15 \pm 0.17 | 0.39 \pm 0.01 | 0.29 \pm 0.38 |
| Carbamate | 0.09 \pm 0.10 | - | 0.00 |
| Diphenyl oxazoline | 0.09 | - | - |
| Neonicotinoid | 0.34 \pm 0.30 | 0.26 \pm 0.26 | 0.14 \pm 0.12 |
| Organophosphate | 0.23 \pm 0.20 | 0.26 \pm 0.42 | 0.42 \pm 0.39 |
| Oxadiazine | 0.26 \pm 0.28 | 0.13 \pm 0.03 | 0.33 \pm 0.23 |
| Pyrethroid | 0.43 \pm 0.39 | 0.28 \pm 0.19 | 0.37 \pm 0.23 |
| Pyridazinone | 0.03 \pm 0.05 | 0.01 \pm 0.01 | 0.01 \pm 0.00 |
| Sulphite ester | 2.40 \pm 0.57 | - | - |
| Aryloxyphenoxypropionate | 0.01 | - | - |

**SD : Standard Deviation*

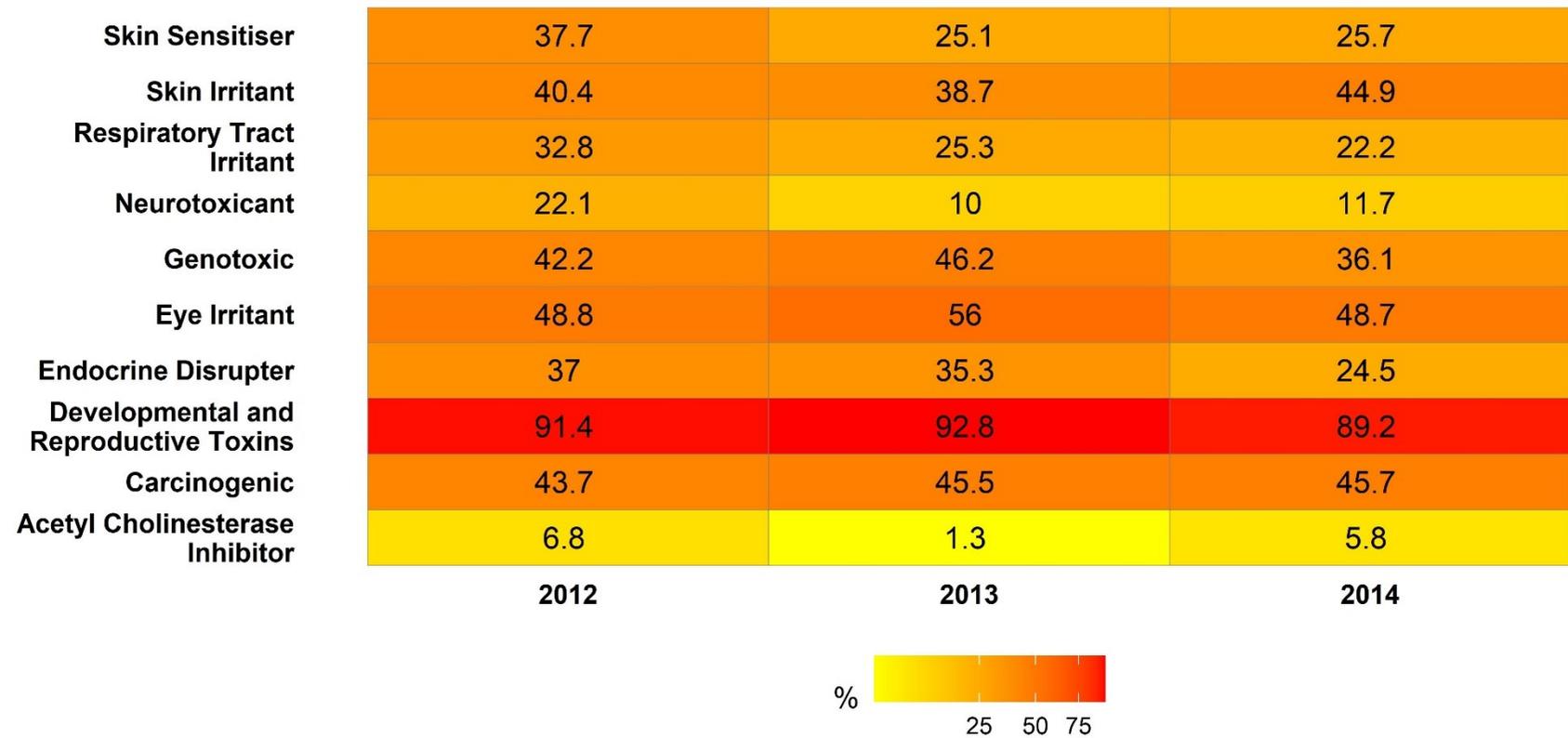


Figure SII - 5: Percentage of the pesticide detected in grapes in 2012, 2013 and 2014 according to risk to human health

Table SII- 5: Occurrences, means, medians and MRL exceedances of pesticides detected in grapes for each region in 2012, 2013, and 2014

| Molecule | BEKAA REGION | | | | | | | | | | | | | | |
|---------------------|--------------|---------------|----------------|--------|-------|------|--------------|----------------|--------|-------|------|-------------|---------------|--------|-------|
| | 2012 | | | | | 2013 | | | | | 2014 | | | | |
| | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL |
| Acetamiprid | 22 | 0.026-0.587 | 0.288 (±0.235) | 0.099 | 10 | 13 | 0.012-0.747 | 0.177 (±0.283) | 0.023 | 3 | 51 | 0.01-0.491 | 0.132±(0.123) | 0.113 | 0 |
| Azoxystrobin | 74 | 0.02-1.109 | 0.445 (±0.328) | 0.326 | 0 | 28 | 0.018-2.51 | 0.286 (±0.516) | 0.082 | 1 | 92 | 0.01-0.92 | 0.106±(0.115) | 0.066 | 0 |
| Boscalid | 55 | 0.04-3.167 | 1.332 (±1.08) | 1.377 | 0 | 13 | 0.021-0.146 | 0.09 (±0.046) | 0.094 | 0 | 98 | 0.036-3.3 | 0.322±(0.425) | 0.196 | 0 |
| Carbendazim | 40 | 0.05-3.1 | 0.937 (±0.753) | 0.770 | 1 | 29 | 0.02-1.4 | 0.366 (±0.349) | 0.294 | 0 | 30 | 0.016-1.23 | 0.238±(0.274) | 0.108 | 0 |
| Carbofuran | 2 | 0.007-0.009 | 0.008 (±0.001) | 0.008 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Chlorpyrifos | 36 | 0.006-0.746 | 0.238 (±0.207) | 0.189 | 3 | 4 | 0.049-1.2 | 0.356 (±0.563) | 0.088 | 1 | 36 | 0.04-2.37 | 0.461±(0.42) | 0.333 | 12 |
| Chlorpyrifos-methyl | 1 | 0.142-0.142 | 0.142 (±0) | 0.142 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cymoxanil | 1 | 0.0089-0.0089 | 0.009 (±0) | 0.009 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cypermethrin | 66 | 0.005-1.09 | 0.394 (±0.355) | 0.285 | 39 | 13 | 0.04-1 | 0.326 (±0.254) | 0.262 | 10 | 29 | 0.039-1.09 | 0.355±(0.221) | 0.271 | 28 |
| Cyproconazole | 8 | 0.0012-0.253 | 0.084 (±0.11) | 0.009 | 3 | 4 | 0.007-0.009 | 0.008 (±0.001) | 0.008 | 0 | ND | ND | ND | ND | ND |
| Cyprodinil | 79 | 0.028-0.875 | 0.379 (±0.269) | 0.345 | 0 | 20 | 0.009-0.875 | 0.182 (±0.22) | 0.100 | 0 | 56 | 0.027-2.46 | 0.275±(0.368) | 0.132 | 0 |
| Deltamethrin | 4 | 0.1-0.246 | 0.164 (±0.062) | 0.155 | 1 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Difenoconazole | 67 | 0.012-0.214 | 0.065 (±0.047) | 0.057 | 9 | 28 | 0.0074-0.772 | 0.122 (±0.172) | 0.058 | 10 | 37 | 0.013-6.143 | 1.396±(1.938) | 0.056 | 13 |
| Diflubenzuron | 1 | 0.008-0.008 | 0.008 (±0) | 0.008 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Dimethoate | 1 | 0.165- | 0.165 (±0) | 0.165 | 1 | 1 | 0.004- | 0.004 (±0) | 0.004 | 0 | ND | ND | ND | ND | ND |

| | | | | | | | | | | | | | | | |
|--------------------|----|---------------|----------------|-------|----|----|--------------|----------------|-------|----|----|---------------|---------------|-------|----|
| | | 0.165 | | | | | 0.004 | | | | | | | | |
| Dimethomorph | 1 | 0.04-0.04 | 0.04 (±0) | 0.040 | 0 | 3 | 0.016-0.04 | 0.025 (±0.013) | 0.020 | 0 | ND | ND | ND | ND | ND |
| Etoazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fenhexamid | 40 | 0.03-1.8 | 0.492 (±0.486) | 0.348 | 0 | 19 | 0.04-2.4 | 0.445 (±0.601) | 0.220 | 0 | 10 | 0.035-1.89 | 0.504±(0.634) | 0.112 | 0 |
| Fluazifop-butyl | 1 | 0.009-0.009 | 0.009 (±0) | 0.009 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flufenoxuron | 4 | 0.141-0.543 | 0.269 (±0.185) | 0.196 | 4 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flusilazole | 12 | 0.023-1.291 | 0.202 (±0.354) | 0.083 | 3 | 4 | 0.018-0.291 | 0.105 (±0.125) | 0.056 | 1 | ND | ND | ND | ND | ND |
| Hexaconazole | 24 | 0.0013-0.0098 | 0.006 (±0.003) | 0.007 | 0 | 5 | 0.005-0.0095 | 0.007 (±0.002) | 0.007 | 0 | 2 | 0.0088-0.13 | 0.069±(0.086) | 0.069 | 1 |
| Imidacloprid | 20 | 0.031-1.29 | 0.46 (±0.364) | 0.329 | 1 | 1 | 0.276-0.276 | 0.276 (±0) | 0.276 | 0 | 5 | 0.115-0.251 | 0.204±(0.056) | 0.217 | 0 |
| Indoxacarb | 40 | 0.02-0.979 | 0.244 (±0.272) | 0.099 | 0 | 2 | 0.141-0.159 | 0.15 (±0.013) | 0.150 | 0 | 2 | 0.169-0.498 | 0.334±(0.233) | 0.334 | 0 |
| Kresoxim-methyl | 1 | 0.652-0.652 | 0.652 (±0) | 0.652 | 0 | 3 | 0.3-1.32 | 0.693 (±0.549) | 0.459 | 1 | 2 | 0.289-0.721 | 0.505±(0.305) | 0.505 | 0 |
| Lambda-Cyhalothrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Lufenuron | 18 | 0.0066-0.404 | 0.13 (±0.167) | 0.008 | 7 | ND | ND | ND | ND | ND | 16 | 0.0033-0.949 | 0.172±(0.292) | 0.009 | 6 |
| Metalaxyl | 6 | 0.02-0.611 | 0.198 (±0.209) | 0.148 | 0 | ND | ND | ND | ND | ND | 4 | 0.036-0.4 | 0.128±(0.182) | 0.038 | 0 |
| Metalaxyl-M | 2 | 0.12-0.237 | 0.179 (±0.083) | 0.179 | 2 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methamidophos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methiocarb | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.0042-0.0042 | 0.004±(0) | 0.004 | 0 |
| Methomyl | 2 | 0.164-0.194 | 0.179 (±0.021) | 0.179 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Myclobutanil | 38 | 0.02-0.945 | 0.319 (±0.301) | 0.187 | 0 | 44 | 0.02-0.985 | 0.218 (±0.215) | 0.176 | 0 | 18 | 0.056-0.531 | 0.24±(0.128) | 0.199 | 0 |
| Penconazole | 11 | 0.01-0.187 | 0.1 (±0.061) | 0.092 | 0 | 5 | 0.017-0.153 | 0.052 (±0.057) | 0.034 | 0 | 7 | 0.0315-0.187 | 0.096±(0.056) | 0.094 | 0 |
| Pirimiphos-methyl | 1 | 0.003-0.003 | 0.003 (±0) | 0.003 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Procyimdone | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

| | | | | | | | | | | | | | | | |
|--------------------|----|------------------|-------------------|-------|----|----|-------------------|----------------|-------|----|----|-------------------|---------------|-------|----|
| Propargite | 2 | 2-2.8 | 2.4 (±0.566) | 2.400 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Propiconazole | ND | ND | ND | ND | ND | 2 | 0.0016- 0.0016 | 0.002 (±0) | 0.002 | 0 | 4 | 0.0037- 0.0066 | 0.005±(0.001) | 0.005 | 0 |
| Pyraclostrobin | 15 | 0.022- 1.083 | 0.249 (±0.342) | 0.105 | 0 | ND | ND | ND | ND | ND | 8 | 0.022- 0.158 | 0.087±(0.049) | 0.074 | 0 |
| Pyridaben | 9 | 0.0016- 0.137 | 0.02 (±0.044) | 0.005 | 1 | ND | ND | ND | ND | ND | 1 | 0.01- 0.01 | 0.01±(0) | 0.010 | 0 |
| Pyrimethanil | 5 | 0.059- 1.19 | 0.423 (±0.485) | 0.195 | 0 | 2 | 0.09- 0.099 | 0.095 (±0.006) | 0.095 | 0 | 2 | 0.113- 0.24 | 0.177±(0.09) | 0.177 | 0 |
| Tebuconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Tetraconazole | 13 | 0.0042- 0.79 | 0.188 (±0.211) | 0.135 | 9 | 3 | 0.007- 0.4 | 0.194 (±0.197) | 0.176 | 2 | 46 | 0.0015- 0.79 | 0.088±(0.157) | 0.008 | 15 |
| Thiamethoxam | ND | ND | ND | ND | ND | 1 | 0.464- 0.464 | 0.464 (±0) | 0.464 | 1 | ND | ND | ND | ND | ND |
| Thiophanate-methyl | 46 | 0.0033- 1.92 | 0.759 (±0.654) | 0.637 | 35 | 28 | 0.0023- 7.2 | 1.359 (±1.707) | 0.811 | 0 | 16 | 0.0046- 11.2 | 1.095±(2.799) | 0.155 | 10 |
| Tolclofos-methyl | 7 | 0.0061- 1.62 | 0.517 (±0.656) | 0.150 | 5 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Triadimenol | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Trifloxystrobin | 35 | 0.01- 0.067 | 0.042 (±0.016) | 0.042 | 0 | 27 | 0.0077- 0.799 | 0.07 (±0.151) | 0.027 | 22 | 19 | 0.01- 0.49 | 0.098±(0.12) | 0.042 | 0 |

