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REVIEW ARTICLE

Eco-friendly alternatives to chemical pest control for sustainable citrus groves in Morocco

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Summary The citrus industry plays a significant socio-economic role in the Moroccan national economy. Seventy phytophagous insect, mite and snail species have been recorded from citrus orchards in Morocco. However, only a few are considered major or occasional pests and may cause economic damage warranting control. Using broad-spectrum pesticides to control insects and mites has resulted in several problems due to human, environmental and eco-toxicological concerns. This paper provides an overview of practical and preventative eco-friendly alternatives to chemical pest control for sustainable citrus groves in Morocco. The role of appropriate biological and cultural methods and safe chemical control in controlling citrus pests is discussed.

Additional keywords: alternative methods, citrus, insects, mites, Morocco, snails

Introduction

In Morocco, the citrus industry plays a significant socio-economic role in the national economy with a citrus area of about 91243 ha and an average annual production of 2 million tons, and it is a source of foreign revenue (Bennani-Smires, 2025). It also generates employment for 13000 families with 32 million workdays per year, including harvesting in orchards, packing and processing and many other related industries. About 70 phytophagous insects, mites and snail species have been recorded on citrus trees in Morocco (Smaili, 2020; Smaili *et al.*, 2022, 2024, 2025; Mrabti *et al.*, 2024; Haddad *et al.*, 2025). However, only a few of them are considered major pests exceeding the economic threshold and warranting reduction or elimination (Smaili, 2016; Garcia-Marí *et al.*, 2018). Some are occasional pests, below the economic threshold with the potential to become major pests, depending on the climate during the year and the citrus growing area and may thus cause economic loss. The California red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), is a major pest in most citrus growing areas throughout the country. It is found on leaves, twigs and fruit on all citrus cultivars. Sometimes, high populations and severe infestations may cause dieback of twigs and branches. Heavy feeding by this scale can reduce the quality of the fruit and the vigor of the citrus tree. Chaff scale *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae) is another key citrus armored scale in northern Morocco (Sidi Kacem province). In many cases, the pest reached the economic injury level causing heavy infestation of fruit. Heavy feeding by this scale can reduce the quality of the fruit but severe infestation on twigs can also be recorded as damaging. This is also the case of the black parlatoria scales *Parlatoria ziziphi* Lucas (Hemiptera: Diaspididae), particularly in orange tree orchards where the pest occasionally reaches the economic injury level during autumn. A severe infestation can occur on leaves, twigs, branches and fruits. Occasional pests may include *Lepidosaphes beckii* Newman (Hemiptera: Diaspididae) found in the Gharb region (North-Western Morocco) and *Lepidosaphes gloverii* Packard (Hemiptera: Diaspididae) in Berkane (North-Estern Morocco). In some years, high infestations of these species are

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recorded on leaves, twigs, branches and fruit.

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) (medfly), causes considerable economic losses for citrus growers in Morocco. The presence of punctures, eggs or larvae in citrus fruit reduces their commercial value. Indeed, the occurrence of this pest in Mediterranean areas remains the main obstacle to the export of fresh citrus fruit to the major world markets (i.e. Japan). The apparent losses from the medfly in citrus orchards may be further exacerbated in fruit packing houses if fruits with eggs and young larvae remain unnoticed during the sorting process. Moreover, many thrips species are associated with citrus trees in Morocco, but few of them are key pests in citrus groves (Smaili *et al*., 2018a; Smaili, 2019). Feeding by thrips larvae, i.e *Scirtothrips* sp. and *Pezothrips kellyanus* Bagnall (Thysanoptera: Thripidae), causes discolored rinds located under the calyces of the young citrus fruitlets and their expansion across the surface of the fruit lead to discolored damage zones during the fruit development process. While such feeding damage does not affect the physico-chemical characteristics of the fruit, it significantly reduces its market value due to consumer and export rejection of the affected fruit. The oriental red mite (Citrus brown mite) *Eutetranychus orientalis* Klein (Acari: Tetranychidae), the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), and the citrus red mite *Panonychus citri* McGregor (Acarina: Tetranychidae) are the key pest mites in Moroccan citrus orchards. *Tetranychus urticae* is a highly polyphagous mite species but *E. orientalis* generally occurs, mainly in citrus trees despite being reported as a polyphagous species. These mite species can be found in citrus orchards between end of April and early January. Currently, *P. citri* population always remains below the economic threshold, while *T. urticae* and *E. orientalis* populations can reach high levels on leaves and fruit. The snail species *Theba pisana* O.F. Müller, *Eobania vermiculata* O.F. Müller, *Helix aspersa* aspersa Müller and *Helix aspersa* maxima Müller (Gastropoda, Helicidae) are all "major pests" in Moroccan citrus groves, mainly in the Gharb region (Smaili, 2013). Once present, the snails consume the fruit epicarp and cause significant damage, which seriously affects citrus productivity. The scars generated by scale feeding on the fruits also provide a means for fungal and bacterial introduction infection, resulting in fruit decomposition. When environmental conditions are favorable for scale activities (mainly wet conditions), snails may cause considerable economic losses for citrus growers. In addition, at packing stations, fruit bearing scars induced by snails are discarded.

In Morocco insect, mite and snail control in citrus orchards is based mainly on pesticides. Chemical control is primarily used for keeping the damage caused by the pests below the economic injury level. However, this strategy is not always effective in reducing damage to leaves, twigs, and fruit. In addition, management based on broad-spectrum insecticides and miticides may cause pesticide-resistance issues, the resurgence of occasional pests and several undesirable side-effects on beneficial insect and mite species. Many efforts have been made by companies, mainly those exporting fruit, to switch from chemical control to the integrated pest management (IPM) approach in citrus groves with and significant progress including surveys, release of natural enemies and efficient use of registered pesticides, with significant progress. In many cases, the exporting compagnies have certified their orchards according to the standards and requirements of importers of fresh citrus fruit with rigorous control requirements. Currently, several factors have greatly encouraged Moroccan citrus producers to search for sustainable alternative methods to chemicals pesticides: i) the limited number of registered insecticides and miticides after the ban of hazardous products (i.e. chlorpyrifos-methyl and spirodiclofen, respectively) from use in citrus groves in the country; ii) new requirements for importers and local market, related to quality of citrus fruit; iii) a decline in bees associated with chemical pesticides use; iv) climate change and mainly variable temperatures inducing occurrence and increased abundance of some insects and mite pests, and v) a noticeable increasing interest in new alternative methods to chemicals, including biological control and organic methods. Here, we discuss the practical and preventative eco-friendly and alternative methods to chemicals pesticides available for the control of key pests in the frame of sustainable citrus grove management in Morocco.

Materials and Methods

The presented and discussed practical, sustainable, preventative and alternative methods to chemicals pesticides against key pests (i.e. armored scales, thrips, medfly and mites) in Moroccan citrus groves considered i) the authors' experience in applied research and extension services conducting field trials over many years, and ii) published peer-reviewed articles. The review reflects the authors' views on the availability, validity and practical use of the plant protection methods in the citrus groves under Moroccan conditions.

Results and Discussion

In Morocco, chemical pesticides and other alternative methods have been explored in experiments to control many pest species in citrus groves (Abbassi, 1990; Benziane, 2003; Smaili, 2016; Smaili *et al*., 2014, 2016). Here we review and propose several practical and preventative eco-friendly and alternative methods to chemical control of key pests in citrus groves in Morocco. The higher the farmers' acceptance of alternative control methods, the healthier the ecosystem, including the biodiversity of natural enemies of pests (Göldel *et al.,* 2020).

Implementation of biological control

Augmentative biological control

Smaili *et al.* (2020) listed the species richness of parasitoids and predators, including those imported for classical biological control, in citrus orchards in Morocco and how to facilitate near future investigations on their potential use as biocontrol agents. The same authors discussed the effects of these beneficial insect communities on citrus pests and concluded that none of the reported taxa are harmful to crops (based on knowledge to date).

Scales: In Moroccan IPM certified citrus orchards, armored scale control is based mainly on monitoring, threshold and efficient insecticide spraying (Smaili *et al*., 2017, 2022). For biological control of the California red scale, *A. aurantii*, the release of the parasitoids, *Aphytis melinus* DeBach (Hymenoptera Aphelinidae) and *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae), is of crucial importance (Table 1). In Moroccan citrus groves, *A. melinus* is most effective during the spring and autumn season while *C. bifasciata* complements during the summer and winter season (Abbassi, 1990; Benziane, 2003; Smaili, 2009, 2016). When the total parasitism reaches an appropriate percentage (i.e. >40%), the scale could be kept below the threshold. *Aphytis. melinus* is commercially mass produced in Morocco. Optimization of this parasitoid's mass rearing facilities should be a priority at a large scale under current conditions, while *C. bifasciata* mass rearing facilities, require a small scale in each citrus producing area. Moreover, there are records of very efficient biological control of *A. aurantii* using the parasitoid *A. melinus* during the fruit growing period in Moroccan citrus growing areas (Abbassi, 1990; Benziane, 2003; Smaili, 2009). In citrus groves where *A. melinus* was released at a high number, significantly higher parasitism rates of *A. aurantii* (64%) were recorded and compared to parasitism rates in control citrus groves (<5%) (Smaili *et al.*, unpublished data). This level of parasitism cannot be reached in many Moroccan citrus regions considering the effect of chemical use, where shifting to IPM may increase parasitism of *A. aurantii* using *A. melinus* releases.

Table 1. Suggested doses, number and periods of release of appropriate biological control agents to control main pests in citrus orchards in Morocco.

Species	Targeted main pests	Relased number	Frequency	Periods
Aphytis melinus	*Aonidiella aurantii*	80000 - 120000/ha	1-2	April-May and October
Comperiella bifasciata	*Aonidiella aurantii*	80000 - 100000/ha	1-2	April-June/ September-November
Aphytis hispanicus	*Parlatoria pergandii*	40000 - 80000/ha	1-2	April-May and October
Aphytis lepidosaphis	*Lepidosaphes beckii*	40000 - 80000/ha	1-2	April-May and October
Orius laevigatus	*Scirtothrips* sp., *Pezothrips kellyanus*	80000 - 100000/ha (10 - 20/m^2 infested trees)	1-2	March-June/ September-October
Amblyseius swirskii	*Scirtothrips* sp., *Pezothrips kellyanus*	100000 - 200000/ha (10 - 40/m^2 infested trees)	1-2	March-June/ September-October
	Panonychus citri, Tetranychus urticae, Eutetranychus orientalis	100000 - 200000/ha	1-2	April-June/ September-October
Phytoseiulus persimilis	*Panonychus citri, Tetranychus urticae, Eutetranychus orientalis*	80000 - 200000/ha	1-2	April-June/ September-October
Neoseiulus californicus	*Panonychus citri, Tetranychus urticae, Eutetranychus orientalis*	80000 - 200000/ha	1-2	April-June/ September-October
Stethorus punctillum	*Panonychus citri, Tetranychus urticae, Eutetranychus orientalis*	10 - 40 /m^2 infested trees	1-2	April-June/ September-October
Chrysoperla carnea	*Panonychus citri, Tetranychus urticae, Eutetranychus orientalis*	60000 - 80000/ha 100 larvae/small area	1-2	April-June/ September-October

Concerning the chaff scale *P. pergandii* and the purple scale *L. beckii* release of the main parasitoids *Aphytis hispanicus* Mercet (Hymenoptera: Aphelinidae) and *Aphytis lepidosaphes* Compere (Hymenoptera: Aphelinidae), respectively, play an important role in reducing their populations (Smaili, 2016; FAO, 2024). Currently, these two parasitoids, acting alone, are not effective in controlling these scales. In Morocco, *A. swirskii* release, may be used to control crawlers of the armored scales, *A. aurantii* and *P. pergandii*, in April–May and May-June, respectively, to avoid the settling in of crawlers on new fruit; thus, offering the possibility of reducing fruit infestation during the growing stage.