NORTH REGION

| Molecule | 2012 | | | | | 2013 | | | | | 2014 | | | | |
|---------------------|------|-----------------|-------------------|--------|-------|------|-----------------|----------------|--------|-------|------|-----------------|---------------|--------|-------|
| | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL |
| Acetamiprid | 12 | 0.022- 0.563 | 0.254 (±0.249) | 0.081 | 5 | 13 | 0.042- 0.637 | 0.311 (±0.265) | 0.100 | 6 | 18 | 0.02- 0.395 | 0.154±(0.126) | 0.150 | 0 |
| Azoxystrobin | 20 | 0.0158- 1 | 0.515 (±0.341) | 0.533 | 0 | 29 | 0.018- 0.68 | 0.109 (±0.137) | 0.056 | 0 | 15 | 0.015- 0.45 | 0.173±(0.146) | 0.130 | 0 |
| Boscalid | 2 | 0.06- 0.356 | 0.208 (±0.209) | 0.208 | 0 | 14 | 0.044- 0.317 | 0.107 (±0.072) | 0.086 | 0 | 15 | 0.056- 0.637 | 0.268±(0.178) | 0.250 | 0 |
| Carbendazim | 33 | 0.036- 1.955 | 0.966 (±0.546) | 1.021 | 0 | 22 | 0.03- 1.67 | 0.272 (±0.388) | 0.089 | 0 | 25 | 0.02- 0.959 | 0.311±(0.307) | 0.186 | 0 |
| Carbofuran | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Chlorpyrifos | 20 | 0.02- 0.73 | 0.252 (±0.196) | 0.217 | 2 | 1 | 0.235- 0.235 | 0.235 (±0) | 0.235 | 0 | 8 | 0.06- 0.63 | 0.269±(0.22) | 0.170 | 2 |
| Chlorpyrifos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cymoxanil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

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|--------------------|----|--------------|----------------|-------|----|----|--------------|----------------|-------|----|----|--------------|---------------|-------|----|
| Cypermethrin | 9 | 0.322-1.774 | 0.808 (±0.525) | 0.567 | 9 | 20 | 0.054-0.72 | 0.248 (±0.142) | 0.240 | 16 | 6 | 0.244-0.83 | 0.457±(0.264) | 0.326 | 6 |
| Cyproconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cyprodinil | 21 | 0.045-1.119 | 0.425 (±0.308) | 0.419 | 0 | 25 | 0.0427-0.526 | 0.154 (±0.105) | 0.129 | 0 | 8 | 0.071-1.19 | 0.412±(0.345) | 0.372 | 0 |
| Deltamethrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Difenoconazole | 18 | 0.015-0.6 | 0.145 (±0.16) | 0.084 | 6 | 20 | 0.01-0.375 | 0.109 (±0.097) | 0.094 | 6 | 9 | 0.014-3.41 | 0.783±(1.471) | 0.049 | 2 |
| Diflubenzuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Dimethoate | 3 | 0.006-0.128 | 0.047 (±0.07) | 0.007 | 1 | 1 | 0.165-0.165 | 0.165 (±0) | 0.165 | 1 | ND | ND | ND | ND | ND |
| Dimethomorph | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Etoxazole | 1 | 0.09-0.09 | 0.09 (±0) | 0.090 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fenhexamid | 21 | 0.06-1.975 | 0.926 (±0.538) | 0.985 | 0 | 16 | 0.03-2.25 | 0.527 (±0.736) | 0.187 | 0 | ND | ND | ND | ND | ND |
| Fluazifop-butyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flufenoxuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flusilazole | 1 | 0.04-0.04 | 0.04 (±0) | 0.040 | 0 | ND | ND | ND | ND | ND | 1 | 0.037-0.037 | 0.037±(0) | 0.037 | 0 |
| Hexaconazole | 13 | 0.0034-0.152 | 0.038 (±0.06) | 0.008 | 3 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Imidacloprid | 4 | 0.031-0.68 | 0.232 (±0.304) | 0.108 | 0 | 2 | 0.145-0.429 | 0.287 (±0.201) | 0.287 | 0 | 1 | 0.441-0.441 | 0.441±(0) | 0.441 | 0 |
| Indoxacarb | 3 | 0.078-0.84 | 0.428 (±0.385) | 0.365 | 0 | 3 | 0.088-0.127 | 0.112 (±0.021) | 0.120 | 0 | ND | ND | ND | ND | ND |
| Kresoxim-methyl | ND | ND | ND | ND | ND | 3 | 0.233-0.652 | 0.381 (±0.235) | 0.259 | 0 | 3 | 0.051-0.164 | 0.101±(0.058) | 0.088 | 0 |
| Lambda-Cyhalothrin | 2 | 0.36-0.4 | 0.38 (±0.028) | 0.380 | 2 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Lufenuron | 1 | 0.266-0.266 | 0.266 (±0) | 0.266 | 1 | 2 | 0.379-0.4 | 0.39 (±0.015) | 0.390 | 2 | 6 | 0.0085-0.769 | 0.433±(0.28) | 0.478 | 5 |
| Metalaxyl | 1 | 0.059-0.059 | 0.059 (±0) | 0.059 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Metalaxyl-M | 1 | 0.237-0.237 | 0.237 (±0) | 0.237 | 1 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methamidophos | 1 | 0.15-0.15 | 0.15 (±0) | 0.150 | 1 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methiocarb | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methomyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

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|---------------------|----------|----------------|-------------------|---------------|-----------------|-------------|----------------|-------------------|---------------|-----------------|-------------|----------------|-------------------|---------------|-----------------|
| Myclobutanil | 7 | 0.063-0.639 | 0.226 (±0.21) | 0.108 | 0 | 11 | 0.03-0.483 | 0.146 (±0.131) | 0.120 | 0 | 12 | 0.04-0.363 | 0.191±(0.088) | 0.189 | 0 |
| Penconazole | 7 | 0.059-0.172 | 0.11 (±0.048) | 0.098 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pirimiphos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Procymidone | 3 | 0.0029-0.0097 | 0.006 (±0.003) | 0.006 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Propargite | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Propiconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pyraclostrobin | 2 | 0.271-0.335 | 0.303 (±0.045) | 0.303 | 0 | ND | ND | ND | ND | ND | 3 | 0.023-0.08 | 0.051±(0.029) | 0.049 | 0 |
| Pyridaben | 7 | 0.0028-0.135 | 0.054 (±0.061) | 0.008 | 3 | 1 | 0.01-0.01 | 0.01 (±0) | 0.010 | 0 | 2 | 0.0087-0.01 | 0.009±(0.001) | 0.009 | 0 |
| Pyrimethanil | 2 | 0.94-1.04 | 0.99 (±0.071) | 0.990 | 0 | ND | ND | ND | ND | ND | 1 | 0.066-0.066 | 0.066±(0) | 0.066 | 0 |
| Tebuconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Tetraconazole | 2 | 0.0043-0.0079 | 0.006 (±0.003) | 0.006 | 0 | 9 | 0.0041-0.549 | 0.132 (±0.211) | 0.009 | 3 | 3 | 0.0023-0.13 | 0.082±(0.07) | 0.115 | 2 |
| Thiamectoxam | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Thiophanate-methyl | 41 | 0.0026-1.92 | 0.456 (±0.55) | 0.167 | 31 | 1 | 3.7-3.7 | 3.7 (±0) | 3.700 | 1 | 3 | 1.84-2.9 | 2.247±(0.571) | 2.000 | 3 |
| Tolclofos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Triadimenol | 2 | 0.7-2.1 | 1.4 (±0.99) | 1.400 | 1 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Trifloxystrobin | 10 | 0.012-0.063 | 0.039 (±0.016) | 0.038 | 0 | 12 | 0.015-0.2 | 0.058 (±0.056) | 0.037 | 0 | 11 | 0.016-0.4 | 0.115±(0.139) | 0.049 | 0 |
| SOUTH REGION | | | | | | | | | | | | | | | |
| 2012 | | | | | | 2013 | | | | | 2014 | | | | |
| Molecule | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL |
| Acetamiprid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 3 | 0.025-0.154 | 0.072 ±(0.072) | 0.036 | 0 |
| Azoxystrobin | 1 | 0.03-0.03 | 0.03(±0) | 0.03 | 0 | 2 | 0.0247-0.199 | 0.112 (±0.123) | 0.112 | 0 | 1 | 0.4-0.4 | 0.4 ±(0) | 0.400 | 0 |
| Boscalid | 1 | 0.066-0.066 | 0.066(±0) | 0.066 | 0 | 3 | 0.084-0.14 | 0.118 (±0.03) | 0.130 | 0 | 3 | 0.061-1.2 | 0.628 ±(0.57) | 0.623 | 0 |
| Carbendazim | ND | ND | ND | ND | ND | 6 | 0.03-0.28 | 0.181 (±0.109) | 0.212 | 0 | 3 | 0.278-1.38 | 0.836 ±(0.551) | 0.850 | 0 |
| Carbofuran | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

| | | | | | | | | | | | | | | | |
|---------------------|----|-------------|-----------|-------|----|----|-------------|----------------|-------|----|----|-------------|----------------|-------|----|
| Chlorpyrifos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Chlorpyrifos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cymoxanil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cypermethrin | ND | ND | ND | ND | ND | 3 | 0.25-0.392 | 0.301 (±0.079) | 0.260 | 3 | ND | ND | ND | ND | ND |
| Cyproconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cyprodinil | 1 | 0.071-0.071 | 0.071(±0) | 0.071 | 0 | 1 | 0.2-0.2 | 0.2 (±0) | 0.200 | 0 | 2 | 0.31-0.416 | 0.363 ±(0.075) | 0.363 | 0 |
| Deltamethrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Difenoconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 3.113-3.113 | 3.113 ±(0) | 3.113 | 1 |
| Diflubenzuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Dimethoate | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.134-0.134 | 0.134 ±(0) | 0.134 | 1 |
| Dimethomorph | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Etoazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fenhexamid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 2 | 0.175-0.335 | 0.255 ±(0.113) | 0.255 | 0 |
| Fluazifop-butyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flufenoxuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flusilazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.07-0.07 | 0.07 ±(0) | 0.070 | 0 |
| Hexaconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.156-0.156 | 0.156 ±(0) | 0.156 | 1 |
| Imidacloprid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Indoxacarb | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Kresoxim-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.383-0.383 | 0.383 ±(0) | 0.383 | 0 |
| Lambda-Cyhalothrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Lufenuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 1.36-1.36 | 1.36 ±(0) | 1.360 | 1 |
| Metalaxyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Metalaxyl-M | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methamidophos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methiocarb | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methomyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Myclobutanil | ND | ND | ND | ND | ND | 3 | 0.093-0.167 | 0.137 (±0.039) | 0.150 | 0 | 1 | 0.341-0.341 | 0.341 ±(0) | 0.341 | 0 |

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|-----------------------------|----------|----------------|-------------------|---------------|-----------------|-------------|----------------|-------------------|---------------|-----------------|-------------|----------------|-------------------|---------------|-----------------|
| Penconazole | ND | ND | ND | ND | ND | 1 | 0.123-0.123 | 0.123 (±0) | 0.123 | 0 | 1 | 0.035-0.035 | 0.035 ±(0) | 0.035 | 0 |
| Pirimiphos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Procymidone | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Propargite | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Propiconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pyraclostrobin | 1 | 0.043-0.043 | 0.043(±0) | 0.043 | 0 | ND | ND | ND | ND | ND | 1 | 0.081-0.081 | 0.081 ±(0) | 0.081 | 0 |
| Pyridaben | ND | ND | ND | ND | ND | 1 | 0.0028-0.0028 | 0.003 (±0) | 0.003 | 0 | ND | ND | ND | ND | ND |
| Pyrimethanil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 1.19-1.19 | 1.19 ±(0) | 1.190 | 0 |
| Tebuconazole | ND | ND | ND | ND | ND | 1 | 0.086-0.086 | 0.086 (±0) | 0.086 | 0 | ND | ND | ND | ND | ND |
| Tetraconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 2 | 0.004-0.008 | 0.006 ±(0.003) | 0.006 | 0 |
| Thiamethoxam | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Thiophanate-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.436-0.436 | 0.436 ±(0) | 0.436 | 1 |
| Tolclofos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Triadimenol | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Trifloxystrobin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 4 | 0.029-0.382 | 0.141 ±(0.163) | 0.077 | 0 |
| MOUNT-LEBANON REGION | | | | | | | | | | | | | | | |
| 2012 | | | | | | 2013 | | | | | 2014 | | | | |
| Molecule | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL | N | Min-Max | Mean (±SD) | Median | N>MRL |
| Acetamiprid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.049-0.049 | 0.049 ±(0) | 0.049 | 0 |
| Azoxystrobin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Boscalid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 7 | 0.099-1.73 | 0.421 ±(0.587) | 0.210 | 0 |
| Carbendazim | ND | ND | ND | ND | ND | 2 | 0.200-0.400 | 0.300(±0.141) | 0.300 | 0 | ND | ND | ND | ND | ND |
| Carbofuran | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Chlorpyrifos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Chlorpyrifos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cymoxanil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

| | | | | | | | | | | | | | | | |
|--------------------|----|----|----|----|----|----|----|----|----|----|----|-------------|----------------|-------|----|
| Cypermethrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.3-0.3 | 0.3 ±(0) | 0.300 | 1 |
| Cyproconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cyprodinil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 3 | 0.155-0.908 | 0.468 ±(0.392) | 0.341 | 0 |
| Deltamethrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Difenoconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 3 | 3.161-4.214 | 3.834 ±(0.584) | 4.127 | 3 |
| Diflubenzuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Dimethoate | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Dimethomorph | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Etoxazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fenhexamid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fluazifop-butyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flufenoxuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Flusilazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Hexaconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Imidacloprid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Indoxacarb | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Kresoxim-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Lambda-Cyhalothrin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Lufenuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Metalaxyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Metalaxyl-M | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methamidophos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methiocarb | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Methomyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Myclobutanil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 0.264-0.264 | 0.264 ±(0) | 0.264 | 0 |
| Penconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pirimiphos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Procyimidone | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Propargite | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Propiconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pyraclostrobin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pyridaben | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pyrimethanil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Tebuconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Tetraconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 3 | 0.0032- | 0.005 ±(0.002) | 0.005 | 0 |

| | | | | | | | | | | | | | | | |
|--|----|----|----|----|----|----|----|----|----|----|----|-----------|-----------|-------|----|
| | | | | | | | | | | | | 0.0072 | | | |
| Thiamethoxam | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Thiophanate-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 1 | 1.21-1.21 | 1.21 ±(0) | 1.210 | 1 |
| Tolclofos-methyl | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Triadimenol | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Trifloxystrobin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| ** the max-min, mean and median are in mg/kg; ND: Not Detected; N: number of samples with the molecule; N>MRL: number of samples where the molecule exceeded its MRL; SD: Standard Deviation | | | | | | | | | | | | | | | |

Annexe II-1 : L'article : "Majed L., Hayar S., Zeitoun R., Maestroni BM & Dousset S. 2022. The effects of formulation on imidacloprid dissipation in grapes and vine leaves and on required pre-harvest intervals under Lebanese climatic conditions. *Molecules*, 27, 252.

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Article

The Effects of Formulation on Imidacloprid Dissipation in Grapes and Vine Leaves and on Required Pre-Harvest Intervals under Lebanese Climatic Conditions

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Abstract: In this study, imidacloprid, a systemic insecticide, currently having a specified European Commission MRL value for vine leaves (2 mg kg⁻¹), was applied on a Lebanese vineyard under different commercial formulations: as a soluble liquid (SL) and water dispersible granules (WDG). In Lebanon, many commercial formulations of imidacloprid are subject to the same critical good agricultural practice (cGAP). It was, therefore, important to verify the variability in dissipation patterns according to matrix nature and formulation type. Random samplings of grapes and vine leaves were performed starting at 2 days until 18 days after treatment. Residue extractions were performed according to the QuEChERS method and the analytical determination using liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS). The SL formulation yielded significantly higher initial deposit than the WDG formulation on grapes and vine leaves. The formulation type did not significantly affect the dissipation rates; the estimated half-lives in grapes and vine leaves were 0.5 days for all imidacloprid formulations. No pre-harvest intervals were necessary on grapes. PHIs of 3.7 days for the SL formulation and 2.8 days for the WDG formulation were estimated on vine leaves. The results showed that the type of formulation and the morphological and physiological characteristics of the matrix had an effect on the initial deposits, and thus residue levels, but not on the dissipation patterns.

Keywords: imidacloprid; vine leaves; grape; QuEChERS; SL and WDG formulation; dissipation; half-life; pre-harvest intervals

1. Introduction

Grapevines are cultivated all over the world, yielding a wide range of products that are part of our daily diet. Grapes, the most economically important product, can be used to make juice, jellies, wine, and pies, and the leaves can be used in cooking [1–3]. In Lebanon and nearby countries, vine leaves are commonly used in the preparation of several traditional dishes, especially the famous dishes in "Lebanese Mezze". As with any other plant, grapevines are vulnerable to fungal and pest infestation and thus the use of

phytosanitary products may be unavoidable in order to prevent and control any occurring disease to increase the yield [1,3].

However, the use of these products may be harmful to final consumers, since they could be exposed to residues of phytosanitary molecules through their daily diet [4]. To overcome this challenge and make good use of pesticides without compromising human and environmental health, national and international bodies, mainly the European Commission (EC) and Codex Alimentarius, have specified legal limits for residues in food, i.e., maximum residues limits (MRLs).

Ensuring that residues are below MRLs is of high importance for producers to meet regulatory and market requirements. Yet such a goal may be impossible to attain for some crops due to lack of specific MRLs. This is the case for vine leaves, for which no specified legal limits for pesticide residue levels have been set by national and international organizations; as a consequence, an MRL that corresponds to the limit of detection (LOD) of the analytical method for the molecules applied on grapevines is assigned for this commodity, i.e., at the European Union level [5–9].

In the field, MRLs are the benchmark against which it is possible to set the preharvest interval values (PHI). PHI corresponds to the time gap between pesticide applications and the crop harvest in order to yield a healthy product that is in compliance with the legal limit [3]. Many studies concluded that the molecules' physicochemical properties, the formulation properties, the local climatic conditions, and the plant physiology could affect the main two parameters used in pesticide residues studies, i.e., dissipation rates and PHIs [3,10–12]. That is why any possible factor affecting these two previously mentioned parameters must be investigated in order to identify the different variables involved and to gain a better understanding of their interactions.

Formulating a pesticide is about combining an active ingredient with compatible "inerts" or "inactive ingredients". Inerts are present to achieve specific results; they can be emulsifiers, petroleum solvents, wetting agents or UV-light blocking chemicals, etc., that increase the persistence of active ingredients and enhance their application and performance [13]. Throughout the industry, pesticide products are marketed as emulsifiable concentrates (EC), microencapsulated formulations (ME), flowable (F), water dispersible granules (WDG), sprayable (S), wettable powders (WP), among others. The type and amount of inert ingredients give the phytosanitary product its uniqueness and thus allow distinction between phytosanitary product lines and markets. Therefore, when selecting which formulation to use, farmers must take into consideration the potential influence of formulations on pesticide efficacy, and more importantly, their potential impact on residues level in crops [14], which is one of the biggest concerns for producers. In fact, non-conclusive results can be found throughout the literature pertaining to the effect of formulation type on residues dissipation. Cabras et al. [15] stated that liquid formulations yield more residues compared to granulated ones and Abdel-Hamid et al. [11] correlated the initial deposit of pesticides on tomato fruits to the variation of physical and chemical properties among pesticide formulations. They demonstrated that EC formulations of fenpyroximate showed higher persistence compared to suspension concentrate (SC) formulations on tomato fruits, likewise for imidacloprid, where they compared four different formulations (SC, WDG, SL and WP) and found lower initial deposits with higher degradation rates for the SC formulation compared to the others [11]. Buzzetti [16] also demonstrated that the pesticide formulation of acetamiprid, imidacloprid and diazinon had an effect on the initial pesticide deposit and persistence on apple samples, but not in the case of l-cyhalothrin. Montemurro et al. [17] compared three different formulations of chlorpyrifos and showed different dissipation rates for EC and WG formulations as compared to ME formulation in orange fruits; however, surprisingly, they observed a similar behavior for the three formulations (EC, WG and ME) in orange leaves and soil. In contrast, after conducting four comparative dissipation studies of three commercial formulations of penconazole 10% EC on four varieties of tomatoes, Abou Zeid et al. [18] concluded that there was no statistically significant difference in rate of dissipation among the three evaluated EC formulations.

Similarly, Alister et al. [12] concluded that formulation type (SC, SL and WP) did not have a significant effect on initial deposit and dissipation rates of acetamiprid, buprofazine and fenhexamid on apple fruits and grape berries.

Imidacloprid, 1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine, a predominantly systemic insecticide, is extensively used for the control of a wide range of insects and pests at various stages of grape cultivation especially thrips and mealybug [19]. It is important to note that imidacloprid is no longer approved for use by the European Commission since 1 December 2020, according to EU resolution EU/2020/1643 [20]. Starting June 2022 import tolerances will be applied, and the applicable MRLs will be 0.7 mg kg^{-1} and 0.01 mg kg^{-1} for grapes and vine leaves respectively; the latter value corresponds to the lower limit of analytical determination for vine leaves [20]. These new MRLs will be replacing the currently approved MRLs of 1 mg kg^{-1} and 2 mg kg^{-1} for grapes and vine leaves, respectively (Reg. (EU) No 491/2014) [21]. As stated by the EU pesticide data base, these modifications were not implemented due to toxicological concerns, but rather due to unavailability of data. In Lebanon, 16 commercial formulation products, registered under different trade names, contain imidacloprid as the main active ingredient. They are subject to the same critical good agricultural practice (cGAP), that is, the same PHIs and the same application rates, despite the fact they differ in the composition of co-formulants [18]. It was, therefore, important to study the variability of the imidacloprid formulation type as soluble liquid concentrate (SL) or as water dispersible granules (WDG) on the dissipation rates and the PHIs on grapes and vine leaves under Lebanese climatic conditions.

2. Results and Discussion

2.1. SL-and WDG-Imidacloprid Dissipation Kinetics

The statistical analysis of imidacloprid residues concentration data showed that the first order decay model according to Equation (1) was a useful approximation of the data up to day 12 (Figure 1a). The data relative to day 18 data did not fit the first order decay model. Therefore, a two-compartment model was formulated; however, there were insufficient data to establish the point of change from the first to the second compartment as well as the rate of decline in the second compartment. Therefore, an alternative statistical model, called the continuous change model, was proposed to fit the data. In this model, the half-life is steadily increases with time. The rate of imidacloprid dissipation after day 12 is very slow, but still occurring according to the model. Such a model was considered satisfactory (Figure 1b), see also Figures S1 and S2 and Tables S1 and S2 in the Supplementary Materials. In this model the slopes of the fitted lines did not differ significantly from each other. The pooled slope of the regression lines (corresponding to the K_{diss}) was -1.269 ± 0.068 . Significant differences were found for the regression intercepts as shown in Table 1.

2.2. Matrix and Residue Levels

Regardless the formulation type, imidacloprid residues were found to be 20 to 70 times higher in vine leaves than in grapes at all sampling times for all of the analyzed samples (Table 2). The literature relates these finding to the morphological and physiological differences between vine leaves and grapes and to the fact that grapes are covered by the leaves i.e., greater contact surface.

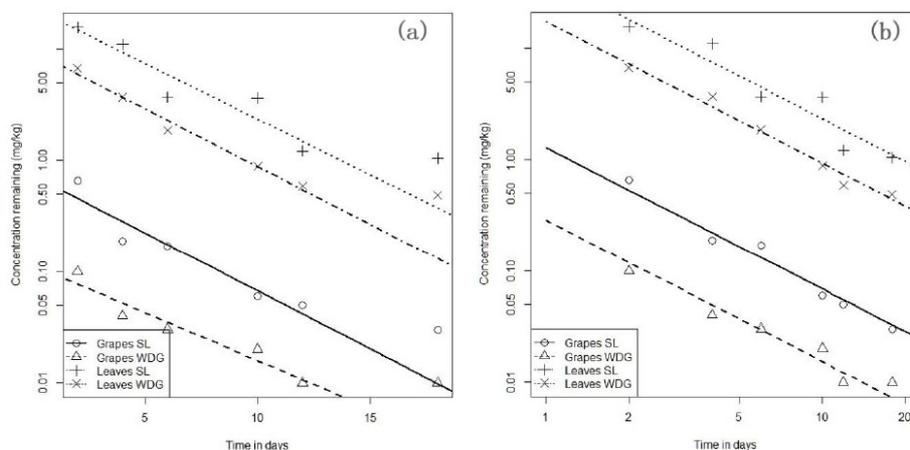


Figure 1. (a) Plot of residual Imidacloprid on a linear-log scale using a first order decay model. (b) Continuous change model of imidacloprid degradation with pooled slope but individual intercepts.

Table 1. Regression equations, dissipation rates, half-life and estimated PHI according to European Union 2021 MRLs (EU 2021) and to European Union 2022 MRLs (EU 2022) for grapes and vine leaves.

| Grapes | | | | | | | | |
|--------------------------|------------------------|--------------------|---------------|-------------------------|------------|---------|----------------------------|---------|
| Imidacloprid Formulation | Regression Equation | Slope (k) | Intercept (b) | DT ₅₀ (Days) | PHI (Days) | | MRL (mg kg ⁻¹) | |
| | | | | | EU 2021 | EU 2022 | EU 2021 | EU 2022 |
| SL | $y = 1.28e^{-1.269x}$ | -1.269 (±0.068) | 0.249 | 0.546 | 0.196 | 0.477 | 1 | 0.7 |
| WDG | $y = 0.29e^{-1.269x}$ | -1.269 (±0.068) | -1.249 | 0.546 | -0.984 | -0.703 | 1 | 0.7 |
| Vine Leaves | | | | | | | | |
| Imidacloprid Formulation | Regression Equation | Slope (k) | Intercept (b) | DT ₅₀ (Days) | PHI (Days) | | MRL (mg kg ⁻¹) | |
| | | | | | EU 2021 | EU 2022 | EU 2021 | EU 2022 |
| SL | $y = 43.55e^{-1.269x}$ | -1.269 (±0.068) | 3.774 | 0.546 | 2.428 | 6.603 | 2 | 0.01 * |
| WDG | $y = 17.37e^{-1.269x}$ | -1.269 (±0.068) | 2.855 | 0.546 | 1.704 | 5.879 | 2 | 0.01 * |

* Indicates the lower limit of detection.

According to Edwards [22], the distribution, retention and ab/adsorption of pesticides in/on plant tissues are greatly influenced by plant morphological and physiological characteristics. In addition, Maclachlan and Hamilton [10] stated that complex factors dictate the quantity of pesticide initially deposited and retained on leaves, i.e., their nature, the phytosanitary molecules' proprieties and abiotic factors such as wind speed, temperature and humidity. Maclachlan and Hamilton [10] also underlined the importance to take canopy density and crop leaf surface into consideration when it comes to spray deposits, given that the canopy acts as a filter of spray droplets and thus deeper parts of the plant far from spray nozzle may receive less spray.

Table 2. Residues of imidacloprid (SL and WDG) in grapes and vine leaves ($n = 5$).

| Imidacloprid Formulation | Grapes | | | | | |
|--------------------------|---|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Mean Concentration (\pm SD) in mg kg ⁻¹ | | | | | |
| | T ₂ | T ₄ | T ₆ | T ₁₀ | T ₁₂ | T ₁₈ |
| SL * | 0.66 (\pm 0.031) ^a (0) ^b | 0.19 (\pm 0.020) (64.1) | 0.17 (\pm 0.012) (67.9) | 0.06 (\pm 0.003) (88.7) | 0.05 (\pm 0.006) (90.5) | 0.03 (\pm 0.008) (94.3) |
| WDG ** | 0.10 (\pm 0.004) (0) | 0.04 (\pm 0.008) (50) | 0.03 (\pm 0.001) (62.5) | 0.02 (\pm 0.018) (75) | 0.01 (\pm 0.001) (87.5) | 0.01 (\pm 0.003) (87.5) |
| Imidacloprid Formulation | Vine Leaves | | | | | |
| | Mean Concentration (\pm SD) in mg kg ⁻¹ | | | | | |
| | T ₂ | T ₄ | T ₆ | T ₁₀ | T ₁₂ | T ₁₈ |
| SL | 15.60 (\pm 0.960) (8.77) | 11.00 (\pm 0.780) (35.7) | 3.69 (\pm 0.510) (78.4) | 3.64 (\pm 0.501) (78.7) | 1.22 (\pm 0.300) (92.8) | 1.05 (\pm 0.230) (93.8) |
| WDG | 6.71 (\pm 0.148) (0) | 3.68 (\pm 0.580) (42.5) | 1.87 (\pm 0.019) (70.8) | 0.89 (\pm 0.090) (86.1) | 0.59 (\pm 0.210) (90.8) | 0.49 (\pm 0.111) (92.3) |

^a Mean \pm standard deviation of five replications. ^b Figures in parentheses indicate cumulative % dissipation through time. * SL: soluble liquid ** WDG: water dispersible granules.

Furthermore, Lichiheb et al. [23] and Fernández and Eichert [24] mentioned that leaf cuticle (permeability of leaf surface) and pesticide lipophilicity are two of the main factors influencing pesticide penetration in plants. Possingham et al. [25] studied wax structure and composition of leaves and fruit of *Vitis vinifera* and found a “considerable qualitative difference between the waxes of leaves and fruits”, where grapes’ cuticular wax consisted of a “hard” wax component (70%); i.e., oleanolic acid; and a “soft” wax component, i.e., mixture of long chain acid, alcohols, aldehydes, ester and hydrocarbons; meanwhile, leaves had only the “soft” fraction.

Since diffusion is the main process for insecticide penetration [26], cuticular waxes affect that process by reducing solutes mobility [27] and pesticide transfer is driven by its lipophilicity and concentration [3]. It is thus harder for molecules with low Kow ($\log P = 0.57$) and high water solubility (610 mg kg^{-1}), such as imidacloprid [28], to move through grapes’ than through leaves’ cuticular waxes, which explains higher residues found in leaves compared to grapes regardless of leaves’ density and vines’ conducting system (pergola).

Hence, our results underline the impact of plants’ nature and morphology on the amount and distribution of residues across plant parts and are in agreement with results obtained by Alister et al. [12], Bletsou et al. [29], Abdallah [30] and Hanafi et al. [31]. Bletsou et al. [29] showed the effect of leaf density, where they used higher application rates of bifenthrin in beans (2.9 kg ha^{-1}) than in peas (2.2 kg ha^{-1}), and found 2.5 times less initial deposit on green beans compared to peas. This result was related to morphological structure differences as green beans did not receive most of the spraying solution due to coverage by their leaves, while pea pods, having smaller leaves, were almost totally exposed to spraying [29].