Mites and thrips: Citrus mites are mainly controlled by predatory mite and insect species, such as phytoseiid mites, lacewings and coccinellids (Smaili, 2020), while thrips are controlled by predatory mites that can easily occupy, the calyx of citrus fruit during fruit development (zones that are preferred by thrips). In this respect, many species can be released in the Moroccan citrus groves to control mites (Smaili *et al.*, 2022). In addition, for mites, the use of a *Beauveria bassiana* (Balsamo) Vuillemin - based product is proposed as foliar treatment when the mite densities are still low (personal research results). This was also the case, after a sudden heavy infestation of fruit by mites (i.e. clementine), just before harvest (mid-October and November).

Neoseiulus californicus McGregor (Mesostigmata: Phytoseiidae), a specialized predator of spider mites and effective predator against phytophagous mites (Pascua *et al.*, 2020) is promoted for biological control against mites in Morocco (Smaili *et al.*, 2020). Abad-Moyano *et al.* (2010a) reported that this phytoseiid mite may be able to persist in the crop when spider mite prey is scarce and addition of pollen could improve *T. urticae* biological control by this beneficial species. However, a study by Abad-Moyano *et al.* (2010b) reports the intraguild effect after the release of *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) and *N. californicus* and their interaction with *E. stipulatus* due to the occurrence of the spider mite webbing, in Valencia (Spain), a region similar to many Moroccan citrus area productions. In Morocco, release of *N. californicus*, to control mites, especially *T. urticae*, would be recommended, when *E. stipulatus* abundance is low, to avoid the lethal effect of intraguild predation.

Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae), a generalist predator, is effective against several species of pests, including mites, i.e. *E. orientalis* (Yalçin *et al.*, 2023) and thrips (Arthurs *et al.*, 2009). In Spain, the augmentative release of *A. swirskii* has been reported as an efficient biological control approach against the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) in citrus (Juan-Blasco *et al.*, 2012).

Phytoseiulus persimilis is a specialized predator of *Tetranychus* species (McMurtry and Croft, 1997) which has been proved highly effective in reducing the damage level caused by mites on citrus plants (Abad-Moyano *et al.*, 2010a; Fadamiro *et al.*, 2013). However, in Morocco, the abundance of this predator is very low in citrus orchards and its resident populations could not maintain *E. orientalis* and *T. urticae* population at acceptable levels (Smaili, 2009; Smaili *et al.*, 2020, 2022). It has been suggested that, when *E. stipulatus* abundance is scarce, at least one release of this predator is required per year to enhance its abundance and confrontation with the mite prey.

In practice, releases of predator species with known positive biocontrol value (Table 1) are preferable, such as *Stethorus punctillum* Weise (Coleoptera: Coccinellidae), both larvae and adult, against spider mites, on the first located infested trees before mite populations reach the threshold, might be useful (Smaili *et al.*, 2022); *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) against spider mites during April-June and September October; *Orius laevigatus* Fieber (Hemiptera: Anthocoridae) against thrips; the generalist *C. carnea* and the specialist *S. punctillum* might provide additional effects to reduce the abundance of the spider mites.

Mediterranean fruit fly: In Morocco, medfly control is based mainly on insecticides (i.e. bait application technique and cover spraying) and mass trapping. Inundative biological control of the medfly using entomopathogenic nematodes (Gazit *et al.*, 2000), has not been sufficiently assessed in the citrus orchards in Morocco to date. However, in Greece, field trials of four species of entomopathogenic nematodes, *Heterorhabditis bacteriophora* Poinar, *H. downesi* Stock (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* Weiser and *S. feltiae* (Rhabditida: Steinernematidae), used at different doses on medfly (Kapranas *et al.*, 2023) have shown that during the early and late season, a single application of *S. feltiae* on soil at a rate 2.5×10^6 *S. feltiae*/m^2, can result in best pest control with 65 % adult medfly suppression. The pathogenicity of the entomopathogenic fungi, *B. bassiana* and *Metarhi-*

zium anisopliae (Metschnikoff) Sorokin to medfly was studied in Morocco (Imoulan *et al*., 2011). Foliar treatments with entomopathogenic fungi during one or two months before harvest might be a suitable method with added value to control medfly in addition to mass trapping.

Spinosad has been successfully used against a large number of insects worldwide, mainly to control medfly (Chueca *et al*., 2007; Smaili *et al*., 2016; Abdel-Razek and Abd-Elgawad, 2021). In Morocco, spinosad is registered and applied to control a wide variety of species in citrus, including thrips species and medfly. Although a bioinsecticide, spinosad can have side effects on natural enemies (D'Ávila *et al*., 2018). Therefore, we suggest using it mainly for medfly control and only during the two weeks before harvest when i.e., using mass trapping techniques may be not sufficient to control medfly in large scale citrus groves. However, regarding medfly control, using a selected mass trapping method via integrating mainly fruit resistance and spinosad-based bait appat technique seem to enhance medfly control (Smaili *et al*., 2016, 2018b).

Mass rearing of biocontrol agents for inoculative biological control

Setting up small or larger scale mass rearing facilities of the key natural enemies to provide/purchase to growers for release in citrus groves may assist in inoculative control but this might be difficult in a of low or middle-income country such as Morocco. Thus, very few beneficial species are mass produced in mass rearing facilities as reported by Smaili *et al*. (2013, 2020; Table 1). In this context, we consider that the inoculative biological control approach may be possible at two levels in the citrus groves of the country:

Small-scale mass rearing of known efficient beneficial species: Smaili *et al.* (2013, 2020) report information on the beneficial species under artificial mass-rearing and release in commercial citrus orchards in Morocco. In practice, it is better to focus on the predator species with known positive biocontrol impact in the citrus groves.

Collect and release natural enemies: Collecting the natural enemies from spontaneously growing weeds and releasing them (with or without small-scale mass rearing) is a common approach used in several citrus groves in Morocco. This is the case with *S. punctillum*, which can be manipulated by collecting its population from spontaneous herbaceous growth areas and easily moved and released on known mite infested citrus plots. This is also the case with several beneficial species, i.e. *Citrostichus phyllocnistoides* Narayanan (Hymenoptera: Eulophidae); *Adalia decempunctata* L. *Coccinella septempunctata* L.; *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae); (Smaili *et al*., 1999a, 2014; Rizqi *et al*., 2003; Nia *et al*., 2008; Nafide *et al*., 2010). This method might be useful in controlling citrus pests in small citrus production areas.

Conservation biological control

The conservation approach could be achieved by managing habitat and food for natural enemies and adjacent shelters/ refuges near to citrus groves as well as selective use of pesticides. Smaili *et al.* (2010, 2020) report 105 species of natural enemies in citrus in Morocco and discuss their potential to enhance biocontrol services in citrus groves. Their conservation is important. For instance, to contribute to the reduction of armored scale populations in citrus orchards, conservation practices are encouraged for *A. melinus*, *C. bifasciata, A. hispanicus, Cybocephalus* spp. and *C. bipustulatus* (for armored scales). For mites, conservation should target to *P. persimilis, E. stipulatus*, *Typhlodromus phialatus* Athias-Henriot, *Euseius rubinia* (Acarida: Phytoseiidae), *S. punctillum, C. carnea, Nephus* sp., *Scymnus* sp. (Smaili *et al*., 2013, 2020). Predator species that attack *T. urticae* and *P. citri* mite populations may also be potential predators against *E. orientalis*. For instance, *E. stipulatus* is the most common and abundant phytoseiid and widespread species in most Moroccan citrus orchards, especially clementine orchards, where the use of chemicals is minimal (Smaili *et al.*, 2013, 2020). Its prey is limited to certain spider mite species, in particular *P. citri* (also *T. urticae* and *E. orientalis*) in Morocco (Smaili *et al.*, 2020, 2022). Compared to other phytoseiid mites, *E. stipulatus* is the species most tolerant to pesticides.

Conservation of potential natural enemies against thrips could include the predators: *O. laevigatus*, *Orius* sp., *Franklinothrips* sp., *Aeolothrips* sp., *Haplothrips* sp., phytoseiids, the lacewings *C. carnea*, *Contwenzia psociformis* Curt (Neuroptera: Coniopterygidae). However, despite the presence of these predators (i.e. *Aeolothrips* sp. and *Haplothrips* sp.) in high abundance in citrus trees, they do not seem to play an important role in limiting thrips populations (Smaili *et al.*, 2018a; Smaili, 2019).

Management of groundcover vegetation to conserve natural enemies

Habitat management involving the manipulation of farmland vegetation can exert direct suppressive effects on pests and promote natural enemies. Indeed, tillage between rows in the groves may affect abundance and diversity of insects, mites, snails, and natural enemies (Gurr *et al.*, 2017). The occurrence and diversity of spontaneous herbaceous plant growth play an important role in conserving beneficial species (Beaumelle *et al.*, 2021). Their inter-row presence constitutes an important source of food and a favorable habitat for their continuous activities during the year. Pollen, nectar, alternative prey, and shelter are the major resources provided by plants to natural enemies (Gurr *et al.*, 2017). On the other hand, mowing and tillage of the grass between citrus rows might have major side effects, i.e. increase of mite and snail abundance in tree canopies. Aguilar-Fenollosa *et al.* (2011a, b) discuss how a conservation approach by means of groundcover offers an interesting alternative to chemical control of the two-spotted spider mite, a key pest of clementine mandarins in Spain. The occurrence of mite populations in spontaneous herbaceous plant growth is considered a primary source of infestation of many pests and may be a source of significant continuous re-infestation of citrus trees throughout the year. If no appropriate measures are taken, their population will move to the trees from the groundcover and their abundance will increase in the canopy, thus exceeding the economic threshold.

Knowledge of the nature of the spontaneous herbaceous plant growth is the first step to an appropriate control of mites (i.e. *T. urticae* and *P. citri*). To not affect citrus trees development, the cutting of spontaneous plant growth under the tree is recommended. However, groundcover-based spontaneous plant growth at 20 cm in height between the citrus rows is preferable as it will provide a conservation habitat, food and shelter for natural enemies. In some cases (i.e. absence of the main predators) the weeds between the citrus rows seem not to contribute to reducing *E. orientalis* populations on the trees (Smaili *et al.*, 2017, 2022). However, these trials carried out mainly in the Gharb area, showed that *T. urtica* abundance on citrus trees is lower in groves with Poaceae as cover vegetation than a mixture of dicotyledonous and monocotyledonous weeds. Several studies have shown that phytoseiid abundance and diversity are strongly influenced by the management of the spontaneous herbaceous plant growth located between the citrus rows in comparison to the bare soil (Aguilar-Fenollosa *et al.*, 2011a). Suitable groundcover management is among the most important cultural practices to regulate the abundance level and movement mainly of thrips and mite species in citrus orchards (Aguilar-Fenollosa *et al.*, 2011a,b; Aguilar-Fenollosa and Jacas, 2013).

Preservation of Poaceae: In Morocco, cultural practices deliberately encouraging and preserving *Poaceae* host plants between rows seems to be a promising solution for reducing the abundance of *T. urticae* and *P. citri* mites on citrus trees. This practice might also increase phytoseiid abundance in the citrus groves.

Deliberate sowing of tall fescue: *Festuca* *arundinacea* Schreber (Poaceae) is widely distributed as a native grass in temperate and cool climates throughout Europe, North Africa, and West and Central Asia (Gibson and Newman, 2001). In Spain, Aguilar-Fenollosa *et al.* (2011b) provide evidence that both bottom-up and top-down regulation processes related to the nature of the ground cover affected the tetranychid mite populations in citrus orchards. The authors concluded that best results were obtained with the ground cover of *F. arundinacea*, which offered a better regulation of both *P. citri* and *T. urticae* than either bare soil or the resident wild cover. In Morocco, planting strips (length; 10-60 m; width: 0.2-1 m) of *F. arundinacea* between the rows of citrus trees might be a new technique to reduce abundance of mites and other insects such as thrips.

The "Predator in First" approach as a preventative biological control technique

The 'predator in first' approach is a new promising approach to control thrips (Kumar *et al.,* 2020; Pasquier *et al.*, 2021). It is a prophylactic control strategy that aims to establish predators before the appearance of pests in an agro-ecosystem (Kumar *et al.,* 2015). This method involves the release of specific predators on un-infested seedlings before transplanting in the field (Kumar *et al.,* 2020; Pasquier *et al.*, 2021). In Morocco, use of host plants, which are already recognized as being attractive to thrips (i.e. peppers, tomatoes, etc.) and which were previously infested at the nursery by an effective predator (i.e. *A. swirskii* or *O. laevigatus*), is preferable. These plants should be planted along the edges of citrus orchards where the predator may become established before the onset of the citrus flowering. This offers the possibility of limiting adult thrips abundance in these plants which will be responsible for the production of the two main first generations of this pest.

'Farming with alternative pollinators' approach as preventative biological control

The 'farming with alternative pollinators' approach (FAP) uses the habitat enhancement zone in fields to attract pollinators and natural enemies i.e., by marketable habitat enhancement plants (MHEPs), so the land serves simultaneously ecological purposes and the interest of farmers to gain income from their entire land. This is one of the advantages of FAP in comparison to wildflower strips, which cause opportunity costs and are not scalable in low- and middle-income countries (Christmann, 2020). FAP measures impacts of habitat enhancement on diversity and abundance of flower visitors, natural enemies, and pests, and net income. In smallholder FAP fields, the main crop is planted in the central area (75%) and 4-12 MHEP cover together 25% of the field area (Christmann and Aw-Hassan, 2012). The mix of MHEP with different colors of petals and different flower types (i.e. oil seeds, spices, food crops, medicinal plants) prolong the flowering period significantly and substantially (Christmann *et al.*, 2021). The 25%-zone hosts also low-cost materials for nesting- and water support. Farmers using FAP approach noticed higher income, an increase in pollinator diversity and abundance, lower pest abundance, and also the impact on the quality of many crops (Christmann *et al.*, 2021, 2022). In FAP plots, predators and parasitoids diversity and abundance were increased (Christmann *et al.*, 2021, 2025). Pollen as food source might help to sustain a population of several beneficial species, i.e. Phytoseiidae mites (Pascua *et al.*, 2020). Combining FAP and augmentative biological control using natural enemies might be another alternative method with additive effects. Such management offers the possibility of increasing both parasitism and predation levels, mainly for armored scale populations during the settling process of crawlers during the fruit growing period.

Use of host plants as traps to reduce thrips population

Commercial host plants of thrips may be used as traps in citrus groves to reduce or to control, i.e., thrips. This strategy can be used at two levels for thrips control.

Host plants deliberately planted as trap

crops: Host plants are planted on the edges of orchards (and not inside the groves as strips) before the onset of the flowering period of citrus trees. Once abundance of thrips becomes significantly higher in trap plants, their suppression via effective and registered products will prevent their moving towards fruits of the citrus canopy. While the trap plant approach might not provide sufficient control to targeted pests, this technique may have positive effects when combined with other techniques.

Spontaneous plant growth managed as host plants: The nature of the spontaneous herbaceous plants around the citrus groves must be known. In our experience, at the edges of citrus orchards, white or yellow flowers of wild plants that precede the blooming phase of citrus trees should be eliminated to prevent thrips populations from moving from those plants to citrus trees. Generally, flowers located in the groundcover are attractive to pests and act as trap crops, mainly for thrips populations. However, during the flowering phase of the spontaneous plants, it is suggested to apply foliar application with an efficient and safe product to reduce their population abundance before they move to the citrus tree (Smaili *et al.*, 2025).

Implementation of cultural practices

Cultivars with host fruit resistance to reduce medfly infestation

Physico-chemical parameters of citrus fruit such as acidity, maturity index, and firmness may determine the level of fruit punctures by medfly, mainly for clementine cultivars (Aluja and Mangan, 2008; Papachristos and Papadopoulos, 2009). Trails conducted in the Gharb region revealed a significant correlation between clementine cultivars and medfly fruit punctures (Smaili *et al.*, 2016, 2017, 2018b), concluding that the main factors affecting the punctures were fruit firmness and maturity index. In Morocco, this use of physico-chemical characteristics of fruit during fruit infestation susceptibility (i.e. two months before harvesting clementine varieties) could contribute to decreasing the number of insecticide applications for the control of this key pest.

Cultural practices improving health and productivity of trees

Water stress combined with heavy infestation by *T. urticae* in citrus trees can result in leaf drop (Aucejo-Romero *et al.*, 2004). Proper irrigation of citrus trees is therefore important.

To achieve an appropriate fruit size, thinning during fruit set is an important cultural practice, mainly for clementine, *Citrus reticulata* var. Bruno, reducing the burden of the tree by eliminating fruits with small size. This practice may also be used to reduce fruit infested with thrips populations. During this phase, it is recommended to target small fruits that can be: i) sensitive to subsequent attacks by thrips, ii) susceptible to host thrips larvae, iii/ close to symptomatic fruits that are visible or not (Smaili *et al.*, 2018a).

Schöneberg *et al.* (2020) reported that pruning small fruit crop canopies alters the microclimate, which in turn may influence insect pest activity. Tree pruning also enables ventilation of the interior and the creation of several openings, so-called external windows. This practice not only enables penetration of bright solar rays to the most shaded places of the tree, but also ventilation of the different external parts of the foliage thus creating an unfavorable environment for different stages of pests, mainly armored scales and mites, hindering their activity in the leaves.

Behavior manipulation for pest control

Behavioral manipulation of a pest includes methods that interfere with their natural behavior and activity (Foster and Harris, 1997). This behavior can be influenced by cues for perception, such as visual, chemical, tactile stimuli, acoustic and vibration waves (Greenfeld, 2016).

Pest monitoring based on growing degree days

Citrus pest activities may vary from one year to another and also within the same year, depending on weather, mainly temperature variation. For citrus trees grown in large areas, monitoring of insects, mainly scale diaspines and thrips species, by collection of samples (leaves, twigs, and fruits) and the beating method (flowers, fruit-set, and fruits, shoots), can be very laborious, time consuming and cost intensive. Using growing degree days between male flights may be a tool to predict the suitable time for application of chemical sprays or the release of natural enemies to control *A. aurantii* (Campos-Rivela *et al.*, 2012). In northern Spain, the same authors, working with Valencia *Citrus sinensis* Osbeck trees, found a thermal constant (615 degree days) across the different development instars of *A. aurantii*, using the lower developmental threshold (11.7°C). Citrus producers can use this technique to identify the male peak flights or the maximum crawler abundance peaks in citrus orchards.

Mating disruption

The mating disruption technique, using synthetically produced chemicals in the orchards to confuse males and limit their ability to perceive calling females, may be effective in controlling *A. aurantii* (Vacas *et al.*, 2009; Leonard, 2019). In Spain, mating disruption techniques using pheromone dispensers reduced the number of downgraded fruits by 60% to 70% (Vacas *et al.*, 2010). In Morocco, the mating disruption technique is a biotechnical method that uses diffusers installed in a citrus orchard at approximately 250 to 300 diffusers/ha. This method is very specific and has no adverse effect on beneficial species and the environment. Such a method can be valid only for isolated orchards, i.e. mandarin Afourer plots. A combination of the mating disruption technique and augmentative biological control using a parasitoid (i.e. *A. melinus*) may be efficient at reducing the occurrence of crawlers on the fruit during the citrus fruit set and can be used to control armored scale between April and June (depending to the zone in the country).

Enhanced mass trapping technology to control medfly and thrips

Mass trapping technology (MTT) can be used to control many citrus pests. In Spain, a combined approach of mass trapping and other methods is used to control mainly populations of medfly (Martinez-Ferrer *et al.*, 2012). In Morocco, producing high fruit quality requires the combination of selected control methods against the targeted pests, mainly medfly and thrips (i.e. *Scirtothrips* sp.). To avoid or to reduce broad-spectrum insecticides, including organophosphate, use of MTT to control *C. capitata* (since 2014) (Smaili *et al.*, 2016) and thrips (since 2021) has increased in Morocco as a new suitable alternative control method to chemical methods. However, during some years, mainly during autumn, despite the use of the MTT, *C. capitata* control method fails to consistently provide sufficient control, particularly in years with high adult medfly abundance in citrus groves. While MTT use in Moroccan orchards is currently efficient, this method could be considerably improved (profitability/cost) in terms of the chosen attractant and the density, maneuverability and location of the traps used. This might depend on the orchard, the prevailing climate conditions of the year and the destination of the production. A study by Smaili *et al.* (2017, 2018b) showed that high medfly population may require addition of complementary methods to mass trapping to control medfly. The authors concluded that BAT (bait appat technique) based on bioinsecticide and fruit resistance seems to improve medfly control using an efficient mass trapping method. Indeed, a current study by Ben-Yazid *et al.* (2020) confirms this funding. MTT combined with another complementary method (i.e. entomopathogen-based biopesticide) as a support might provide additional effects in controlling such dipteral by reducing punctures on fruits.

First attract and kill approach-based mass trapping for thrips species, showed little or non-lasting success coupled with high costs (Smaili, 2019, 2020). However, field trials conducted in lemon citrus, during 2024 (spring and autumnal) and 2025 (spring), showed that installation of 1–2 yellow sticky traps (baited with or without pheromone)/4 trees, proved effective in mass trapping of *Scirtothrips* sp. by reducing fruit infestation under large scale field conditions (Smaili *et al.*, 2025). Therefore, set up of yellow sticky traps-based MTT at suggested rate are recommended for management of this thrips in citrus orchards.

Sterile insect technique to reduce medfly infestation

The sterile insect technique (SIT) is an environmentally friendly pest control method that involves sterilizing male insects using radiation, and their release in target areas, resulting in lower or no fruit infestation. The SIT can be applied in integration with other control methods against the medfly. Indeed, Morocco recently completed the construction of a new mass-rearing facility to apply the SIT to protect citrus produce (IAEA, 2025). The facility will eventually be capable of producing 130 million sterile medfly per week. which will allow releases to cover approximately 55,000 hectares of citrus growing area (Souss and Oriental areas) (Zouhry *et al.*, 2025). The first releases of sterile males were conducted in September 2024 in 7,000 ha in Ouled Aissa area, Taroudante province.

Alternative substances to chemical pesticides

Water and potassium-soap foliar applications: A strong jet of water can remove insects like aphids and spider mites of plant leaves and stems (Adhikari, 2022). Foliar spraying with high pressure of water can reduce the abundance of mite populations by mechanical effect, disrupting their activities and making the environment unsuitable for them, especially on the exposed fruit and leaf surface (Smaili *et al.*, 2022). It applies to all spider mites, especially for *E. orientalis*, that try to settle on the exposed leaves and fruit surface; a single application is sufficient to increase the time between at least two peaks of abundance on leaves and fruit. Aqueous solution of potassium soap might provide additional impact in reducing mite population (Smaili *et al.*, 2022).