As in our paper, Abdallah [30] also found higher residues of chlorfenapyr and difenconazole in vine leaves compared to grapes. Cuticular wax chemistry and structural arrangement, which influence pesticide penetration [26,32], change according to fruit type and growth stage. Alister et al. [12] endorsed the effect of cuticular wax on pesticide penetration, where they concluded that fruit growth stage was the predominant parameter affecting pesticide initial deposit and dissipation rate, and that the effects of environmental parameters, such as rain, are important to consider; however, ultimately, it is the fruit type that determines the amount of pesticide penetration. Finally, Hanafi et al. [31] used the same application rate of imidacloprid (0.625 kg ha^{-1}) and oxamyl (1.8 kg ha^{-1}) on green beans and chili peppers and found residue level for both molecules higher in green

beans compared to chili peppers; similarly, they attributed these findings to morphological characteristic of each plant and to the so-called “dilution-effect” related to the growth stage.

2.3. Formulation and Residues Level

As shown in Table 2, despite a lower application amount per unit area (0.07 kg ha^{-1} for SL and 0.21 kg ha^{-1} for WDG), higher initial residues levels were found in vine leaves and grapes treated with SL- imidacloprid compared to WDG- imidacloprid. The finding that the SL formulation yielded more residues than the WDG formulation is in accordance with the results obtained by Buzzetti [16], where, in her work on apples, higher residue levels, initial and final deposits, of imidacloprid were found when applied as SL formulation (initial: 1.20 mg kg^{-1} , final: 0.47 mg kg^{-1}) compared to WP (initial: 0.90 mg kg^{-1} , final: 0.30 mg kg^{-1}) and soluble concentrate (SC) (initial: 0.89 mg kg^{-1} , final: 0.29 mg kg^{-1}) formulations. The author inferred that the variations of the ratio and nature of the other components of the formulated product (adjuvants, surfactant, inerts . . .) were behind the variation of the level of residues detected between SL, WP and SC formulations, despite the fact that all the treatments were performed in a way to obtain the same dose of active ingredient per hectare [16]. Moreover, Buzzetti [16] explained the similarity of the level of residues of the WP and SC formulations to be due to the fact that both have in common that they form suspensions on water compared to the SL formulation that forms a solution. This is a similar situation as the study described in this paper where a SL formulation and another suspension forming formulation (WDG) are compared.

Abdel-Hamid et al. [11] also reported a great influence of the formulation type on the residue level, more precisely on the initial deposits, when comparing 4 different formulations (SL, WDG, SC and WP) of imidacloprid in their two consecutive year study (2009 and 2010). However, and contrary to Buzzetti [16] and with the results presented in this study, among the four formulations they studied, they reported higher residues level in tomatoes for imidacloprid WDG formulation (initial: 4.55 mg kg^{-1} in 2009 and 3.68 mg kg^{-1} in 2010, final: 0.51 mg kg^{-1} in 2009 and 0.30 mg kg^{-1} in 2010) compared to the SL formulation (initial: 3.11 mg kg^{-1} in 2009 and 2.49 mg kg^{-1} in 2010, final: 0.05 mg kg^{-1} in 2009 and below the detection limit in 2010) [11]. Taken together, these findings support the hypothesis that the formulation type has an impact on the level of residues on vine leaves and grapes according to the Food and Agriculture Organization (FAO) statement: “While having the same concentration of an active ingredient two products are not considered similar if they have different formulations or have different synthesizing methods” [16].

The dissipation rates (k) of SL and WDG formulations are shown in Table 1. Despite the previously discussed higher initial deposits of the SL formulation compared to WDG's, and the higher residues found on vine leaves than on grapes, the two formulations followed the same dissipation patterns and had quite similar dissipation rates of 1.269 day^{-1} on grapes and vine leaves, leading to similar half-lives of 0.5 day for the two formulations.

Pre-harvest intervals were estimated according to the MRLs set by the European Commission on vine leaves and grapes (Table 1). For grapes, no PHIs were necessary since all estimated PHIs values were less than one day for the two formulations, which could be related to the aforementioned low initial deposits of imidacloprid on grapes. In the case of vine leaves, when using the currently applicable EU MRL (2 mg kg^{-1}) for the calculations, the calculated PHIs were 2.4 and 1.7 days for the SL and the WDG imidacloprid formulation, respectively. Whereas, when the new EU MRL (0.01 mg kg^{-1}) that is approved for application starting June 2022 was used, the PHIs were 6.6 and 5.9 days for the SL and the WDG imidacloprid formulation, respectively.

Furthermore, it was noticeable that after only 12 days post-treatment nearly 90% of imidacloprid residues had dissipated in grapes and vine leaves for the two formulations (Table 2), which is consistent with previous studies where 98% of imidacloprid dissipated after 6 days in sugar beet and where total imidacloprid dissipation was observed after 15 days in broad bean [11]. Likewise, imidacloprid rapid dissipation was widely discussed in the literature and short PHIs were reported in various matrix, e.g., vine leaves and grapes,

tomatoes, okra, rocket, parsley, green beans, chili peppers, zucchini, etc. [11,19,30,31,33–35]. It was found to be due to imidacloprid's high sensibility to photodegradation, even under low light intensity conditions [36,37]. Altogether, these studies demonstrated a significant PHI dependence on climatic conditions (sunlight, humidity, temperature, etc.) and they underlined the need to determine PHIs on a regional scale to ensure their accuracy and reliability.

3. Materials and Methods

3.1. Chemicals and Reagents

Analytical imidacloprid standard was purchased from Dr. Ehrenstorfer. Analytical grade solvents and reagents, acetonitrile, methanol and ammonium acetate were purchased from Sigma-Aldrich International GmbH (Munich, Schnellendorf, Germany). Laboratory ultra-pure water was obtained using Milli-Q water purification system (Millipore, Billerica, MA, USA). NaCl, anhydrous MgSO₄, PSA, and GCB were purchased from Agilent technologies (Santa Clara, CA, USA). The two commercially formulated imidacloprid products used in field trials Diclean 20% (SL) and Pilarking Plus 70% (WDG) were officially registered in the Ministry of Agriculture of Lebanon and were purchased from Amalia, S.A.L., Verdun, Rabah Center 5th floor, Beirut, Lebanon and the National Development and General Trading Co., Bank Street, Tyre, Lebanon (Table 3).

Table 3. Pesticides active ingredients and phytosanitary commercial products used for the experimental treatment of vines.

| Trade Name | Active Substance (%) Formulation Type | Recommended Dose (L ha ⁻¹ –Kg ha ⁻¹) | PHI (Days) | Supplier Country | Importer |
|-----------------|--|--|---------------|--|--|
| Pilarking® Plus | Imidacloprid 70% WDG | 0.3 | 14 | Zhejiang Hisun Chemical Co., LTD Zhejiang, China | Rmail Trading Est. |
| Diclean | Imidacloprid 20% SL | 0.35 | 14 | Hailir Pesticides and Chemicals Group Co., LTD, Chengyang, China | National Development and General Trading Co. |

3.2. Site Location and Specification

The vineyard of local Tfeifih variety (*Vitis vinifera*) (1200 vines–12 years old–conduction system: pergola) was located in Tamnine-El-Tahta, Governorate of Baalbeck Hermel [33°52'43.8" N 36°00'13.9" E] at an altitude of 960 m and has an area of 5000 m². No imidacloprid treatments were performed on the target vineyard before the study.

3.3. Pesticide Application and Sampling

Imidacloprid was applied in the first week of July 2018, when the temperature was 31 °C with passing clouds and the wind speed was 2 km/h blowing from 270° West to East with a relative humidity of 39%. During the sampling period, the temperature varied between 18 and 32 °C, the relative humidity ranged between 27 and 65% and no precipitations were recorded.

As shown in Figure 2, the field was divided into two equal plots of 2500 m², each plot was treated according to the OECD guidelines for crop field trials [38], with one imidacloprid commercial product per plot, Diclean 20% (SL, rate 0.35 L ha⁻¹) and Pilarking Plus 70% (WDG, rate 0.30 L ha⁻¹). A back sprayer calibrated according to FAO guidelines on good practice for ground application of pesticides (nozzle calibrated to 200–400 µm with a spray pressure of 40 PSI) was used for applications [39]. A buffer zone of 2000 m² consisting of treated but unsampled vines was established to separate the sampling zones (1200 m² ~ 144 vines) delimited in each plot. Label indications were followed meticulously to prepare the imidacloprid formulations and extra care was taken to make sure the products were homogeneously dissolved. For WDG treatments, continuous agitation of the tank was done during spraying to keep the imidacloprid ingredient suspended in water.

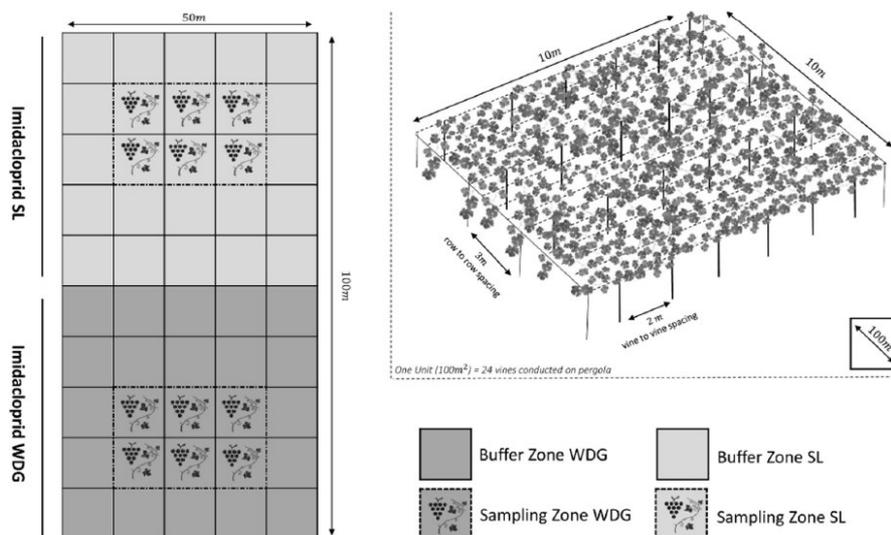


Figure 2. On the left: layout of the field experimental design showing the surface area treated with imidacloprid SL (light grey), the surface area treated with imidacloprid WDG (dark grey), buffer zones and sampling zones. On the top right: an overview of vines conduction system (pergola) and canopy density per unit.

Sampling Procedure for Grapes and Vine Leaves

Randomized sampling of vine leaves was conducted every 2 days, from 2 days up to 18 days after treatment. Sampling was implemented according to FAO guidelines (CAC/GL 33–1999) [40]. For each sampling date, one composite field sample of 2 kg of vine leaves and grapes was collected. From the composite sample a laboratory sample of 1 kg was subsampled and weighed, kept in polyethylene bag and sent directly to the laboratory for residue analysis. The laboratory sample (1 kg sample) was homogenized using a VCM4 Waring Vertical Cutter Blender/Mixer 309 (Halldé, Sweden) and 5 replicate analytical portions of 10 g were taken for analysis.

3.4. Residue Extraction and Clean-Up

Residue extraction was performed following the original unbuffered QuEChERS method, which is widely used for pesticide residue extraction. QuEChERS is an abbreviation for quick, easy, cheap, effective, rugged and safe, and it was developed and first published by Anastassiades et al. [41]. Ten grams of a homogenized sample were weighed in a 50 mL polypropylene centrifuge tube, 10 mL of acetonitrile (ACN) were added. The mixture was shaken by hand for 1 min, followed by addition of 4 g of $MgSO_4$ and 1 g of NaCl. The tube was manually shaken again for 1 min. Afterwards, the tube was subjected to centrifugation, for 10 min at 2066 g. One ml of the supernatant was isolated and put in a dispersive solid-phase extraction (d-SPE) tube containing 150 mg $MgSO_4$, 25 mg primary secondary amine (PSA) and, only for vine leaves samples, 50 mg graphitized carbon black (GCB). The tube was shaken for 1 min and then subjected to centrifugation for 10 min at 3000 rpm. The extract was isolated in a 15 mL polypropylene tube and put in the refrigerator overnight. Finally, the supernatant was filtered using a 0.20 μm PTFE filter. Different levels of dilutions (100 times and 200 times dilution) were performed in acetonitrile in order to minimize the matrix effect and to reduce the concentration level to a level that would

fall within the validated analytical range. The final extract was transferred into a glass vial to be directly analyzed by liquid chromatography mass spectrometry (LC-MS/MS).

3.5. Instrumentation and LC-MS/MS Analytical Conditions

The LC-MS/MS analysis was performed using Agilent Technologies 1200 Infinity Series liquid chromatograph coupled to 3200 QTrap Triple Quadrupole Mass Spectrometer (AB Sciex, Dublin, CA, USA). The unit was equipped with a Phenomenex Analytical, C18 Synergi Fusion 150 × 0.25 mm × 2.5 μm, separation column and a guard column. The injection volume of 5 μL was delivered using an automatic injector with a flow rate of 0.4 mL/min. The eluent was composed of a solvent water (A)-methanol (B) gradient (MeOH), which was buffered with 5 mM ammonium acetate. The gradient program was as follows: 2% B to 100% of B over 12 min, held at 100% B until 20 min then decreased to 2% B at 25.01 min. The total run time was 30 min. The retention time of imidacloprid was 9.47 min. The equipped mass spectrometer provides the capability of combining positive and negative ionization modes by ESI. It was operated in positive ion mode; MRM (multiple reaction monitoring) mode was used for data acquisition (Figure 3).

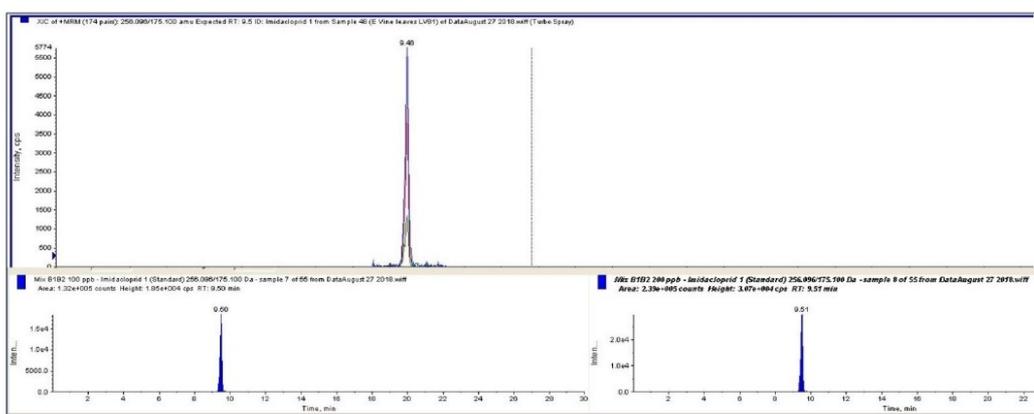


Figure 3. Total ion chromatogram (TIC) of the MRM of imidacloprid and the extracted ion chromatogram for imidacloprid in vine leaves at 100 μg/L (lower left figure) and 200 μg/L (lower right figure).

Table 4 shows the optimized parameters used for imidacloprid qualification and quantification. The source temperature and the ion spray voltages were 500 °C and 5000 v respectively. The ions underwent fragmentation by collisions with nitrogen (inert gas) that was also used as nebulizer curtain gas. Pre-configured iMethod™ Application (AB Sciex) and associated libraries designed for quantitative and qualitative screening using QTRAP® technology were used. EU SANTE/12682/2019 guidelines were followed for imidacloprid identification and quantification [42].