Kaolin: Smaili *et al.* (2014, 2016) studied the effects of foliar application with kaolin Under Moroccan conditions and reported that kaolin (99.9 %) protects citrus shoots and fruits against aphids and medfly, suggesting its integration in the IPM approach against citrus pests. Recently, Salerno *et al.* (2020) studied the mechanism of action of kaolin particle film and how this natural product can contribute to developing future physical control barriers against pest insects. We propose kaolin-based foliar application throughout the year and just before the fruit harvesting period to avoid any pesticide residues remaining after foliar application.

Botanical products: Botanical extracts have emerged as another safe alternative to chemical pesticides as biodegradable and with little side effects on natural enemies. Guleria and Tiku (2009) and Ngegba *et al.* (2022) studied the effect of derived plant-based pesticides extracted from many plants, such as neem (from *Azadirachta indica* A. Juss), *Capsicum frutescens* L., pyrethrin (from flower *Chrysanthemum cinerariaefolium);* pyrethrum (from *Tanacetum cinerariifolium*), tobacco (from *Nicotiana tabacum* L.), rotenone (from *Derris* spp., *Lonchocarpus* spp. and *Tephrosia* spp.), sabadilla (from *Schoenocaulon* spp. (*S. officinale*)), thyme essential oil (from *Thymus vulgaris L., Thymus* spp.) and other products. Guleria and Tiku (2009) reported the efficiency of these botanical products and concluded that since botanical insecticides are fast acting and degrade rapidly in sunlight and moisture, frequent foliar applications during precise timing might be necessary. To date, only neem is the main botanical product used for pest control in practice in Morocco. A review of Isman (2020) showed that insecticides based on pyrethrum and neem (azadirachtin) are high performing botanical products, due to the introduction of new jurisdictions. Nevertheless, it must be noted that some botanical components, i.e. essential oils, may have a harmful effect on natural enemies. In our opinion, botanical insecticides need to be carefully selected in relation to possible adverse effects on beneficial species and pollinators, but they must be used more frequently as alternative pest control methods to chemicals in the near future.

Control of snails

In Morocco, methaldehyde-based formulation, a chemical molluscicide, is typically applied to control snails in citrus orchards, mainly *T. pisana*. Indeed, *T. pisana* is the only snail species with known biological cycle in citrus groves in the country: spawning periods, the structure of the populations as well as the movements of this snail between citrus trees and the groundcover. Using this biological data, an appropriate control method could be used to block adults from laying their eggs (i.e. collection of adults or using safe chemical control) during fall and when snail adults located in the tree canopy try to reach ground cover. This measure may decrease the snail population abundance during the following spring.

Physical – cultural control

Hand collection is an important method that enables collecting a high number of snails located on groundcover between the rows of citrus trees (Smaili, 2013; Smaili *et al.*, 2017). The snail collection may continue throughout the year, focusing on snails with large diameter (shell width) and the young stages. In practice, snail collection should be a permanent activity and not limited to occasional collectors who only collect snail species with commercial interest (i.e. *E. vermiculata* and not *T. pisana*). We suggest keeping spontaneous herbaceous plants, when available, between rows as groundcover (not under canopy as this will drive a high snail population to move to the citrus trees) for a long period, during autumn and spring, the seasons with high humidity (and frequent rains, in some years). During autumn, especially for *T. pisana*, when the population becomes active with a high risk of infesting citrus fruit, collecting on canopy is possible only for medium-sized trees as well as for young plantations. Unfortunately, this technique cannot be used in trees with large canopy which would induce high costs and difficult operation during collection.

New trap

The Regional Center of Agricultural Research of Kenitra, Morocco (National Institute of Agricultural Research) developed a new trap for keeping snails out of citrus orchards in 2011 for the first time in the country (Smaili, 2013). The potential use of this trap for keeping snails out (i.e. *T. pisana*) has been recognized as a promising new technology and snail control method in many citrus orchards in the country, mainly in the Gharb region (Smaili, 2017). These traps, settled in the trunk (at least 40-60 cm from soil), catch the snails trying to climb up the tree during their movement from the soil to the tree canopy. Especially for young plantations and small trees, trap use is suggested during February and May (early June in some years). The traps may be improved by using nets with enamel instead of plastic. When the citrus growing area is very large, this method combined with other techniques such as continuous survey and physical control, cover ground management, and biological control, might have an additional positive effect on snails.

Biological control

Duck have been used as a non-chemical control method against the golden apple snail *Pomacea canaliculata* Lamarck (Architaenioglossa: Ampullariidae) in wetland rice ecosystems in China (Liang *et al.*, 2013). Currently, duck production is a common activity in rural Moroccan households to enhance farmers' income as well as food security. Ducks can be used as preventative pest control in the context of natural biological control approaches to reduce snail abundance in citrus orchards. A group made up of 10-20 individuals placed early in the morning in an infested area when snails are active, can provide an appropriate method to reduce snail populations. However, when the citrus area production is very large, a consistent survey of the ducks is necessary to prevent their disappearance.

Integrated Pest Management

Efficient use of selective, appropriate, safe and effective chemical products are major components of the IPM approach which provides an alternative to conventional chemical control. The Economic threshold and the Economic injury level are the basis for pest control decision making, mainly for pesticide applications in IPM citrus groves, while reflecting economic considerations (Pascual-Ruiz *et al.*, 2014). In addition, two key aspects for the success of chemical spraying to control scales are the developmental stages that predominate in the population and the time of invasion by crawlers of the citrus fruitlets (Rodrigo *et al.*, 2004).

In Moroccan citrus groves, the economic threshold may depend on many parameters, mainly biology and population dynamics of the targeted key pest, parameters used for monitoring and the nature of market access and safe product exports. To avoid the settling of a maximum of new crawlers of armored scales (i.e. California red scale), under Moroccan conditions, Smaili *et al.* (2017) discussed various technologies of pest control of armored scales in citrus trees and concluded that many parameters need to be assessed. According to the authors during the process of invading fruit between fruitlets and the fruit growing period, these parameters include i) monitoring of male flight numbers in pheromone-based sticky traps and ii) moving of first crawlers, percentage of highly susceptible armored scales (mainly crawlers, first, second stage and female first instar), iii) percentage of newly infested fruit by crawler during fruitlets period. For each region, the economic threshold must be established before any decision is taken regarding pesticide application. Also, small trials should be performed to ensure and implement the economic threshold. For instance, *E. orientalis* causes discolouration of leaves and fruit where it feeds, while *T. urticae* displays chlorotic spots on the leaves and scars on the fruit. In Morocco, noticeable damage is observed on leaves and later on fruit when the mite abundance increases and reaches 10% of infested leaves (i.e. 10-16 moving nymphs or 4 females of *E. orientalis*/leaf; 3 moving *T. urticae*/leaf) (Smaili *et al.*, 2022). In Spain, chemical control measures are recommended once population of *Eutetranychus* spp. reach the economic injury level of 20% of leaves infested during August to October (Monzó *et al.*, 2016).

Conservation of beneficial species through judicious chemical control

Efficient use of safe pesticides is one of the major components of the IPM approach as well as the knowledge of the effects of pesticides on biological control agents (Bozhgani *et al.*, 2018). Some insecticides and miticides, including insect growth regulators, are toxic and harmful (direct and indirect impact) for one category of beneficial species but safe and selective for another. Biondi *et al.* (2012) discussed the acute and sublethal toxicity of 14 pesticides on the adults of the generalist predator *O. laevigatus* under laboratory conditions and concluded that these pesticides greatly differed in their toxicity, both in terms of lethal and sub-lethal effects, as well as in their persistence. Broad-spectrum insecticides have direct and indirect effects on spider mites that can promote mite outbreaks (Rebek *et al.*, 2012). This is the case in Morocco with, i.e. control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and medfly with pyrethroid insecticides that can cause a resurgence of mites (Smaili *et al.*, 1999b). Similar cases were reported with neonicotinoids that are applied as foliar application to control several pests in the citrus orchards leading to a resurgence of cottony cushion scale *Icerya purchasi* Maskel (Hemiptera: Monophlebidae) (Smaili, personal observations).

According to a study by Torres and Bueno (2018), choosing a selective insecticide or selectively applying one is an important decision for conserving natural enemies. Basically, reducing pesticide interventions means applying an IPM approach, with appropriate application of chemicals only when needed via efficient monitoring of pest population thresholds and efficient product use. Efficient pesticide use intends to avoid broad-spectrum application with persistent compounds and to consider priority and selection of pesticides with high direct and indirect toxicity on pests but without any side effects on natural enemies. Several research studies have provided evidence that the use of many products, including hazardous pesticides, may have side effects on a range of non-target insects, including natural enemies, pollinators, and soil-beneficial insects (Biondi *et al*., 2012; Goulson, 2013; Serrão *et al*., 2022). In Morocco, in the context of good phytosanitary practices, safe pesticides are selected and carefully applied only when needed in commercial citrus orchards certified for Good Agricultural Practices (Smaili *et al*., 2017, 2018b).

Conclusion

In view of the non-target effects of chemical pest control methods and their costs, new approaches are required for sustainable citrus production Morocco. More appropriate solutions using cultural and biological control methods to reduce chemical control could contribute to this direction as well as suitable alternative substances to chemical pesticides. However, in citrus orchards, these alternative pest control approaches may have low direct control effects in the short term compared to conventional chemicals pesticides, due to the need for technical support for application, additional inputs and their variable efficacy.

Conflict of interest
The authors declare no conflict of interest.

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Οικολογικές εναλλακτικές μέθοδοι φυτοπροστασίας στη χημική καταπολέμηση για βιώσιμους οπωρώνες εσπεριδοειδών στο Μαρόκο

M.C. Smaili, N. Haddad, O. Chetto, H. Benyahia και H. Benaouda

Περίληψη Η βιομηχανία των εσπεριδοειδών διαδραματίζει σημαντικό κοινωνικοοικονομικό ρόλο στην εθνική οικονομία του Μαρόκου. Εβδομήντα είδη φυτοφάγων εντόμων, ακάρεων και σαλιγκαριών έχουν καταγραφεί σε εσπεριδοειδώνες στο Μαρόκο. Ωστόσο, μόνο λίγα από αυτά θεωρούνται σοβαροί ή περιστασιακοί εχθροί και ενδέχεται να προκαλέσουν οικονομική ζημία οπότε απαιτούν αντιμετώπιση. Η χρήση ευρέως φάσματος χημικών εντομοκτόνων και ακαρεοκτόνων για την καταπολέμηση εντόμων και ακάρεων έχει δημιουργήσει αρκετά προβλήματα που σχετίζονται με ζητήματα για την ανθρώπινη υγεία, το περιβάλλον και την οικοτοξικότητα. Η παρούσα εργασία παρέχει μια επισκόπηση πρακτικών και προληπτικών, φιλικών προς το περιβάλλον εναλλακτικών λύσεων έναντι της χημικής φυτοπροστασίας, για τη βιώσιμη καλλιέργεια των εσπεριδοειδών στο Μαρόκο. Συζητείται ο ρόλος κατάλληλων βιολογικών και καλλιεργητικών μεθόδων, καθώς και της ασφαλούς χημικής καταπολέμησης, στην αντιμετώπιση των εχθρών των εσπεριδοειδών.

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SHORT COMMUNICATION

New record of *Hylesinus varius* (Coleoptera: Curculionidae: Scolytinae) on olive trees in Insular Greece

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Summary This is a new record of the ash bark beetle, *Hylesinus varius* (Olivier), traditionally known as a pest of *Fraxinus* (ash) and other Oleaceae species, on olive trees (*Olea europaea*) on Milos Island of the Cyclades Group, Greece, after an old report on Crete. Adult specimens were found in galleries of living branches during the olive fruit harvest. This new record emphasizes the need for intensified monitoring of the insect and further research to assess its dispersal and potential impact on olive production.

Additional keywords: Ash bark beetle, *Fraxinus*, galleries, *Hylesinus fraxini, Olea europaea*

Hylesinus varius (= *H. fraxini*) (Olivier 1800) (Coleoptera: Curculionidae: Scolytinae), commonly referred to as the ash bark beetle, is chiefly known for attacking members of the Oleaceae family, such as *Olea* and *Fraxinus* (Stark, 1952). Nonetheless, its host range also includes species in Betulaceae, Fabaceae, Fagaceae, Juglandaceae, Rosaceae, and Sapindaceae (Modarres Awal, 2012).

In October 2024, characteristic bark beetle galleries were discovered in living branches of several olive trees on Milos Island in the Southern Aegean Sea, Greece (36°44′03 N, 24°28′44 E). These galleries became evident when branches were inadvertently damaged during olive fruit harvesting (Fig. 1). Adult beetles retrieved from beneath the bark were transported to the Laboratory of Agricultural Zoology and Entomology at the Agricultural University of Athens (AUA) for identification. Twelve (12) specimens were collected and identified using standard morphological keys (Wood and Bright, 1992; Knížek, 2011), focusing on diagnostic traits such as body size, coloration, and gallery patterns. This process confirmed the presence of *H. varius*, representing the first documented occurrence of the species infesting olives in Greece (Fig. 2). Voucher specimens are preserved in the Laboratory of Agricultural Zoology and Entomology, AUA.

Hylesinus varius has been referred (as *Hylesinus fraxini* and *Leperesinus fraxini*) in Continental Greece (one specimen was recorded at Ouranoupolis, Central Macedonia with no reference to the host-plant), in Northern Aegean (one specimen was recorded at Mytilene with no reference to the host-plant), and in Crete (one specimen was recorded at Rethymnon, with reference to olive as a host-plant) (Peffer, 1995; Knížek, 2011).

Although often regarded as a secondary pest, *H. varius* can become more damaging under suitable conditions. In *Fraxinus*, its infestations may exacerbate crown dieback, particularly when compounded by fungal pathogens (Skovsgaard *et al.*, 2010). High population densities enable *H. varius* to colonize not only stressed or weakened hosts but also healthier, younger, and newly planted trees (Pfister, 2012). Such outbreaks have implications for forest management, as reported in Central Europe (Teusdea *et al.*, 2012), and the beetle's distribution may extend to novel hosts if environmental conditions prove favorable (Lieutier *et al.*, 2004).

Hylesinus varius is widely distributed throughout Europe with olive as a host-

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Figure 1. Olive trees infested by *Hylesinus varius,* Island of Milos, Greece, October 2024.

Figure 2. Adult of *Hylesinus varius,* collected from galleries of infested branches of olive trees, Island of Milos, Greece, October 2024.

plant (Peffer, 1995; Knížek, 2011), extending into parts of Asia (as far as China) and North Africa (Modarres Awal, 2012; Knížek and Modlinger, 2013). Infestation of olive trees in Insular Greece, long after the first report from Crete, raises concerns over the pest's spread and potential impacts on both tree health and production in olive, holding 75% of fruit tree plantations in the country (Hellenic Statistical Authority, 2022). The beetle's propensity to infest weakened bark and young trees suggests that recently established orchards could be particularly vulnerable, while several infestations might threaten older, established groves.

Management strategies have involved sanitation measures, prompt removal of infested branches, and the utilization of natural enemies. Two hymenopteran parasitoids, *Cheiropachus quadrum* (Pteromalidae) and *Dendrosoter protuberans* (Braconidae) which attack the olive bark borer, *Phloeotribus scarabaeoides,* are known to parasitize the ash bark beetle, *H. varius,* (Campos and Lozano, 1994). This shared enemy complex may offer potential avenues for biocontrol or integrated pest management although further study is required.

In conclusion, documenting this new record of *H. varius* infesting olive trees in Insular Greece reinforces the importance of active pest surveillance in agricultural systems. Future research should prioritize understanding its distribution, population dynamics, and interplay with local biotic and abiotic conditions in the olive crop for pest risk assessment, safeguarding the sustainability and productivity of Greek olive orchards.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Νέα καταγραφή του εντόμου *Hylesinus varius* (Coleoptera: Curculionidae: Scolytinae) σε ελαιόδενδρα στη Νησιωτική Ελλάδα

Δ. Ατματζίδης και Α.Ε. Τσαγκαράκης

Περίληψη Η παρούσα ανακοίνωση αφορά σε νέα καταγραφή του σκολύτη του φράξου, *Hylesinus varius* (Olivier), επιβλαβούς εντόμου κυρίως για τον φράξο (*Fraxinus*) και άλλα είδη της οικογένειας Oleaceae, σε ελαιόδενδρα στη Νήσο Μήλο των Κυκλάδων μετά από παλαιά αναφορά για το έντομο στην Κρήτη. Ενήλικα άτομα βρέθηκαν εντός στοών σε κλάδους μετά από τη θραύση τους κατά την διάρκεια της ελαιοσυλλογής. Η νέα αυτή καταγραφή υπογραμμίζει την ανάγκη για εντατικοποίηση της παρακολούθησης και περαιτέρω έρευνα για να κατανοηθεί η διασπορά του εντόμου και δυνητική επίδρασή του στην ελαιοπαραγωγή.

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Toxicity of ozone fumigation on *Galleria mellonella* larvae and latent effects on pupation and adult emergence

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Summary The greater wax moth *Galleria mellonella* L. is a serious pest of honey bee combs in hives. Ozone fumigation was applied on fourth instar larvae at three concentrations, 0.5, 1.0 and 2.0 g/m^3 for 30, 60, 90 and 120 min. Our findings showed that the two concentrations of 0.5 and 1.0 g/m^3 were less effective than that of 2.0 g/m^3 on larval mortality with 40 and 60%, respectively, at 120 min of exposure time after 7 days. Full larval mortality (100%) was obtained by 2.0 g/m^3 of ozone at 90 min exposure time in 3 and 7 days post treatment. Our findings demonstrated that ozone had latent effects on pupation as well as adult emergence from treated larvae of *G. mellonella*. Exposure to 0.5 g/m^3 for 120 min, 1.0 g/m^3 for 120 min and 2.0 g/m^3 for 60 min resulted in the lowest pupation percentage (60, 40 and 0%, respectively) and strong inhibition of adult emergence (16.7, 0 and 0% adult emergence, respectively. Our research indicates that ozone fumigation is a useful strategy for the control of the greater wax moth.

Additional keywords: adult emergence, fumigation, greater wax moth, larval mortality, ozone, pupation

Introduction

The wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), is a major insect pest of honey bee combs in hives (Hanley *et al.,* 2003). Colony losses associated with the pest are considerable in a lot of places; the effect is stronger in weaker colonies and stored combs (Graham 1992; Ellis *et al.,* 2013; Sohail *et al.,* 2017). The larvae consume pollen, honey, wax combs, and the skins of cast honey bee larvae. The presence of larval wax moth in combs is indicated by their production of silken threads, which are frequently tunnel-like inside the combs.

Management of *G. mellonella* is applied by synthetic fumigants which question the potential risks on honey bee and human health, and the environment (Desneux *et al.,* 2007). In this respect, safe and effective control methods such as ozone fumigation and freezing have been tested for the control of *G. mellonella* (Zhu *et al.,* 2016; Nofal *et al.,* 2024). Ozone (O_3) is an unstable gas created when oxygen is exposed to a high voltage electric discharge (Kim *et al.,* 1999). It has been suggested as an excellent alternative for conventional fumigants such as phosphine gases since it has a short half-life (20–50 min) due to its rapid breakdown into oxygen, and therefore does not leave any residues on treated stored products, significantly deplete nutrients in commodities or cause insect resistance. (Mendez *et al.,* 2003).

Several studies illustrate how ozone applied to stored goods effectively combated a range of insect pests (Isikber *et al.,* 2007; Niakousari *et al.,* 2010; James, 2011; Jemni *et al.,* 2015; Ayad, 2019; Gad *et al.,* 2021a, b; Ingegno and Tavella, 2022; Sitoe *et al.,* 2024; Metwaly *et al.,* 2024). The mortality of *G. mellonella* caused by ozone gas has been reported by other studies (James, 2011; Nofal *et al.*, 2024). However, the latent effect of ozone on biological aspects such as pupation and adult emergence from treated larvae is limited. As a result, the current study focuses on studying the susceptibility of fourth instar larvae to ozone and their latent effects on pupation and adult emergence.

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Materials and Methods

Greater wax moth rearing

Galleria mellonella was mass cultured in the laboratory using larvae that were collected from infested beehives. Fourth instar larvae which were raised on bee wax for many generations at 26°C and 65% R.H. were used in the tests.

Ozone production

The ozone was generated using techniques reported by Gad *et al.* (2021b) at the Centre of Plasma Technology, Al-Azhar University, Nasr City, Cairo, Egypt.

Exposure of larvae of greater wax moth to ozone fumigation

To determine the effect of ozone on fourth instar larvae of the greater wax moth, 20 larvae were introduced inside small plastic tubes (5 cm high and 3 cm diameter) which were then placed into glass fumigation jars (35 cm high and 20 cm diameter). A metal lid with an inlet and an exit was used to cover the fumigation jar. The ozone generator was connected to the fumigation jars through a plastic tube extending from the entrance to the bottom of the fumigation jar. Both openings of the lid were secured with a valve. The larvae were exposed to the desired gas concentrations (0.5, 1.0 and 2.0 g/m^3) for 30, 60, 90 and 120 min and then the treated larvae were moved to 500 ml glass jars. For the control group, the larvae were exposed to air without ozone. For each ozone concentration and the control, four replicates (jars of 20 larvae) were set up. To determine the percentage of mortality, the mortality of larvae was recorded 3 and 7 days post exposure. After that, treated larvae were transferred into glass jars (3 cm diameter and 5 cm depth) with muslin cloth coverings for incubation under the same conditions mentioned above. They were checked every day until pupation and adult emergence (Al-Ayat *et al.,* 2025). Percent pupation, adult emergence and survival were calculated using the following formulas:

Pupation (%) = (Number of pupae/Total number of larvae) × 100

Adult emergence (%) = (Number of moths/Total number of pupae) × 100

Survival (%) = (Number of moths/Total number of larvae) × 100

Data analysis

The data of mortality, pupation adult emergence were transformed Arcsine. ANOVA was performed and the means were compared using Tukey's test ($P < 0.05$) in SPSS version 21 (Haynes, 2013).

Results

Larval mortality of *G. mellonella*

The mortality percentage of treated larvae of *G. mellonella* increased gradually with an increase in either concentration of ozone or exposure time. The two concentrations of 0.5 and 1.0 g/m^3 were less effective than that of 2.0 g/m^3 on larval mortality with 30 and 40% ($P < 0.01$; $F = 2.5$; df = 3, 12), respectively (Table 1) at 120 min of exposure time and 3 days post treatment. After 7 days of treatment, the two concentrations of 0.5 and 1.0 g/m^3 induced larval mortalities (40 and 60%) ($P < 0.01$; $F = 3.1$; df = 3, 12), respectively at 120 min of exposure time (Table 1). The highest concentration (2.0 g/m^3) induced the maximum larval mortalities (80 and 90%) at 60 min of exposure, 3 days ($P < 0.01$; $F = 763.7$; df = 3, 12) and 7 days after treatment ($P < 0.01$; $F = 907.6$; df = 3, 12), respectively. Full larval mortality (100%) was obtained by 2.0 g/m^3 of ozone at 90 min exposure time, 3 and 7 days post treatment.

Latent effects of ozone fumigation on *G. mellonella*

The delayed effect of ozone fumigation on pupation, adult emergence and survival percentage of *G. mellonella* is shown in Table 2. The lowest pupation percentage (60, 40 and 0%) was obtained from treated larvae by 0.5, 1.0 and 2.0 g/m^3 at 120, 120 and 60 min exposure time, respectively ($P < 0.01$; $F = 209.0$; df = 4, 15), ($P < 0.01$; $F = 38.9$; df = 4, 15) and ($P < 0.01$; $F = 600.0$; df = 4, 15).