Table 4. Precursor, transition ions and source parameters for imidacloprid residues analyzed by the LC-MS/MS method.

| Condition | | Content | | | | | | | | | | |
|----------------------|----------|---|---------------------|----|------------|-----|---------------------|----|------------|-----|------------|------------|
| Instrument: | | Model AB Sciex 3200 QTRAP LC-MS/MS SYSTEM | | | | | | | | | | |
| Column: | | C ₁₈ column, Phenomenex Analytical Synergi, 150 × 2 mm, 2.5 μm particle size | | | | | | | | | | |
| Column Flow: | | Gradient elution program at 0.4 mL·min ⁻¹ | | | | | | | | | | |
| Source temperature: | | 500 °C–5000 v | | | | | | | | | | |
| Ion Spray-Potential: | | Electron Spray Ionization, | | | | | | | | | | |
| Mode: | | Positive Mode | | | | | | | | | | |
| Molecule | RT (min) | Precursor ion (m/z) | Transition Q1 (m/z) | DP | CE (Volts) | CXP | Transition Q2 (m/z) | DP | CE (Volts) | CXP | LOD (ng/g) | LOQ (ng/g) |
| Imidacloprid | 9.47 | 256 | 209 | 51 | 21 | 7 | 175.0 | 46 | 25 | 7 | 1.93 | 6.45 |

RT, retention time; Q1, first quadrupole; DP, declustering potential; CE, collision energy; CXP, collision cell exit potential; Q2 second quadrupole.

3.6. Method Validation for Grapes and Vine Leaves

Method validation was implemented according to Hayar et al. [7]. The following parameters, as required by EU SANTE/12682/2019 [42], were established: linearity (R^2), recovery (RM%), within-laboratory repeatability (RSD_r%) and reproducibility (RSD_{RW}%) and the limit of detection and quantification (LOD and LOQ, respectively).

As per Hayar et al. [7], linearity was performed by first preparing a stock solution of 1000 mg kg⁻¹ of imidacloprid standard in acetonitrile. Afterwards, aliquot solutions were obtained by serial dilution with 6 concentrations ranging from 5 to 500 μg kg⁻¹. These solutions were later used to build standard calibration curves. Similarly, matrix-matched standard solutions were prepared by adding an imidacloprid standard to blank sample extracts, previously prepared, of grapes and vine leaves. Linear regression of all calibration curves had regression coefficient R^2 greater than 0.99. The limits of detection and quantification (LOD and LOQ) were 1.93 and 6.45 μg kg⁻¹, respectively, in vine leaf matrix. In grape matrix, the limits of detection and quantification (LOD and LOQ) were 1.08 and 5.03 μg kg⁻¹, respectively.

Recovery and % RSD were determined by fortifying matrix blanks (10 g) with three concentration levels (0.01; 0.05; and 0.1 mg kg⁻¹) of imidacloprid standard mixture. Five replicates of each fortification level were prepared on three different days. After fortification, the samples were left at room temperature for 30 min to allow the pesticide to be evenly incorporated into the matrix. Later, QuEChERS extraction procedure was performed and followed by LC-MS-MS analysis, as described in Section 2.3, respectively.

For the method to be satisfactory for imidacloprid analysis, EU SANTE/12682/2019 guidelines [42] require recovery values between 70% and 120% with a relative standard deviation (% RSD) less than 20%, for samples tested on the same day (expressed as repeatability % RSD_r) and for samples analyzed on three different days (expressed as reproducibility % RSD_{RW}). In our study, recovery means were greater than 92% and 80% for grapes and vine leaves, respectively, with RSD % < 20% for all values (Table 5).

Table 5. Method validation results showing the average of recovery data (RM%), repeatability (RSD_r%) and reproducibility (RSD_{RW}%) for imidacloprid at the three fortification levels, 0.01, 0.05 and 0.1 mg kg⁻¹ (*n* = 5 at each level) in grapes and vine leaves samples.

| Matrix | Level of Spiking (mg kg ⁻¹) | Recovery Mean (RM%) | Repeatability (RSD _r %) | Reproducibility (RSD _{RW} %) |
|-------------|---|---------------------|------------------------------------|---------------------------------------|
| Grapes | 0.01 | 96.5 | 16.6 | 12.1 |
| Vine leaves | | 92.0 | 17.0 | 19.0 |
| Grapes | 0.05 | 92.6 | 13.3 | 9.5 |
| Vine leaves | | 84.0 | 7.0 | 8.0 |
| Grapes | 0.1 | 98.5 | 1.2 | 2.3 |
| Vine leaves | | 82.0 | 11.0 | 13.0 |

3.7. Statistical Analysis

The data was subjected to statistical analysis using R free software [43] to give regression equations and half-life (*DT*₅₀) (Tables 1 and 2) and was fit to a first order kinetic dissipation model (Maclachlan and Hamilton [10]) according to Equation (1):

$$C_t = C_0 e^{-kt} \quad (1)$$

where *C_t* represents the residual concentration at sampling time *t*, *C₀* represents the initial concentration and *k* represents the dissipation rate of the molecule and at the same time the slope of the exponential regression curve that is used for the determination of the half-life which is the time required for imidacloprid to decrease to half of its initial concentration after application [3]. The following Equation was used:

$$DT_{50} = \frac{\ln 2}{k} \quad (2)$$

Pre-harvest intervals (*PHI*) were estimated as the time needed for the residues to fall to their specified EU MRL (see Table 1) and were derived from Equation (3) PHIs were estimated as the time needed for the residues to dissipate to values equivalent to MRL after pesticide application (time 0) using an established regression model. The Equation used was:

$$PHI = \left[\frac{\text{intercept} - \ln(\text{MRL value})}{k} \right] \quad (3)$$

For data visualization, R software version 3.6.3 packages were used [43].

4. Conclusions

The effect of formulation type on imidacloprid residues in vine leaves and grapes was investigated. Higher residue levels were detected when grapevines were treated with Diclean 20% (SL) than when treated with Pilarcking Plus 70% (WDG). The type of formulation and the morphological and physiological characteristics of the matrix were found to have an impact on initial deposits, and thus on residue levels, but not on the dissipation patterns.

Since each product formulation is unique, the designers of pesticide formulations have a wide territory to innovate out of the traditional basic roles of adjuvants as carriers, penetrants, stickers, buffers, etc., and move towards more holistic approaches when developing new products that encompass all the legal, economical, ecological and safety challenges from farm to fork. Consequently, the improvement in formulation and inert compositions will enable new phytosanitary products to meet regulatory authorities' requirements, which are becoming more and more restrictive especially when it comes to pesticide residues in food products and safety to applicators.

Further field studies need to be conducted under Lebanese pedoclimatic conditions in order to set more accurate and reliable PHIs, specific to the local environmental conditions,

and to provide farmers with the knowledge they need to choose the appropriate pesticide formulation for their crop variety (e.g., vines, apple), targeted matrix (e.g., berries or leaves), the plants' growth stage (e.g., grapes and leaves diameters) and local climatic conditions (e.g., temperature, humidity).

In this context, this work may be considered as a pilot study for other future ones that will involve other phytosanitary molecules used on grapevines and in which the effect of pesticide application frequencies will be evaluated.

Supplementary Materials: The following are available online, Table S1: Summary of two compartment models, Figure S1: Two compartmental model for each treatment combination, Table S2: Summary of model of imidacloprid decomposition with pooled slope but individual intercepts, Figure S2: Model of imidacloprid degradation with separate slopes and intercepts.

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Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

References

1. Sannakki, S.; Rajpurohit, V.; Nargund, V.; Kulkarni, P. Diagnosis and classification of grape leaf diseases using neural networks. In Proceedings of the Fourth International Conference on Computing, Communications and Networking Technologies (ICCCNT), Tiruchengode, India, 4–6 July 2013; pp. 5–8, ISBN 978-1-4799-3926-8.
2. Lacerda, D.S.; Costa, P.C.; Funchal, C.; Dani, C.; Gomez, R. Benefits of Vine Leaf on Different Biological Systems. *Grape Wine Biotechnol.* **2016**, *19*, 125–143. [CrossRef]
3. Maestroni, B.M.; Ghanem, I.; Correll, R.; Alnaser, A.A.; Islam, M.; Cesio, V.; Heinzen, H.; Cannavan, A. Required Withholding Period for Vine Leaves Following Spraying with Pesticide. *J. Health Environ. Res.* **2018**, *4*, 140. [CrossRef]
4. Mdh, P.; Mw, A.; Sn, A. Determination of Pre-Harvest Interval for Quinalphos, Malathion, Diazinon and Cypermethrin in Major Vegetables. *J. Environ. Anal. Toxicol.* **2018**, *8*, 100163. [CrossRef]
5. EU Pesticides Database (v.2.1) Details. Available online: <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/products/?event=details&p=163> (accessed on 4 April 2021).
6. Katalinic, V.; Mozina, S.S.; Generalic, I.; Skroza, D.; Ljubenkov, I.; Klancnik, A. Phenolic Profile, Antioxidant Capacity, and Antimicrobial Activity of Leaf Extracts from Six *Vitis vinifera* L. Varieties. *Int. J. Food Prop.* **2013**, *16*, 45–60. [CrossRef]
7. Hayar, S.; Zeitoun, R.; Maestroni, B.M. Validation of a Rapid Multiresidue Method for the Determination of Pesticide Residues in Vine Leaves. Comparison of the Results According to the Different Conservation Methods. *Molecules* **2021**, *26*, 1176. [CrossRef] [PubMed]
8. Hayar, S.; Al Hadi, J.; Merhi, A.; Majed, L.; Saba Ayoun, L. L'effet de 3 modes de conservation sur la dissipation des résidus de pesticides dans les feuilles de vigne *Vitis vinifera*. In Proceedings of the 47ème Congrès du Groupe Français des Pesticides: Regards Transfrontaliers sur les Pesticides, Nancy, France, 15 May 2017; pp. 65–75.
9. Sandikly, N.; Hayar, S.; Millet, M. Monitoring Survey of Pesticide Residues in Table Grapes and Risk Assessment to Human in Lebanon. In Proceedings of the 49ème Congrès du Groupe Français des Pesticides, Montpellier, France, 21 May 2019.

10. Maclachlan, D.J.; Hamilton, D. A New Tool for the Evaluation of Crop Residue Trial Data (Day-Zero-plus Decline). *Food Addit. Contam. Part A* **2010**, *27*, 347–364. [CrossRef] [PubMed]
11. Abdel-Hamid, M.; El-Sayed, W.; Ahmed, N. The Relationship Between Different Formulation Types And The Residue Levels Of Pesticides On Tomato Fruits. *Res. J. Agric. Biol. Sci.* **2013**, *9*, 8–16.
12. Alister, C.; Araya, M.; Becerra, K.; Saavedra, J.; Kogan, M. Preharvest Interval Periods and Their Relation to Fruit Growth Stages and Pesticide Formulations. *Food Chem.* **2017**, *221*, 548–554. [CrossRef]
13. Cress, D. Factors Affecting Pesticide Behavior and Breakdown. Available online: <https://bookstore.ksre.ksu.edu/pubs/MF958.pdf> (accessed on 17 April 2021).
14. Ad Meijs, W.H.M. New Type of Formulations and New Application Techniques; Consequences for the Authorisation of Pesticides. *Environmentalist* **2008**, *28*, 5–8. [CrossRef]
15. Cabras, P.; Gennari, M.; Meloni, M.; Cabitza, F.; Cubeddu, M. Pesticide Residues in Lettuce. 2. Influence of Formulations. *J. Agric. Food Chem.* **1989**, *37*, 1405–1407. [CrossRef]
16. Buzzetti, K. Role of the Formulation in the Efficacy and Dissipation of Agricultural Insecticides. *Insectic. Agric. Toxicol.* **2018**, 43–64. Available online: <https://www.intechopen.com/chapters/58195> (accessed on 19 October 2021).
17. Montemurro, N.; Grieco, F.; Lacertosa, G.; Visconti, A. Chlorpyrifos Decline Curves and Residue Levels from Different Commercial Formulations Applied to Oranges. *J. Agric. Food Chem.* **2002**, *50*, 5975–5980. [CrossRef] [PubMed]
18. Abou Zeid, M.; Jammoul, A.; Melki, K.; Abou-Jawda, Y.; Kallassy, M. Dissipation of 3 Commercial Formulations of Penconazole 10% EC on Greenhouse Tomatoes in Lebanon. *Res. J. Chem. Environ.* **2020**, *13*, 2230.
19. Mohapatra, S.; Ahuja, A.; Sharma, D.; Manthirachalam, D.; Prakash, G.; Kumar, S. Residue Study of Imidacloprid in Grapes (*Vitis vinifera* L.) and Soil. *Qual. Assur. Saf. Crops Foods* **2011**, *3*, 24–27. [CrossRef]
20. EU. Commission Implementing Regulation (EU) 2020/1643 of 5 November 2020 Amending Implementing Regulation (EU) No 540/2011 as Regards the Approval Periods of the Active Substances Calcium Phosphide, Denathonium Benzoate, Haloxifop-P, Imidacloprid, Pencycuron and Zeta-Cypermethrin (Text with EEA Relevance); EU: Brussels, Belgium, 2020; Volume 370.
21. EU Pesticides Database (v.2.2) Pesticide Residue(s) and Maximum Residue Levels (Mg/Kg). Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=details&pest_res_ids=326&product_ids=&v=1&e=search.pr (accessed on 19 October 2021).
22. Edwards, C.A. Factors that affect the persistence of pesticides in plants and soils. *Pure Appl. Chem.* **1975**, *42*, 39–56. [CrossRef]
23. Lichiheb, N.; Bedos, C.; Personne, E.; Benoit, P.; Bergheaud, V.; Fanucci, O.; Bouhlel, J.; Barriuso, E. Measuring Leaf Penetration and Volatilization of Chlorothalonil and Epoxiconazole Applied on Wheat Leaves in a Laboratory-Scale Experiment. *J. Environ. Qual.* **2015**, *44*, 1782–1790. [CrossRef]
24. Fernández, V.; Eichert, T. Uptake of Hydrophilic Solutes Through Plant Leaves: Current State of Knowledge and Perspectives of Foliar Fertilization. *Crit. Rev. Plant Sci.* **2009**, *28*, 36–68. [CrossRef]
25. Possingham, J.; Chambers, T.; Radler, F.; Grncarevic, M. Cuticular Transpiration and Wax Structure and Composition of Leaves and Fruit of *Vitis vinifera*. *Aust. J. Biol. Sci.* **1967**, *20*, 1149. [CrossRef]
26. Chowdhury, A.; Jepson, P.; Ford, M.; Frampton, G. The Role of Cuticular Waxes and Surface Roughness in Determining the Insecticidal Efficacy of Deltamethrin and Dimethoate Applied as Emulsifiable Concentrates to Leaf Surfaces. *Int. J. Pest Manag.* **2005**, *51*, 253–263. [CrossRef]
27. Baur, P.; Marzouk, H.; Schönherr, J.; Bauer, H. Mobilities of Organic Compounds in Plant Cuticles as Affected by Structure and Molar Volumes of Chemicals and Plant Species. *Planta* **1996**, *199*, 404–412. [CrossRef]
28. Lewis, K.A.; Tzilivakis, J.; Warner, D.J.; Green, A. An International Database for Pesticide Risk Assessments and Management. *Hum. Ecol. Risk Assess. Int. J.* **2016**, *22*, 1050–1064. [CrossRef]
29. Bletsou, A.A.; Hanafi, A.H.; Dasenaki, M.E.; Thomaidis, N.S. Development of Specific LC-ESI-MS/MS Methods to Determine Bifenthrin, Lufenuron, and Iprodione Residue Levels in Green Beans, Peas, and Chili Peppers Under Egyptian Field Conditions. *Food Anal. Methods* **2013**, *6*, 1099–1112. [CrossRef]
30. Abdallah, O. Dissipation Behavior of Chlorfenapyr and Difenoconazole Residues in/on Grapes (*Vitis vinifera* L.). *Nat. Sci.* **2014**, *12*, 49–54.
31. Hanafi, A.; Dasenaki, M.; Bletsou, A.; Thomaidis, N.S. Dissipation Rate Study and Pre-Harvest Intervals Calculation of Imidacloprid and Oxamyl in Exported Egyptian Green Beans and Chili Peppers after Pestigation Treatment. *Food Chem.* **2018**, *240*, 1047–1054. [CrossRef]
32. Rogiers, S.Y.; Hatfield, J.M.; Jaudzems, V.G.; White, R.G.; Keller, M. Grape Berry Cv. Shiraz Epicuticular Wax and Transpiration during Ripening and Preharvest Weight Loss. *Am. J. Enol. Vitic.* **2004**, *55*, 121–127.
33. Arora, P.K.; Jyot, G.; Singh, B.; Battu, R.S.; Singh, B.; Aulakh, P.S. Persistence of Imidacloprid on Grape Leaves, Grape Berries and Soil. *Bull. Environ. Contam. Toxicol.* **2009**, *82*, 239–242. [CrossRef]
34. Karthik, P.; Venugopal, S.; Datchina Murthy, K.; Lokesh, S.; Karthik, G.; Sharmila, U.; Paramasivam, M.; Senguttuvan, K.; Gunasekaran, K.; Kuttalam, S. Bioefficacy, Phytotoxicity, Safety to Natural Enemies and Residue Dynamics of Imidacloprid 70 WG in Okra (*Abelmoschus esculenta* (L) Moench) under Open Field Conditions. *Crop Prot.* **2015**, *71*, 88–94. [CrossRef]
35. Oliva, J.; Cermeño, S.; Cámara, M.A.; Martínez, G.; Barba, A. Disappearance of Six Pesticides in Fresh and Processed Zucchini, Bioavailability and Health Risk Assessment. *Food Chem.* **2017**, *229*, 172–177. [CrossRef]