Table 1. Percentage larval mortality of *Galleria mellonella,* 3 and 7 days after fumigation with different ozone concentrations for different exposure times.

Concentration (g/m^3)	% Larval mortality (mean ± SE) after 3 days				% Larval mortality (mean ± SE) after 7 days			
	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Control	0.0±0.0c	0.0±0.0d	0.0±0.0d	0.0±0.0d	0.0±0.0b	0.0±0.0d	0.0±0.0d	0.0±0.0d
0.5	5.0±2.9bc	17.5±2.5c	25.0±1.2b	30.0±1.2c	10.0±1.4b	27.5±2.5c	30.0±1.6c	40.0±4.1c
1.0	10.0±2.0b	30.0±2.6b	40.0±1.0b	40.0±2.9b	30.0±2.0a	40.0±2.6b	50.0±1.0b	60.0±4.1b
2.0	20.0±2.0a	80.0±2.2a	100.0±0.0a	100.0±0.0a	40.0±4.0a	90.0±2.0a	100.0±0.0a	100.0±0.0a
F	15.0	763.7	883.0	2.5	40.0	907.6	8.6	3.1
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

There is no significant difference between the mean values in each column that are followed by the same letter (df = 3, 12, $P < 0.05$).

Table 2. Latent effects of ozone fumigation at different concentrations and different exposure times, on pupation, adult emergence and survival of *Galleria mellonella* treated as fourth instar larvae.

Exposure times (min)	Ozone concentration of 0.5 g/m^3			Ozone concentration of 1.0 g/m^3			Ozone concentration of 2.0 g/m^3		
	Pupation (%)	Adult emergence (%)	Survival (%)	Pupation (%)	Adult emergence (%)	Survival (%)	Pupation (%)	Adult emergence (%)	Survival (%)
0	100±0.0a	100±0.0a	100±0.0a	100±0.0a	100±0.0a	100±0.0a	100±0.0a	100±0.0	100±0.0
30	90.0±0.8b	88.9±3.4b	80.0±1.9b	70.0±4.0b	28.9±1.8b	20.0±4.1b	50.0±3.5b	0.0±0.0	0.0±0.0
60	72.5±2.5c	55.4±1.8c	40.0±2.2c	60.0±1.2bc	16.9±1.0c	10.0±2.0c	0.0±0.0c	0.0±0.0	0.0±0.0
90	70.0±1.2c	25.0±3.6d	17.5±2.5d	50.0±4.0cd	0.0±0.0d	0.0±0.0d	0.0±0.0c	0.0±0.0	0.0±0.0
120	60.0±4.1d	16.7±1.0e	10.0±1.7e	40.0±4.1d	0.0±0.0d	0.0±0.0d	0.0±0.0c	0.0±0.0	0.0±0.0
F	209.0	433.0	1231.0	39.8	1997.0	9.4	600.0		
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		

There is no significant difference between the mean values in each column that are followed by the same letter (df = 4, 15, $P < 0.05$).

The lower concentration (0.5 g/m^3) caused strong suppression of adult emergence and survival (16.7 and 10.0%), ($P < 0.01$; $F = 433.0$; $df = 4, 15$) and ($P < 0.01$; $F = 1231.0$; $df = 4, 15$), respectively. The two concentrations (1.0 and 2.0 g/m^3) induced full suppression (0%) of adult emergence and survival ($P < 0.01$; $F = 1997.0$; $df = 4, 15$) and ($P < 0.01$; $F = 9.4$; $df = 4, 15$), respectively.

Discussion

Since methyl bromide was added to the list of chemicals that cause ozone depletion, many fumigants have been proposed as an alternative to methyl bromide (UNEP, 1995). Ozone is one of these fumigants, which has been demonstrated to have some potential for controlling postharvest insects (Mahroof *et al.,* 2018). Our investigation demonstrated that the larvae of *G. mellonella* were highly sensitive to ozone fumigation. The larval mortality increased with increasing ozone concentration and exposure time. The ozone concentration of 2.0 g/m^3 was effective to reach complete larval mortality in two exposure times, 90 and 120 min. Our findings are in line with several reports on other insects indicating that ozone fumigation was effective on larvae of postharvest insects. Kells *et al.* (2001) showed that ozone induced higher larval mortality of *Plodia interpunctella* (Hübner) by 50 ppm after three days of treatment. Isikber *et al.* (2007) reported that 300 ppm of ozone for 2 h of exposure time caused complete larval mortality of *Ephestia kuehniella* (Zeller). Osman (2009) found that 1 g/m^3 of ozone caused complete mortality of *E. kuehniella* larvae after 6 days of exposure. Hussain (2014) stated that larvae of *Ephestia cautella* (Walker) treated with 80 ppm caused 100% mortality after 5 h of exposure. Ibrahim and Al-Ahmadi (2014) observed that 80 ppm for 1 h caused the larval mortality of *Phthorimaea operculella* (Zeller) (80.0%). Keivanloo *et al.* (2014) demonstrated that the larval mortality of *P. interpunctella* (85.0%) was achieved by ozone treatment (5 ppm) for 2 h. Husain *et al.* (2015) ozone fumigation caused complete larval mortality of *E. cautella* by 22 ppm for 24 h of exposure time. Zinhoum and El-Shafei (2019) showed that complete larval mortality of *P. interpunctella* was obtained after 90 min of exposure to 1.0 g/m^3 of ozone. Mahmoud *et al.* (2023) revealed that the LC_{50} values were 275.3 and 446.8 ppm for the second and fifth larval instars of the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermetstidae), after 2 h of exposure time.

The high toxicity of ozone against larvae of *G. mellonella* found in the present investigation may be due to the strong oxidizing properties of the gas. According to Hermes-Lima (2004), when ozone disintegrates into dioxygen, free radicals of reactive oxygen species (ROS) are produced which can significantly change DNA and protein molecules as well as oxidize polyunsaturated fatty acids. As a result, according to Holmstrup *et al.* (2011), these effects may damage cells and kill insects.

Moreover, our findings demonstrated full suppression of pupation, adult emergence and survival, by ozone fumigation at the concentration of 2.0 g/m^3. Similarly, Bonjour *et al.* (2011) demonstrated that ozone induced full inhibition of *Tribolium castaneum* (Herbest) progeny by 70 ppm treatment for four days. Abd El-Ghaffar *et al.* (2016) mentioned the progeny of *Sitotroga cerealella* (Olivier) was completely suppressed by treatment of the larval stage with 5 g/m^3 for 5 h. Abd El-Ghaffar *et al.* (2017) showed that the complete suppression of pupation of red flour beetle from treated larvae with ozone was achieved after 5 h exposure of 5 g/m^3. Subramanyam *et al.* (2017) indicated that the ozone fumigation was effective in progeny suppression of *Rhyzopertha dominica* (F.) at lower concentrations (0.42 and 0.84 g/m^3). The same findings were obtained by Xinyi *et al.* (2019).

Finally, the present study provided valuable and promising data on the application of ozone fumigation against the greater wax moth under laboratory conditions. However, there are some limitations to the application of ozone gas as a fumigant to control this insect pest in stored hives such as the difference between fumigation chambers used in this study and places of stored hives, which are far more complex, with irregular internal structures, variable airflow, and potentially porous materials like wax, wood, and propolis that could absorb or react with ozone. These factors may lead to uneven ozone distribution, reducing its efficacy against pests in some parts of the hive. Also, field trials are needed to determine whether ozone-treated comb is acceptable to bees, and if adequate fumigation conditions can be achieved on a large field-use scale, where the woodenware will slow the accumulation of ozone within a chamber (James, 2011). Also, the cost of ozone generators is still high for beekeepers. However, the sustainable application for ozone fumigation will reduce the cost and might become cheaper if the application of this technology increases under field conditions.

Conclusion

Based on our findings, the ozone fumigation can cause full larval mortality of *G. mellonella* after exposure for 90 min to 2.0 g/m^3 while lower time exposure of 30 min at this concentration can cause full inhibition of adult emergence similarly with 90 min exposure to 1.0 g/m^3, supporting the potential use of ozone in IPM strategies against the pest. Further studies are needed on the efficacy of ozone against the pest under field conditions and its impact on treated stored hives in terms of delay in the development of insect resistance as well as to reduce the risk to honeybees, humans and the environment.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Τοξικότητα του υποκαπνισμού με όζον στις προνύμφες του κηρόσκωρου, *Galleria mellonella*, και λανθάνουσες επιδράσεις στη νύμφωση και την έξοδο των ενηλίκων

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Περίληψη Ο κηρόσκωρος, *Galleria mellonella* L., είναι ένας σοβαρός εχθρός των κηρηθρών των μελισσών στις κυψέλες. Ο υποκαπνισμός με όζον εφαρμόστηκε σε προνύμφες τέταρτης ηλικίας σε τρεις συγκεντρώσεις, 0,5, 1,0 και 2,0 g/m^3 για 30, 60, 90 και 120 λεπτά. Τα αποτελέσματα έδειξαν ότι οι συγκεντρώσεις των 0,5 και 1,0 g/m^3 ήταν λιγότερο αποτελεσματικές από αυτή των 2,0 g/m^3, με 40 και 60% θνησιμότητα των προνυμφών, αντίστοιχα, σε χρόνο έκθεσης 120 λεπτών, 7 ημέρες μετά την εφαρμογή. Καθολική θνησιμότητα (100%) των προνυμφών επιτεύχθηκε με 2,0 g/m^3 όζοντος σε χρόνο έκθεσης 90 λεπτών, 3 και 7 ημέρες μετά την εφαρμογή. Τα αποτελέσματα έδειξαν ότι το όζον είχε λανθάνουσες επιδράσεις στη νύμφωση καθώς και στην έξοδο των ενηλίκων του *G. mellonella*. Τα χαμηλότερα ποσοστά νύμφωσης και εξόδου ενηλίκων καταγράφηκαν μετά από έκθεση σε 0,5 g/m^3 για 120 λεπτά, 1,0 g/m^3 για 120 λεπτά και 2,0 g/m^3 για 60 λεπτά και ήταν 60, 40 και 0% για τη νύμφωση, αντίστοιχα, και 16,7, 0 και 0% για την έξοδο των ενηλίκων, αντίστοιχα. Συμπερασματικά, φαίνεται ότι ο υποκαπνισμός με όζον είναι μια χρήσιμη πρακτική για την αντιμετώπιση του κηρόσκωρου.

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SHORT COMMUNICATION

First record of the poinsettia thrips *Echinothrips americanus* Morgan (Thysanoptera: Thripidae) in Greece

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Summary The presence of the poinsettia thrips, *Echinothrips americanus* Morgan (Thysanoptera: Thripidae), is reported for the first time in Greece. In spring 2023, specimens of *E. americanus* were collected from infested *Rhoicissus rhombifolia* plants in Attiki.

Additional keywords: *Echinothrips americanus*, Greece, *Rhoicissus rhombifolia*

Thrips are one of the most harmful crop pests worldwide, not only by reducing yield via direct feeding damage to foliage and fruits but also because there are vectors of important plant viruses. Because of the aesthetic damage that thrips cause, especially to ornamental crops, the tolerable population threshold is usually at a very low level. Thrips' feeding causes silver-grey patches and black dots of their excreta on leaves as well as streaking of petal tissues that are unacceptable to consumers (Lewis, 1997; Ullman *et al*., 1997).

In March 2023, heavily infested leaves of *Rhoicissus rhombifolia* (Vitaceaea) by thrips were sent to the Laboratory of Agricultural Entomology of Benaki Phytopathological Institute (BPI) from a private garden in Vouliagmeni, Attiki (37°49′52.03″N, 23°46′36.99″E) (Fig. 1). Adult thrips were collected and stored in 70% alcohol. Samples submerged in 10% KOH at room temperature overnight to clear and were mounted in Hoyer medium. Voucher specimens have been deposited in the collection of BPI. For specimen identification, the zur Strassen's dichotomous taxonomic key was used. The species has body brown to dark brown, antennal segment III and basal halves of segments IV-V white (Fig. 2). The forewings are grey-brown, basally paler, with vein setae blunt to weakly capitate (Fig. 3 and 4). The inner surface of reticulations on head and pronotum minutely wrinkled (Fig. 5). Tergites laterally with numerous microtrichiae (Fig. 6). In male, sternites III-VIII densely covered on entire surface by numerous small rounded areae porosae (Fig. 7) (zur Strassen, 2003). Photographs were taken with an EVOS XL Core imaging system. All collected thrips, 9 females and 5 males in total, were identified as *Echinothrips americanus* Morgan (Thripidae). This is the first record of this species in Greece.

All *Echinothrips* species are native to the Americas. *Echinothrips americanus* was originally described in Florida and it is known to be native throughout eastern North America (Mound and Marullo, 1996; Vierbergen, 1998). It is the only *Echinothrips* species that has become widespread established outside its native range, primarily as a green-

Figure 1. Feeding damage of *Echinothrips americanus* on *Rhoicissus rhombifolia* (Vitaceae).

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Figure 2. The poinsettia thrips *Echinothrips americanus* Morgan (Thysanoptera: Thripidae).

Figure 3. *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Forewing basally paler.

Figure 4. *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Forewing vein setae blunt to weakly capitate.

Figure 5. *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Minute wrinkled of inner surface reticulations on head and pronotum.

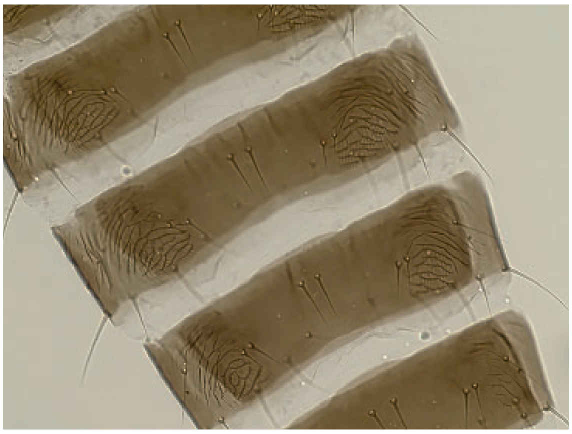

Figure 6. *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Tergites with numerus microtrichiae laterally.

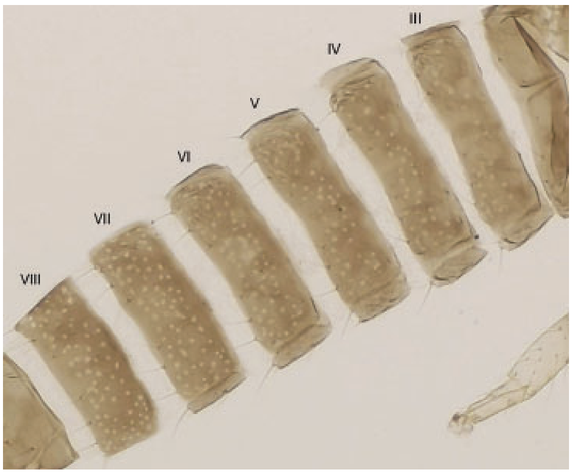

Figure 7. *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Sternites III-VIII of male with numerous area porosae.

house pest over Canada, Asia, and Northern Australia (Hoddle *et al*., 2012; Krueger *et al*., 2015; EPPO, 2024). The first interception of the species in Europe was in United Kingdom in 1989 (Collins, 1998) whereas the first report of crop infestation was in 1993 in the Netherlands, where the species was found on plants belonging to the genera *Syngonium* and *Homalomena* (Cevat and Roosjen, 1994; Vierbergen, 1998). It has spread rapidly across Europe since 1995 and among other countries it has been found in France (Reynaud, 1998), Italy (Marullo and Pollini, 1999), Bulgaria (Karadjova and Krumov, 2003), Serbia (Andjus *et al*., 2009) and Malta (Degabriele *et al*., 2023). It is currently reported in at least 22 countries (Vierbergen *et al.,* 2006; EPPO, 2024).

Its rapid spread suggests that *E. americanus* is a successful opportunist, easily overlooked during import inspections due to the subtle nature of the damage (Vierbergen *et al*., 2006; Mirab-Balou *et al*., 2010). EPPO first included *E. americanus* on its alert list in 1995. Over the next few years, there was little evidence of significant damage from this species in the countries where it had been detected. In 2000, it was concluded that the species did not meet the criteria for classification as a quarantine pest and that sufficient alerts had already been issued (EPPO, 2000).

The host range of *E. americanus* is remarkably broad, making it the most economically significant species within its genus. It has been recorded on 41 plant genera from 27 different families (Vierbergen *et al*., 2006; Mound, 2021; Mound *et al*., 2022). According to Collins (1998), it is mainly associated with species of the family Araceae which are commonly grown as ornamental plants. It can also develop high population densities on many other plants, including *Capsicum* and various weeds (Vierbergen, 1998).

The poinsettia thrips causes significant damage to various vegetable and ornamental crops through extensive foliage feeding (Vierbergen *et al*., 2006; Varga *et al*., 2010). In ornamental plants, it leads to silver leaf damage, chlorotic lesions, and leaf deformations causing aesthetic deterioration (Oetting *et al*., 1993). Due to the generally low cosmetic damage thresholds, the market value of affected plants may be severely impacted (Kaas, 2001). Considering the polyphagous status of the pest, plant protection measures against thrips in greenhouse and ornamental plant production in Greece should include the potential presence of *E. americanus*. Heteroptera bugs of the genus *Orius* (Mouratides *et al*., 2022) and the phytoseid mite *Amblyseius swirskii* (Athias-Henriot) (Ghasemzadeh *et al*., 2017) are the most significant generalist predators which could contribute to the biological control of the poinsettia thrips.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του θρίπα *Echinothrips americanus* Morgan (Thysanoptera: Thripidae) στην Ελλάδα

Ι.Χ. Λύτρα, Δ.Μ. Μαρκογιαννάκη και Π.Γ. Μυλωνάς

Περίληψη Ο θρίπας *Echinothrips americanus* Morgan (Thysanoptera: Thripidae) βρέθηκε για πρώτη φορά στην Ελλάδα τον Μάρτιο του 2023. Το έντομο βρέθηκε στο καλλωπιστικό φυτό του είδους *Rhoicissus rhombifolia* (E.Mey. ex Harv.) Planch (Vitaceae: Vitales) στην Αττική.

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Dissipation patterns and characteristics of four pesticides in sandy and clay soil under controlled conditions

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Summary The dissipation of pesticides significantly influences their behaviour in soil, which is crucial for evaluating their stability and safety. This study investigated the dissipation patterns and half-lives of four pesticides—ametryn, bentazone, carbofuran, and oxamyl—in sandy and clay soils at two concentration levels (25 $mg \cdot kg^{1}$ and 100 $mg \cdot kg^{1}$). The experiment was conducted at 26°C with a 60% water-holding capacity. First-order kinetics effectively described the dissipation ($R^2 > 0.92$). After 60 days, pesticide dissipation exceeded 97% in sandy soil, while remaining residues were 80–86% for ametryn, 80–89% for bentazone, and 85-88% for carbofuran and oxamyl. In clay soil, dissipation was initially slower (<8% for all pesticides), but subsequently accelerated. The quantity of pesticides declined sharply in the first month, followed by a gradual decrease in the second month. Ametryn exhibited the longest half-life, whereas bentazone had the shortest. Overall, pesticide loss correlated with decreased concentrations and organic matter content.

Additional keywords: dissipation, half-life, pesticides, residue, soil

Introduction

A significant challenge confronting modern agriculture is the necessity to secure sufficient food supplies for the world's expanding population. As a result, pesticides are designed to enhance agricultural production (Marín-Benito *et al.*, 2019). Approximately 3.0 million tons (Mt) of active ingredients and 7.0 Mt of formulated products are utilised annually for crop protection (Silva *et al.*, 2019; FAO, 2022).

Herbicides play a crucial role in enhancing agricultural productivity by efficiently controlling the rivalry between crops and weeds for essential nutrients in the soil (FAO, 2018). Their application facilitates the optimisation of resource availability, thereby enhancing crop yields and ensuring efficient nutrient utilization in diverse agricultural practices systems. The ideal herbicide should effectively target only the weed and stay active in the environment for a suitable period (Marín-Benito *et al.*, 2019). Ametryn and bentazone are two selective herbicides commonly used to control weeds in many crops in Egypt. These herbicides are typically applied either by pre-plant incorporated treatments or at pre-emergence stage. Their interactions and behaviour within the soil significantly affect their effectiveness in controlling weeds and their environmental impact persistence.

Carbofuran and oxamyl are pesticides frequently used to control insects and nematodes on several crops because they have a wide-range biological action (Subramanian and Muthulakshmi, 2016; Kuswandi *et al.*, 2017). As systemic insecticides, they enter the plant through its roots and are then distributed throughout its organs to reach insecticidal concentrations. These pesticides are applied directly to the soil surface or plants (Alvarez *et al.*, 2022). Despite their effectiveness, they are among the most dangerous pesticides (Kuswandi *et al.*, 2017).

Once pesticides reach the soil, they dissipate through various processes, includ-

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ing surface runoff, volatilization, chemical hydrolysis, leaching, and microbial degradation (Davie-Martin *et al.*, 2015). The contribution of each dissipation process varies depending on the pesticide, soil type, and environmental factors. Sorption and desorption, along with degradation, are the primary processes that influence the behavior of pesticides in soils (Vagi *et al.*, 2010; Marín-Benito *et al.*, 2019). Research on the environmental behavior of pesticides has indicated that certain pesticides remain in the soil for an extended period, while others degrade quickly and do not persist (Rahman *et al.*, 2020). There is a strong correlation between environmental pollution by pesticides and their persistence. Nonetheless, during breakdown processes, new chemicals that could be either more or less toxic than the original compound may be generated (El-Aswad *et al.*, 2024). The half-life of a pesticide is a crucial factor in determining whether it is likely to accumulate in the soil.

To effectively assess environmental safety, it is crucial to gather data on the rate of pesticide degradation. This information is needed to understand how the fate of applied pesticides changes, taking into account potential variations in degradation factors (Purnama *et al.*, 2014; Rani and Sud, 2015). At present, the nematicides carbofuran and oxamyl, along with the herbicides ametryn and bentazone, are being utilised across various agricultural crops in Egypt. Nonetheless, information regarding the dissipation rates of these pesticides in Egyptian clay and sandy soils remains insufficient. The half-lives of these pesticides can exhibit considerable variation influenced by soil properties and environmental conditions. Accordingly, it is essential to establish these parameters within controlled laboratory environments, which are both cost-efficient and labour-effective (Purnama *et al.*, 2014).

The present research focused on the dissipation trends and persistence of four commonly used pesticides—ametryn, bentazone, carbofuran, and oxamyl—within two prevalent soil types in Egypt: clay and sandy soil. Clay soil, renowned for its exceptional water retention and nutrient-rich properties, was contrasted with sandy soil, which offers improved drainage but has a lower nutrient content. Conducted under controlled conditions, the study aimed to closely monitor the degradation of these pesticides over time in each soil type. Understanding their environmental behavior is essential for promoting sustainable agricultural practices and assessing their potential impact on the ecosystem.