36. Scholz, K.; Reinhard, F. Photolysis of Imidacloprid (NTN 33893) on the Leaf Surface of Tomato Plants. *Pestic. Sci.* **1999**, *55*, 652–654. [CrossRef]
37. Dikshit, A.K.; Pachauri, D.C.; Jindal, T. Maximum Residue Limit and Risk Assessment of Beta-Cyfluthrin and Imidacloprid on Tomato (*Lycopersicon esculentum* Mill). *Bull. Environ. Contam. Toxicol.* **2003**, *70*, 1143–1150. [CrossRef] [PubMed]
38. OECD Guidance Document on Crop Field Trials. Available online: https://read.oecd-ilibrary.org/environment/guidance-document-on-crop-field-trials_2794fdd6-en (accessed on 19 October 2021).
39. Food and Agriculture Organization of the United Nations Guidelines on Good Practice for Ground Application of Pesticides. Available online: <http://www.fao.org/3/y2767e/y2767e00.htm> (accessed on 19 March 2021).
40. FAO. Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance With MRLs: CAC/GL 33-1999. Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXG%2B33-1999%252FCXG_033e.pdf (accessed on 19 March 2021).
41. Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D.; Schenck, F.J. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce. *J. AOAC Int.* **2003**, *86*, 412–431. [CrossRef] [PubMed]
42. Guidance SANTE/12682/2019—Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed. Available online: <https://www.accredia.it/en/documento/guidance-sante-12682-2019-guidance-document-on-analytical-quality-control-and-method-validation-procedures-for-pesticides-residues-analysis-in-food-and-feed/> (accessed on 19 October 2021).
43. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.

Annexe II-2 : Matériel supplémentaire de l'article : "Majed L., Hayar S., Zeitoun R., Maestroni BM & Dousset S. 2022. The effects of formulation on imidacloprid dissipation in grapes and vine leaves and on required pre-harvest intervals under Lebanese climatic conditions. *Molecules*, 27, 252. <https://doi.org/10.3390/molecules27010252>"

Supplementary Materials

The effects of formulation on imidacloprid dissipation in grapes and vine leaves and on required pre-harvest intervals under Lebanese climatic conditions

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Summary: The statistical analysis of imidacloprid residues concentration data is presented in this document. First order decay was a useful approximation of the data up to Day 12 but the day 18 data did not fit the model. A two compartment model was formulated but there were insufficient data to establish the change from the first to second compartment as well as the rate of decline in the second compartment. There was little difference between the Day 12 and Day 18 data. An approximation resolved the difference between the transition time from one compartment and the next as opposed to the rate of decomposition within the second compartment by assuming no decomposition occurred in the second compartment. An alternative model, where the half-life continually increased was proposed. Such a model is simpler in that it only involved a single slope and four intercepts.

1. Data

A summary of the data is given in Appendix A. The data suggest that the 5 concentrations are analysis replicates of the same incubation.

2. Statistical Methods

The data were plotted on a linear-log scale that is typical for first order decay data. The result was a curved plot. A log-log plot produced a good approximation to a linear result.

3. Results

3.1. Experimental unit

An analysis which assumed there were incubations from which were taken 5 aliquots for assay. Analysis of variance was performed using incubations as 'main plots' and within incubations as 'subplots'. The treatment effects were removed and the day effects removed of covariates based on the logarithm of the day. The within incubation had a residual mean square of 0.0153 whereas the model using the means of the concentrations had a residual mean square of 0.0542. This confirmed that the modelling should be made on the means of the five assays.

3.2. First order decay model

A plot of residual Imidacloprid against day is shown in Figure III - 1. Plot of residual Imidacloprid on a linear-log scale is shown in Figure 1. There was a steady decline in concentration until Day 12. After Day 12 there was perhaps some loss, but the amount of loss was not statistically significant. The Day 18 data do not fit a first order decay model. The overall the quadratic term to Day 12 was not statistically significant but the quadratic term was negative for each treatment. There was therefore suggestion of curvature between Day 2 and Day 12.

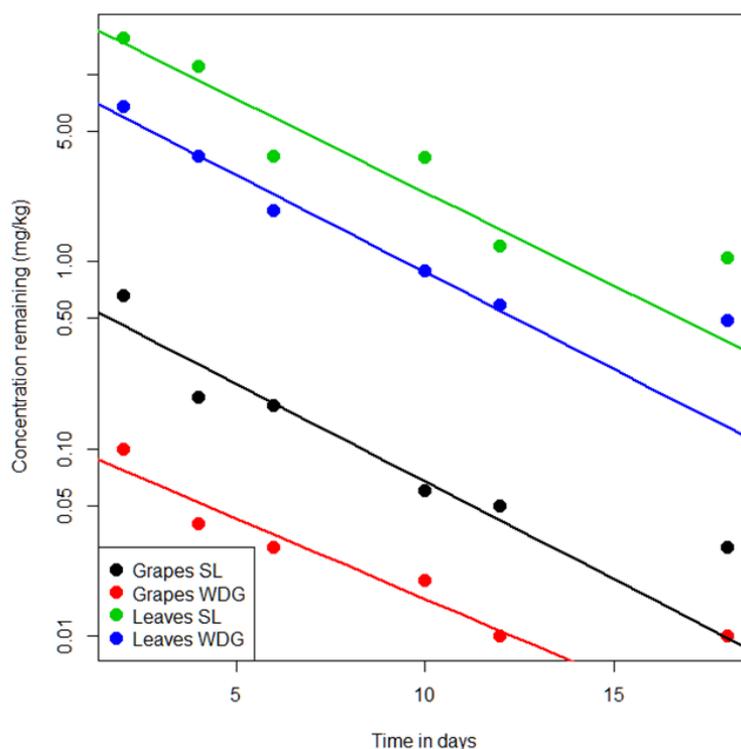


Figure III - 1: Plot of residual Imidacloprid on a linear-log scale using a first order decay model

3.3. Two compartment model

A plot of two compartment models is shown in Figure SIII - 1. The position of the change from one compartment to the other is confounded with the slope or rate of decay in the second

compartment. The slope of the second compartment could be defined using information based on the last two observations. Some of those estimates did not have a consistent sign. An approximation, based on the available data, is that there was effectively no decomposition in the second compartment as shown in Figure S1. Details of the model are given in Table SIII-1.

Table SIII - 1: Summary of two compartment models

| | Intercept | Initial | Half-lives (days) | Last observed ($\mu\text{g}/\text{kg}$) | Duration (days) |
|------------|-----------|---------|-------------------|---|-----------------|
| Grapes SL | -0.299 | -0.240 | 0.74 | 2.89 | 0.03 |
| Grapes WDG | -2.165 | -0.198 | 0.115 | 3.51 | 0.01 |
| Leaves SL | 3.148 | -0.229 | 23.3 | 3.02 | 1.05 |
| Leaves WDG | 2.259 | -0.238 | 9.6 | 2.91 | 0.49 |

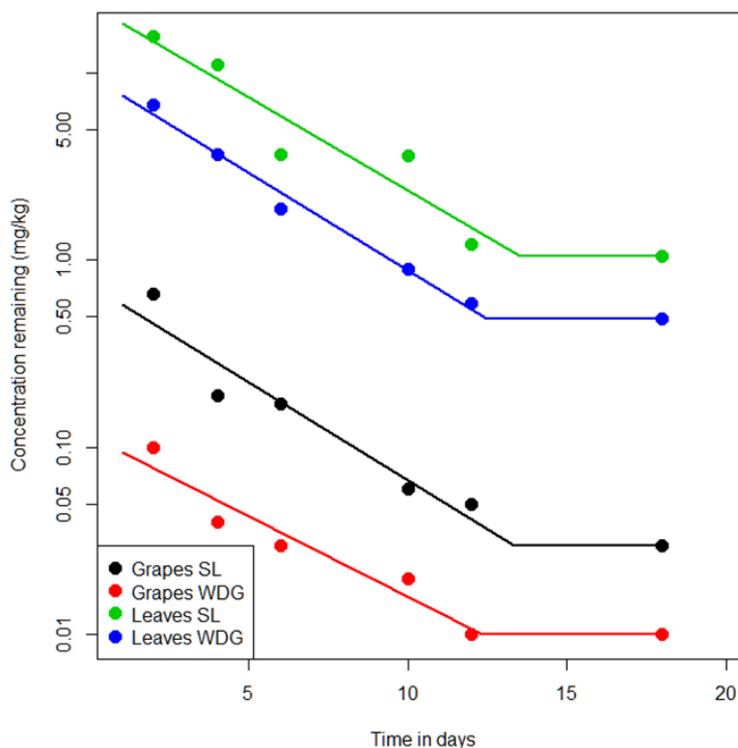


Figure SIII - 1: Two compartmental model for each treatment combination

3.4. Continuous change model

A plot on a log-log scale (Figure SIII - 2) showed no sign of curvature. This was considered a satisfactory model. There was no significant difference in the slopes between the treatments. This enabled a simplification of the model where there was a common slope and separate intercepts. A summary of that model is given in Table SIII - 2.

A plot on a log-log scale (Figure III- 2) showed no sign of curvature. The slopes of the fitted lines did not differ significantly but there were marked differences in the intercepts. The

pooled slope was -1.269 ± 0.068 .

Table SIII - 2: Summary of model of imidacloprid decomposition with pooled slope but individual intercepts

| | Treatment | Estimate | Std. Error |
|-----------|------------|----------|------------|
| Slope | All | -1.269 | 0.068 |
| Intercept | Grapes SL | 0.249 | 0.166 |
| Intercept | Grapes WDG | -1.249 | 0.166 |
| Intercept | Leaves SL | 3.774 | 0.166 |
| Intercept | Leaves WDG | 2.855 | 0.166 |

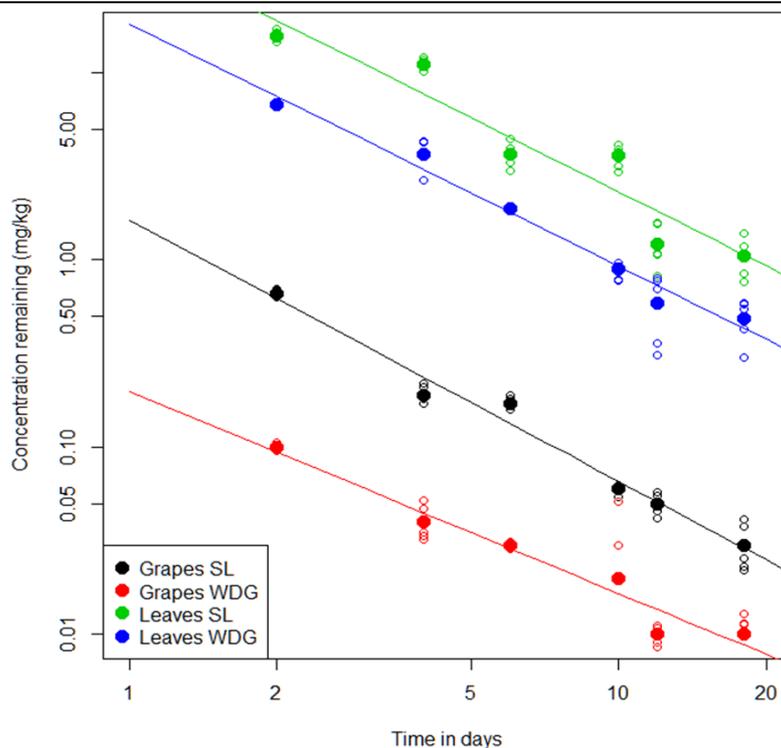


Figure SIII - 2: Model of imidacloprid degradation with separate slopes and intercepts

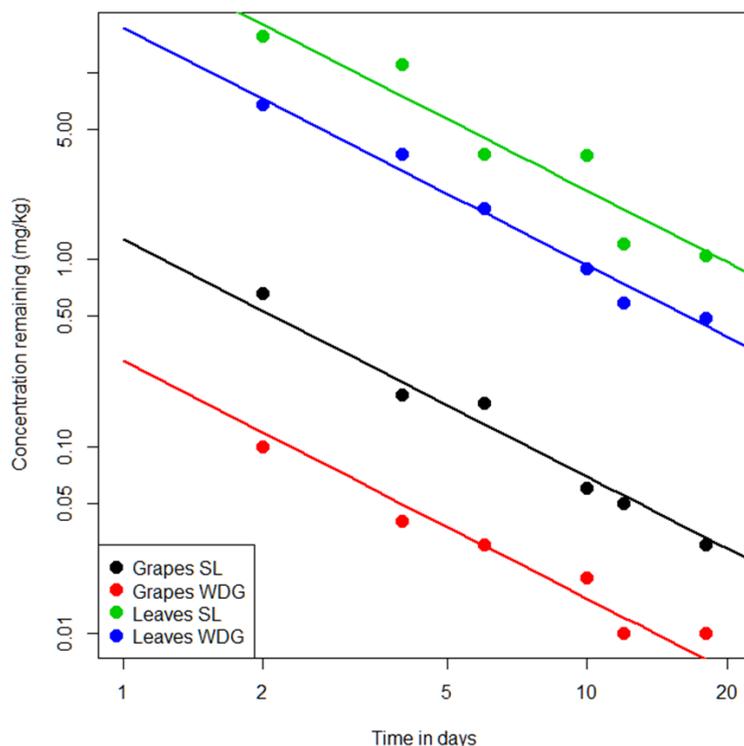


Figure III- 2. Model of imidacloprid degradation with pooled slope but individual intercepts

4. Discussion

The continuous change model implies that the half-life is steadily increasing with time. The rate of loss after Day 12 is very slow but was still occurring according to that model. By contrast, the two-compartment model had potentially no breakdown after Day 12.

There was no formal estimate available for the rate of breakdown beyond Day 12 for the two-compartment model. The continuous change model does give a measure for that. The continuous change model has five parameters (4 intercepts and a common slope) whereas the two compartment model requires 4 intercepts and four slopes, and four change points from one compartment to the next and four slopes within the second compartment.

Table III - 3: Appendix A Raw Data Supplied

| Matrix | Formulation | Time (days) | Concentration C1 (mg/kg) | Concentration C2 (mg/kg) | Concentration C3(mg/kg) | Concentration C4 (mg/kg) | Concentration C5 (mg/kg) | Mean CONCENTRATION | SD |
|--------|-------------|-------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------|-------|
| Leaves | SL | 2 | 14.68 | 15.18 | 16.46 | 14.68 | 17.01 | 15.60 | 0.96 |
| Leaves | SL | 4 | 11.99 | 11.75 | 10.2 | 10.08 | 10.97 | 11.00 | 0.78 |
| Leaves | SL | 6 | 3.79 | 3.94 | 2.98 | 3.3 | 4.44 | 3.69 | 0.51 |
| Leaves | SL | 10 | 4.1 | 3.16 | 4.14 | 3.87 | 2.93 | 3.64 | 0.50 |
| Leaves | SL | 12 | 1.07 | 0.82 | 1.08 | 1.55 | 1.59 | 1.22 | 0.30 |
| Leaves | SL | 18 | 0.76 | 1.39 | 1.07 | 0.85 | 1.18 | 1.05 | 0.23 |
| Leaves | WDG | 2 | 6.67 | 6.95 | 6.81 | 6.56 | 6.57 | 6.71 | 0.15 |
| Leaves | WDG | 4 | 3.63 | 2.67 | 3.59 | 4.23 | 4.28 | 3.68 | 0.58 |
| Leaves | WDG | 6 | 1.85 | 1.87 | 1.85 | 1.9 | 1.89 | 1.87 | 0.02 |
| Leaves | WDG | 10 | 0.97 | 0.97 | 0.77 | 0.96 | 0.79 | 0.89 | 0.09 |
| Leaves | WDG | 12 | 0.36 | 0.8 | 0.77 | 0.7 | 0.31 | 0.59 | 0.21 |
| Leaves | WDG | 18 | 0.55 | 0.3 | 0.59 | 0.43 | 0.58 | 0.49 | 0.11 |
| Grapes | SL | 2 | 0.69 | 0.65 | 0.63 | 0.63 | 0.7 | 0.66 | 0.03 |
| Grapes | SL | 4 | 0.18 | 0.17 | 0.22 | 0.17 | 0.21 | 0.19 | 0.02 |
| Grapes | SL | 6 | 0.16 | 0.16 | 0.19 | 0.18 | 0.17 | 0.17 | 0.012 |
| Grapes | SL | 10 | 0.0625 | 0.062 | 0.0546 | 0.0588 | 0.0621 | 0.06 | 0.003 |
| Grapes | SL | 12 | 0.0551 | 0.0499 | 0.0579 | 0.0416 | 0.0455 | 0.05 | 0.006 |
| Grapes | SL | 18 | 0.0232 | 0.0254 | 0.0221 | 0.0413 | 0.0379 | 0.03 | 0.008 |
| Grapes | WDG | 2 | 0.0976 | 0.0954 | 0.0976 | 0.1036 | 0.1059 | 0.10 | 0.004 |
| Grapes | WDG | 4 | 0.0321 | 0.0467 | 0.0352 | 0.0523 | 0.0338 | 0.04 | 0.008 |
| Grapes | WDG | 6 | 0.031 | 0.0291 | 0.0288 | 0.0313 | 0.0299 | 0.03 | 0.001 |
| Grapes | WDG | 10 | 0.0058 | 0.0064 | 0.051 | 0.0299 | 0.0069 | 0.02 | 0.018 |
| Grapes | WDG | 12 | 0.0111 | 0.0086 | 0.0109 | 0.0091 | 0.0103 | 0.01 | 0.001 |
| Grapes | WDG | 18 | 0.0115 | 0.0043 | 0.0128 | 0.0101 | 0.0113 | 0.01 | 0.003 |

Table SIII - 4: Appendix B| Precursor, Transition ions and Source Parameters for imidacloprid residues analyzed by the LC-MS/MS method.

| Condition | | Content | | | | | | | | | |
|------------------------------|---|------------------------|----|---------------|-----|------------------------|----|---------------|-----|------|---------------|
| Instrument : | Model AB Sciex 3200 QTRAP LC-MS/MS SYSTEM | | | | | | | | | | |
| Column : | C ₁₈ column, Phenomenex Analytical Synergi, 150 x 2 mm, 2.5 μm particle size | | | | | | | | | | |
| Column Flow : | Gradient elution program at 0.4 ml/min | | | | | | | | | | |
| Source temperature: | 500 °C – 5000 v | | | | | | | | | | |
| Ion Spray- Potential: | Electron Spray Ionization, | | | | | | | | | | |
| Mode : | Positive Mode | | | | | | | | | | |
| Molecule | RT (min) | Transition Q1 (m/z) | DP | CE (Volts) | CXP | Transition Q2 (m/z) | DP | CE (Volts) | CXP | LOD | LOQ (ng/g) |
| Imidacloprid | 9.47 | 256.0 --> 209 | 51 | 21 | 7 | 256.0 --> 175.0 | 46 | 25 | 7 | 1.93 | 6.45 |

Annexe III-1 : L'article : "Majed L., Hayar S., Dousset S., Maestroni B.M. & El Omari K. 2024. Effect of vine leaves processing on azoxystrobin, fenazaquin and indoxacarb residues dissipation: processing factors and consumer safety assessment. Food Chemistry, 447, 139065.

<https://doi.org/10.1016/j.foodchem.2024.139065>" comme affiché sur le site web de la revue Food Chemistry – Elsevier



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Effect of vine leaves processing on Azoxystrobin, Fenazaquin and Indoxacarb residues dissipation: processing factors and consumer safety assessment

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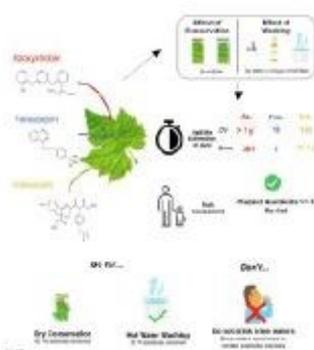
Highlights

- Dry conservation is preferred to brine conservation with 60 % residue removal.
- An average of 2% of the pesticide residues passed into the brine.
- Washing with boiling water is recommended with 30 % residue removal.
- Health risk assessment shows no risk in extreme scenarios, with Hazard Quotients<1.

Abstract

The effect of vine leaves processing techniques on Azoxystrobin, Fenazaquin, and Indoxacarb residues was investigated. Residue extraction following field application of pesticides and leaf processing was carried out using the QuEChERS method, with analysis conducted by LC-MS/MS. In dry conservation, Azoxystrobin's half-life was estimated to exceed a year, Fenazaquin's was 18 days, and Indoxacarb's was 142 days. Azoxystrobin had a half-life of 261 days, Fenazaquin had a half-life of 9 days, and Indoxacarb's half-life exceeded a year in brine conservation. It is recommended to use dry conservation because it results in an average 60% reduction in residue levels for the three pesticides. Boiling water significantly reduced pesticide residues (Azoxystrobin –40.3%, Indoxacarb –22.4%, and Fenazaquin –28.8%). It is recommended to use boiling water for washing, as it shows an average removal rate of approximately 30%. The health risk assessment indicated that consuming vine leaves posed no health risk for consumers, but overall exposure to residues must be considered.

Graphical abstract



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Introduction

Being the main ingredient of one of the most iconic Mediterranean dishes, vine leaves are consumed throughout the year by the local populations. Whether stuffed with meat, rice or vegetables, vine leaves entered the diet of many countries and were subject to diverse

culinary practices to enhance their organoleptic proprieties and to extend their shelf life (Cantwell, Hong, Albornoz & Berlanga, 2022). Therefore, many conservation methods were traditionally used to preserve vine leaves' appealing sensory proprieties: e.g., the dry, the brining, the pickling, and the blanching/freezing conservation methods (Cantwell et al., 2022). As a result, this commodity is marketed in various forms to meet its worldwide growing demand and expanding market.

Still, vine leaves may contain harmful residues from pesticides used in vineyards, posing a challenge for international trade. Notably, specific phytosanitary regulations, such as Maximum Residue Limits (MRLs), have not been established for this commodity leading to border rejections of vine leaves exports when residues are quantified in this commodity (Hayar, Zeitoun & Maestroni, 2021). Thus reducing the amount of these residues or completely removing the residues is of outmost importance to protect human health and facilitate vine leaves trade. In fact, the marginalization of vine leaves as a minor crop on the international level, resulted in a limited number of accessible studies on pesticide residues levels or removal methods in this food (Cangi, Yanar & Dülgeroğlu, 2019). Most of these studies have primarily focused on the effects of brining on residue reduction and have suggested potential benefits of brining and hot water washings in reducing residues (Maestroni et al., 2018; Cangi et al. 2019; Hayar et al., 2021). Where more than 97 and 82% of Azoxystrobin and Triadimenol residues respectively were removed by hot water brining in Cangi et al. (2019) work. Hayar et al. (2021) reported lower residue contaminations in brined vine leaves compared to dry or stuffed from samples collected from the Lebanese market. Indeed, in their review on the effect of processing and washing on residues in food, Bajwa and Sandhu (2014) have demonstrated that nearly all food processing techniques (e.g., washing, peeling, pasteurization, boiling, cooking, pickling and drying) altered pesticide residue levels. However, the amount of pesticide residue in the finished product depends on factors such as the type of pesticide molecule, location, commodity, processing steps, and product preparation (Bajwa et al., 2014; Gao et al., 2020).

Since the existing literature do not comprehensively investigate and compare the effectiveness of various processing/washing methods for vine leaves, the aim of this study was to investigate whether two industrial preservation methods (in brine and dry) and three commonly used industrial/household washing methods (tap water, boiling water or blanching, and a 10% vinegar solution) have varying effects on pesticide residue levels. Three pesticides (Azoxystrobin, Fenazaquin, and Indoxacarb) were chosen for this study due to the frequent detection of their residues in national grape monitoring, which reported MRLs exceedances in Lebanon (unpublished results). This selection is further supported by documentation from the Rapid Alert System for Food and Feed (RASFF) of the European

Union, which identifies these pesticide residues in rejected exports of vine leaves (RASFF, 2021). Azoxystrobin, Fenazaquin and Indoxacarb have no Codex Alimentarius MRL for vine leaves (Codex Online Databases|CODEXALIMENTARIUS FAO-WHO), meanwhile, in the European Union the MRLs on vine leaves are set at the limits of detection (LODs), for Azoxystrobin and fenazaquin (0.01 mg.kg^{-1}) and for indoxacarb (0.02 mg.kg^{-1}) (EU Pesticides Database).

Additionally, this study sought to determine if any of the studied methods could be recommended as a universal approach for effectively reducing the residues of all three molecules, thereby facilitating their removal by industrialists for pesticide residue control. Processing factors and health risk assessments were estimated to support this objective.

Section snippets

Chemicals and reagents

The analytical standards for the three compounds –Indoxacarb, Fenazaquin, and Azoxystrobin—were bought from Dr. Ehrenstorfer. Acetonitrile, methanol, and ammonium acetate were acquired from Sigma-Aldrich International GmbH as analytical grade solvents and reagents (Munich, Schnellendorf, Germany). The Milli-Q water purification system was used to obtain ultra-pure laboratory water (Millipore, Billerica, MA, USA). These items were purchased from Agilent Technologies: NaCl, Anhydrous MgSo₄, and PSA ...

Dissipation kinetics of the residues in dry and brined vine leaves

The dissipation of Azoxystrobin, Fenazaquin and Indoxacarb residues over 60 days is presented in Fig. 3. The dissipation rates (k), their uncertainties (estimated for the slopes of the regressions), intercepts, half-lives and confidence intervals are given in Table 1. The results from the industrial study showed that for Azoxystrobin, other than the residues detected in T8, the difference between in residue levels between the two processing techniques was not statistically significant for all ...

Conclusion

This study confirms that different preservation and washing methods have varying effects on pesticide residues in vine leaves. The mode of action and physicochemical properties of the molecules contributed to dissipation rate variations, influencing residue levels. The

dissipation rates of Azoxystrobin (DT50=261 days in brine conservation and exceeding one year in dry conservation) and Indoxacarb (DT50 exceeding one year in brine conservation and of 142 days in dry conservation) were lower ...

CRediT authorship contribution statement

Liliane Majed: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Salem Hayar:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Sylvie Dousset:** Writing – review & editing, Supervision. **Britt Marianna Maestroni:** Writing – review & editing, Validation. **Khaled El Omari:** Formal analysis. ...

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. ...

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References (36)

Annexe III-2 : Matériel supplémentaire de l'article : "Majed L., Hayar S., Dousset S., Maestroni B.M. & El Omari K. 2024. Effect of vine leaves processing on azoxystrobin, fenazaquin and indoxacarb residues dissipation: processing factors and consumer safety assessment. Food Chemistry, 447, 139065. <https://doi.org/10.1016/j.foodchem.2024.139065>"

Table SIV - 1: Phytosanitary products, percentage of active substance, application rate, pre-harvest intervals (PHI) and restricted entry interval (REI) as mentioned on the product label

| Commercial name | Active substance (%) & Type of formulation | Recommended Dose (L/ha-Kg/ha) | Active Substance Applied Dose (Kg/ha) | PHI (days) | REI (hours) | Supplier Country | Importer |
|--------------------------|--|-------------------------------|---------------------------------------|------------|-------------|---|--|
| Magister | Fenazaquin 20% w/v SC | 0.4 | 0.08 | 30 | 12 | Gowan Comercio Internacional Services LDA – France | Société AGROMECA S.A.R.L. |
| Dexter | Azoxystrobin 25 % w/v SC | 0.75 - 1 | 0.25 | 21 | 4 | Hailir Pesticides and Chemicals Group Co., LTD. – China | National Development and General Trading Co. |
| Indoxam Super | Indoxacarb 30 % w/w WDG | 0.12 | 0.036 | 10 | 12 | Nantong Shizhuang Chemical Co., LTD- China | Amalia S.A.L. |

Table SIV - 2: Physico-chemical and toxicological proprieties of azoxystrobin , fenazaquin and indoxacarb1

| Molecule Name | Azoxystrobin | Fenazaquin | Indoxacarb |
|---|---|--|--|
| Chemical Formula | C ₂₂ H ₁₇ N ₃ O ₅ | C ₂₀ H ₂₂ N ₂ O | C ₂₂ H ₁₇ ClF ₃ N ₃ O ₇ |
| Family | Strobilurin | Quinazolin | Oxadiazin |
| Molecular mass (g.mol ⁻¹) | 403.4 | 306.4 | 527.83 |
| Chemical Structure | | | |
| Mode of action | Systemic | Contact | Contact |
| ADI (mg. kg ⁻¹ .day ⁻¹) | 0.2 | 0.005 | 527.83 |
| LogP (Kow) | 2.5 | 5.51 | 4.65 |
| Solubility in water (20 °C) in mg.l ⁻¹ | 6.7 | 0.102 | 0.2 |
| Vapour pressure (20 °C) in mPa | 1.1x10 ⁻⁰⁷ | 1.90 x10 ⁻⁰² | 9.8 x 10 ⁻⁰⁶ |
| Dissociation constant (pKa) at 25 °C | No dissociation | 2.44 (Strong Acid) | No dissociation |
| Boiling point (°C) | 360 | Decomposes before boiling | Decomposes before boiling |
| Degradation point (°C) | 345 | 307 | 235 |
| Dissipation rate RL ₅₀ on plant matrix (half life on the surface) | 7.6 | - | 3.4 |
| Dissipation rate RL ₅₀ on/ and in plant matrix (half life in the matrix) | 8 | 3.1 | 1.6 |
| Aqueous photolysis DT ₅₀ (days) at pH 7 | 8.7 (Moderately Fast) | 15 (Slow) | 4.5 (Moderately Fast) |
| Aqueous hydrolysis DT ₅₀ (days) at 20 °C and pH 7 | Hydrolytically Stable (Not pH sensitive) | 130 (Persistent) | 17.6 (Non-persistent) |
| Mammals -Acute oral LD ₅₀ (mg.kg ⁻¹) | >5000 (Low) | 134 (Moderate) | 179 (Moderate) |

| | | | |
|--|----------|-----------------------------|----------------------|
| Bio-concentration factor BCF (l.kg ⁻¹) | Low risk | 699 (Threshold for concern) | 77.3 (Low potential) |
|--|----------|-----------------------------|----------------------|

Table SIV - 3: Method validation results for the molecules at the three fortification (n = 5 at each level) in vine leaves

| Molecule | LOD (µg/g) | LOQ (µg/g) | Matrix | Level of Spiking (µg/g) | Recovery Mean (RM%) ±SD | Repeatability (RSD _r %) | Reproducibility (RSD _{RW} %) |
|--------------|------------|------------|----------------|-------------------------|-------------------------|------------------------------------|---------------------------------------|
| Azoxystrobin | 0.002 | 0.006 | Vine leaves | 0.01 | 85 ±3.75 | 12 | 13 |
| | | | Brine Solution | | 76 ±4.61 | 9 | 10 |
| | | | Vine leaves | 0.05 | 84 ±1.40 | 7 | 8 |
| | | | Brine Solution | | 77 ±1.47 | 3 | 5 |
| | | | Vine leaves | 0.1 | 82 ±1.33 | 8 | 7 |
| | | | Brine Solution | | 79 ±3.51 | 4 | 9 |
| Fenazaquin | 0.002 | 0.007 | Vine leaves | 0.01 | 101 ±5.07 | 12 | 8 |
| | | | Brine Solution | | 79 ±2.06 | 10 | 16 |
| | | | Vine leaves | 0.05 | 104 ±3.08 | 15 | 16 |
| | | | Brine Solution | | 80 ±1.65 | 7 | 7 |
| | | | Vine leaves | 0.1 | 100 ±1.62 | 12 | 12 |
| | | | Brine Solution | | 81 ±2.02 | 5 | 12 |
| Indoxacarb | 0.001 | 0.004 | Vine leaves | 0.01 | 83 ±7.11 | 17 | 19 |
| | | | Brine Solution | | 109 ±6.79 | 9 | 17 |
| | | | Vine leaves | 0.05 | 84 ±5.77 | 13 | 12 |
| | | | Brine Solution | | 104 ±0.54 | 5 | 7 |
| | | | Vine leaves | 0.1 | 80 ±3.09 | 14 | 12 |
| | | | Brine Solution | | 103 ±3.23 | 7 | 9 |

Table SIV - 4: Mean residue concentrations, half-lives (DT50), processing factors (PF) and Student's t-test p-values of the 3 pesticides for the two industrial processing techniques, brining and drying

| Molecule | T ₀ Concentration before processing (±SD) in mg.kg ⁻¹ | Conservation Method | Mean Concentration (±SD) in mg.kg ⁻¹ | | | | | | | | |
|--------------|--|------------------------|---|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | | | T ₀ | T ₂ | T ₄ | T ₈ | T ₁₂ | T ₂₀ | T ₂₈ | T ₄₄ | T ₆₀ |
| Azoxystrobin | 12.82 (±0.002) ^a | Brined | 9.67 (±0.186) (0.75) ^b | 9.65 (±0.368) (0.75) | 9.58 (±0.256) (0.75) | 9.03 (±0.002) (0.70) | 9.05 (±0.022) (0.71) | 8.64 (±0.071) (0.67) | 7.99 (±0.525) (0.62) | 8.59 (±0.330) (0.67) | 8.24 (±0.252) (0.64) |
| | | Dry | 9.34 (±0.027) (0.73) | 9.25 (±0.194) (0.72) | 9.26 (±0.813) (0.72) | 9.86 (±0.049) (0.77) | 7.90 (±0.451) (0.62) | 8.24 (±0.292) (0.64) | 9.14 (±0.377) (0.71) | 9.10 (±0.023) (0.71) | 8.05 (±0.326) (0.63) |
| | | p-value* | 0.130 | 0.173 | 0.700 | 0.002 | 0.063 | 0.148 | 0.188 | 0.147 | 0.108 |
| Fenazaquin | 3.69 (±0.002) | Brined | 2.00 (±0.000) (0.54) | 2.08 (±0.052) (0.56) | 1.26 (±0.031) (0.34) | 1.16 (±0.016) (0.31) | 0.86 (±0.020) (0.23) | 0.49 (±0.014) (0.13) | 0.08 (±0.001) (0.021) | 0.07 (±0.006) (0.018) | 0.02 (±0.000) (0.005) |
| | | Dry | 1.57 (±0.039) (0.43) | 1.21 (±0.015) (0.33) | 1.11 (±0.015) (0.30) | 0.73 (±0.018) (0.20) | 0.74 (±0.007) (0.20) | 0.63 (±0.056) (0.17) | 0.44 (±0.004) (0.12) | 0.27 (±0.024) (0.073) | 0.11 (±0.011) (0.03) |
| | | p-value | 0.004 | 0.002 | 0.007 | 0.002 | 0.009 | 0.105 | 0.000 | 0.010 | 0.008 |
| Indoxacarb | 3.39 (±0.177) | Brined | 3.39 (±0.297) (1) | 2.85 (±0.014) (0.84) | 2.88 (±0.049) (0.85) | 2.87 (±0.077) (0.85) | 2.62 (±0.084) (0.77) | 3.46 (±0.112) (1.02) | 3.25 (±0.074) (0.96) | 2.74 (±0.014) (0.81) | 2.92 (±0.025) (0.86) |
| | | Dry | 2.37 (±0.115) (0.70) | 2.30 (±0.160) (0.69) | 2.19 (±0.046) (0.65) | 2.17 (±0.160) (0.64) | 2.28 (±0.067) (0.67) | 1.93 (±0.005) (0.57) | 2.25 (±0.030) (0.66) | 1.80 (±0.008) (0.53) | 1.70 (±0.133) (0.50) |
| | | p-value | 0.068 | 0.046 | 0.009 | 0.052 | 0.021 | 0.003 | 0.005 | 0.000 | 0.005 |

^a Mean ± standard deviation of three replications

^b Figures in parentheses indicate: Processing factors (PF= C_{Processed leaves}/C_{Unprocessed leaves}); PF<1 reduction factor ; PF>1 concentration factor

*For each sampling date, significance level: α = 0.05;

Table SIV - 4 Description

T0 represents the sampling time just after the conservation process; T2 represents the sampling date after two days of storage; T4: after 4 days; T8: after 8 days; T12: after 12 days; T20: after 20 days; T28: after 28 days; T44: after 44 days; and T60: after 60 days of storage. The “p-values” of the Student's t-test were calculated to assess whether the differences in the average concentrations between the two conservation techniques, at each sampling date, are statistically significant or not. When the p-value is larger than 0.05, no significant difference exists between the two conservation techniques. Residue dissipations were also calculated for each sampling date and expressed as the ratio of the residue concentration at the sampling time versus the residue concentration before processing. The concentration before processing represents the residue concentration of the studied pesticide after 12 hours from the formulation application in the respective field.

Table SIV - 5: Analysis of brine waters for azoxystrobin, fenazaquin and indoxacarb (n= 3)

| Molecule | Time (In days) | T ₀ | T ₂ | T ₄ | T ₈ | T ₁₂ | T ₂₀ | T ₂₈ | T ₄₄ | T ₆₀ |
|--------------|------------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Azoxystrobin | MC (±SD) in mg.kg ⁻¹ | 0.595 (±0.0376) | 0.603 (±0.0381) | 0.433 (±0.0237) | 0.436 (±0.0281) | 0.492 (±0.0339) | 0.538 (±0.0041) | 0.308 (±0.0144) | 0.565 (±0.0204) | 0.247 (±0.0174) |
| | Ratio B/L(±SD) | 0.062 (±0.004) | 0.062 (±0.003) | 0.045 (±0.004) | 0.048 (±0.003) | 0.054 (±0.004) | 0.062 (±0.000) | 0.039 (±0.004) | 0.066 (±0.002) | 0.030 (±0.003) |
| Fenazaquin | MC (±SD) in mg.kg ⁻¹ | 0.002 (±0.0001) | 0.002 (±0.0001) | 0.002 (±0.0000) | 0.002 (±0.0000) | 0.002 (±0.0000) | 0.002 (±0.0000) | 0.002 (±0.0001) | 0.002 (±0.0000) | 0.002 (±0.0000) |
| | Ratio B/L (±SD) | 0.001 (±0.000) | 0.001 (±0.000) | 0.001 (±0.000) | 0.001 (±0.000) | 0.002 (±0.000) | 0.003 (±0.000) | 0.019 (±0.001) | 0.023 (±0.002) | 0.064 (±0.001) |
| indoxacarb | MC (±SD) in mg.kg ⁻¹ | 0.007 (±0.0001) | 0.004 (±0.0001) | 0.004 (±0.0000) | 0.024 (±0.0009) | 0.005 (±0.0001) | 0.020 (±0.0004) | 0.004 (±0.0003) | 0.007 (±0.0001) | 0.013 (±0.0034) |
| | Ratio B/L (±SD) | 0.002 (±0.000) | 0.001 (±0.000) | 0.001 (±0.000) | 0.008 (±0.001) | 0.002 (±0.000) | 0.006 (±0.000) | 0.001 (±0.000) | 0.003 (±0.000) | 0.004 (±0.000) |

MC, Mean Concentration measured in brine in mg.kg⁻¹; ±SD: standard deviation of three replications ; Ratio B/L = Cbrine/Cleaves : ratio between the concentration of residues in the brine (Cbrine) and the concentration of residues in the vine leaves (Cleaves)

Table SIV - 6: Mean residue concentrations in mg.kg-1 before and after the washing procedures of vine leaves samples (n=3), processing factors and Tukey's test comparing these different procedures

| Molecule | Washing Method | Mean Concentration (mg.kg ⁻¹) (±SD) | %Reduction (±SD) | PF (±SD) | ANOVA* (α = 0.05) | Tukey** (α = 0.05) |
|---------------|----------------|--|------------------|---------------|----------------------------|-----------------------|
| Azoxystrobine | Control | 12.82 (±0.002) | 0 (±0) | 1 (±0.000) | $p = 7.19 \times 10^{-10}$ | a |
| | Tap water | 8.95 (±0.235) | 30.21 (±1.838) | 0.70 (±0.018) | | b |
| | Boiling water | 7.65 (±0.056) | 40.34 (±0.432) | 0.60 (±0.004) | | c |
| | Vinegar 10% | 8.91 (±0.066) | 30.53 (±0.508) | 0.69 (±0.005) | | b |
| Fenazaquin | Control | 3.69 (±0.002) | 0 (±0) | 1 (±0.000) | $p = 1.99 \times 10^{-10}$ | a |
| | Tap water | 2.09 (±0.001) | 43.38 (±0.062) | 0.57 (±0.001) | | b |
| | Boiling water | 2.63 (±0.013) | 28.82 (±0.398) | 0.71 (±0.004) | | c |
| | Vinegar 10% | 2.43 (±0.064) | 34.24 (±1.729) | 0.66 (±0.017) | | d |
| Indoxacarb | Control | 3.39 (±0.177) | 0 (±0) | 1 (±0.000) | $p = 0.00047$ | a |
| | Tap water | 3.26 (±0.217) | 3.79 (±1.412) | 0.96 (±0.014) | | a |
| | Boiling water | 2.62 (±0.053) | 22.39 (±5.532) | 0.78 (±0.055) | | b |
| | Vinegar 10% | 3.71 (±0.003) | 0 (±5.682) | 1.10 (±0.057) | | a |

**Same letter no significant difference (p-value > 0.05).

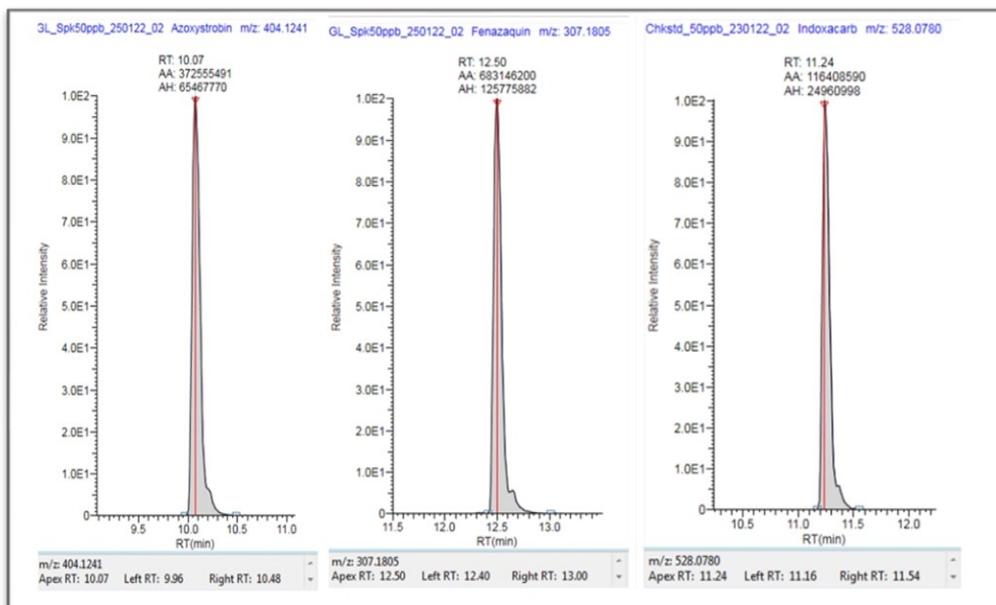


Figure SIV- 1: the extracted ion chromatogram for azoxystrobin, fenazaquin and indoxacarb in vine leaves at 50µg/L

Annexe III-3 : Résumé graphique de l'article : "Majed L., Hayar S., Dousset S., Maestroni B.M. & El Omari K. 2024. Effect of vine leaves processing on azoxystrobin, fenazaquin and indoxacarb residues dissipation: processing factors and consumer safety assessment. Food Chemistry, 447, 139065. <https://doi.org/10.1016/j.foodchem.2024.139065>"