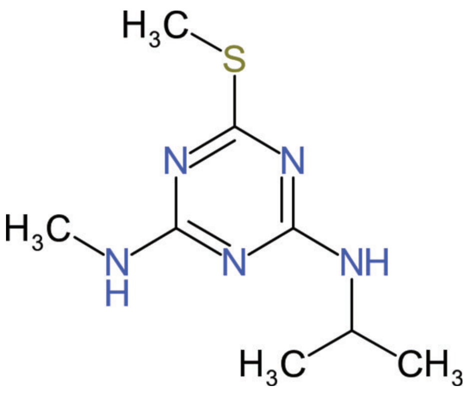
Materials and Methods

Chemicals and reagents

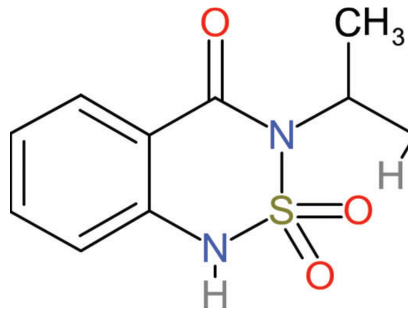
Ametryn, bentazone, carbofuran, and oxamyl (purity > 99%) reference standards were obtained from Dr Ehrenstorfer GmbH in Augsburg, Germany. The chemical structures of these pesticides are depicted in Figure 1. All reagents and chemicals used in the experiment were supplied by Supelco (Bellefonte USA). Stock solutions of ametryn, bentazone, carbofuran, and oxamyl were meticulously prepared at a concentration of 1000 $\mu g \cdot mL^{-1}$ using acetonitrile as solvent and stored at a temperature of -20°C. Subsequently, appropriate stock solutions were diluted in acetonitrile to generate working standard solutions for the studied pesticides. Calibration standards were then created by combining the working standards with acetonitrile and water in a 60:40 (v/v) ratio, covering a concentration range from 1 to 1000 $ng \cdot mL^{-1}$. The working standard solutions also underwent serial dilutions with blank soil extracts to establish a matrix-matched calibration standard.

Experimental soil

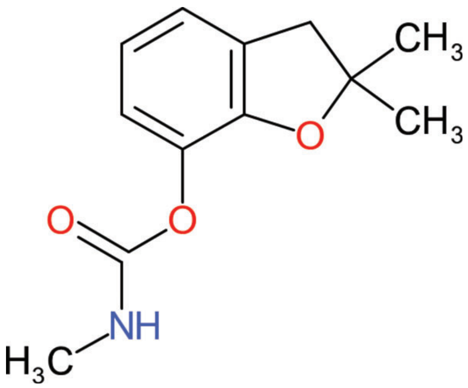
The clay soil utilised in this study was sourced from the experimental farm at Menofyia University's Faculty of Agriculture. The sandy soil was acquired from the Sadat area in the Menofyia Governorate of Egypt. Soil samples were collected from 0 to 15 cm depth, air-dried, and ground to pass through a 2 mm sieve to remove larger fragments and undecomposed plant material.



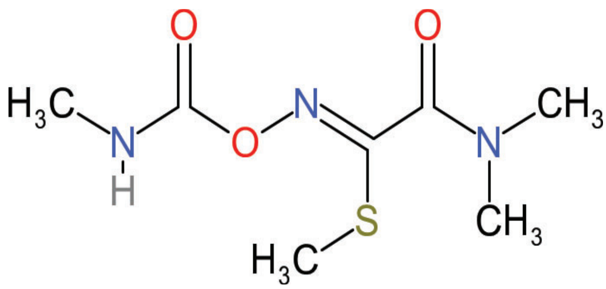
Ametryn



Bentazone



Carbofuran



Oxamyl

Figure 1. Chemical structure of the pesticides ametryn, bentazone, carbofuran, oxamyl.

The processed soil samples were carefully stored in plastic bags to prevent contamination and kept at room temperature throughout the study. To ensure accurate analysis, a representative subsample was meticulously extracted from each batch of soil samples for detailed physicochemical testing. The results of this analysis are presented in Table 1, which offers a comprehensive overview of several key characteristics, including the distribution of soil particle sizes, the concentration of total organic carbon, the pH level of the soil, and the cation exchange capacity, which indicates the soil's ability to hold and exchange essential nutrients. This information is essential in evaluating the soil's quality and potential agricultural productivity. The amount of water added to the soil was determined using a water retention curve established after one year of monitoring field soil moisture. The calculated gravimetric water content (w) was determined using the following equation:

$$W = \frac{\textit{wet soil weight - dry soil weight}}{\textit{wet soil weight}} \quad (1)$$

The collected soil samples were oven-dried at 105°C for 24 h to obtain the dry weight.

Pesticides dissipation studies

The degradation rates of ametryn, bentazone, carbofuran, and oxamyl were studied in two different soil types (clay and sandy soils) following the OECD (Organization for Economic Co-operation and Development) Guideline for the Testing of Chemicals (OECD, 2025). Prior to the application of pesticides, 500 g of air-dried soil was positioned within a glass container, subsequently rehydrated to reestablish microbial activity, and

Table 1. Physical and chemical properties of the under study soils.

Parameters	Sandy soil	Clay soil
Depth (cm)	0-15	0-15
pH (H_2O)	7.93	7.82
Organic carbon content (%)	0.30	1.10
Organic matter content (%)	0.51	1.89
Electrical conductivity (ds/m)	29.53	1.91

incubated for a duration of two weeks at a temperature of 26°C in the absence of light to facilitate seed germination and removal. Both soil samples were contaminated with ametryn, bentazone, carbofuran, and oxamyl in glass bottles at 25 and 100 mg·kg^{-1} concentrations. The contaminated soils were thoroughly mixed with pre-incubated samples before applying water treatments.

The soil specimens were kept in an incubator at a constant temperature of 26°C, maintaining the moisture level at 60% of the soil's water-holding capacity (WHC). Every 2 days, the samples were exposed to ensure adequate aeration and to regulate the moisture levels by replenishing the lost water. At the commencement of the experiment, soil samples were collected 0, 3, 7, 15, 30, and 60 days after treatment with the test pesticide.

Analytical procedures

Sample extraction

Five grams of soil samples (± 0.1g) were procured from a glass container at regular intervals. These samples were subsequently processed in a 50-mL centrifuge tube by combining them with 10 mL of acetonitrile and 7 mL of distilled water; vigorous agitation followed for 15 minutes and sonication for 10 minutes (Asensio-Ramos *et al*., 2010). Next, 1 g of sodium chloride, 4 g of anhydrous magnesium sulfate, 0.5 g of disodium hydrogen citrate sesquihydrate, and 1 g of trisodium citrate dehydrate were added to the extraction tube, and the mixture was once again vigorously shaken for 10 minutes and centrifuged at 5000 rpm for 5 minutes. Then, 1 mL of the resulting supernatant was collected, filtered using a polytetrafluoroethylene (PTFE, 0.22 µm) syringe filter, and transferred to a glass vial for analysis via LC-MS/MS.

Chromatographic conditions

The Exion liquid chromatography system, interfaced with a 6500 + AB SCIEX QTRAP mass spectrometer, was employed to analyze residues of ametryn, bentazone, carbofuran, and oxamyl in samples soil. The separation process was conducted on a Synergi C18 column (2.5 µm Fusion-RP 100 Å, 3.0 × 50 mm) from Phenomenex, with the column temperature maintained at 40°C. The mobile phase consisted of (a) 10 mM ammonium formate in water (pH=4) and (b) methanol. The total runtime was 15 minutes, following a gradient elution method: 0 min at 100% A; from 1 to 10 min, a transition from 100% to 0% A; from 10 to 12 min at 0% A; and from 12 to 15 min back to 100% A. Injection volume was 2 µL, with a 15 min runtime. Ionization was achieved through electrospray (ESI) in the positive ion mode using multiple reaction monitoring (MRM). Details of the quantification and validation of critical parameters such as retention time, collision energy potentials (CE), collision cell exit potential (CXP), declustering potential (DP), and entrance potential (EP) for the tested compounds are provided in Table 2. Optimizations were made for gas sources and parameters: ion spray voltage set at 5500 v for ESI (+); ion source temperature at 400°C; curtain gas maintained at 20 psi; and adjustment of collision gas medium.

Method validation

The proposed method for analyzing residues was assessed following the EU SANTE/11312 guidelines for validating residue analytical methods (SANTE, 2021). Linearity was evaluated using linear regression analysis on calibration curves of standard solutions and matrix-matched samples. The complexity of the soil matrix may influence the analytical process by either suppressing

Table 2. LC-MS/MS parameters for determination of the pesticides ametryn, bentazone, carbofuran, oxamyl.

Compound	Retention Time (Min)	Precursor (m/z)	Product (m/z)	Declustering potential (Volts)	Entrance potential (Volts)	Collision energy (Volts)	Collision cell exit potential (Volts)
Ametryn	10.2	228.1	186.2	11.0	25.0	6.5	11.0
	10.2	228.2	96.1	4.5	33.0	4.0	4.5
Bentazone	5.9	241.0	199.0	10.0	19.0	10.0	10.0
	5.9	241.0	107.0	10.0	39.0	10.0	10.0
Carbofuran	7.9	222.1	165.2	9.5	17.0	6.5	9.5
	7.9	222.1	123.0	10.0	29.0	2.0	10.0
Oxamyl	2.7	237.1	90.1	4.0	11.0	3.0	4.0
	2.7	237.1	72.0	5.5	21.0	6.5	5.5

or enhancing the response, potentially compromising the accuracy, selectivity, and sensitivity of the method. To evaluate possible interferences in the chromatographic response, a matrix effect study was conducted in the range from 1 to 100 $\mu g \cdot L^{-1}$. Calibration curves, prepared in triplicate either in the matrix extract or in the solvent for LC-MS/MS, were utilised. The matrix effect (ME) was assessed by comparing the response obtained for each analyte in the soil extract with that in the solvent at the same concentration in LC-MS/MS, after subtracting the background signal of the soil matrix. No significant matrix effects were deemed present when ME ranged between 80 and 120%. The predominant trend observed for ME in LC-MS/MS was a tendency towards signal suppression, although this effect was not statistically significant for the compounds tested. Nevertheless, to enhance the accuracy of quantification and to streamline the procedure, the decision was made to employ matrix-matched calibration LC-MS/MS.

Accuracy and precision were determined through recovery experiments conducted at three spiking levels (10, 30, and 60 $\mu g \cdot kg^{-1}$) in the soil (Figure 2). These tests were performed over three consecutive days with six replicates each day. The limit of quantification (LOQ) was defined as the lowest concentration at which the recovery percentage falls between 80% and 120% and the relative standard deviation (RSD) remains below 20% (SANTE/11312/2021, 2021).

Statistical analysis

The degradation of ametryn, bentazone, carbofuran, and oxamyl in the soil is presumed to follow the first-order kinetics model. A first-order rate equation determined the dissipation rate constant and half-life (DT_{50}).

$$C_t = C_0 e^{-kt} \qquad (2)$$

The concentrations of ametryn, bentazone, carbofuran, and oxamyl residues ($mg \cdot kg^{-1}$) at time t after treatment and the initial pesticide concentrations are denoted by C_t and C_0, respectively. The constant k represents the rate of dissipation. Furthermore, the half-lives (DT_{50}) of ametryn, bentazone, carbofuran, and oxamyl in the soil are calculated using the formula.

$$DT_{50} = \frac{ln\ (2)}{k} \qquad (3)$$

Results and Discussion

Method validation

The conclusions of this examination were obtained from measuring peak areas

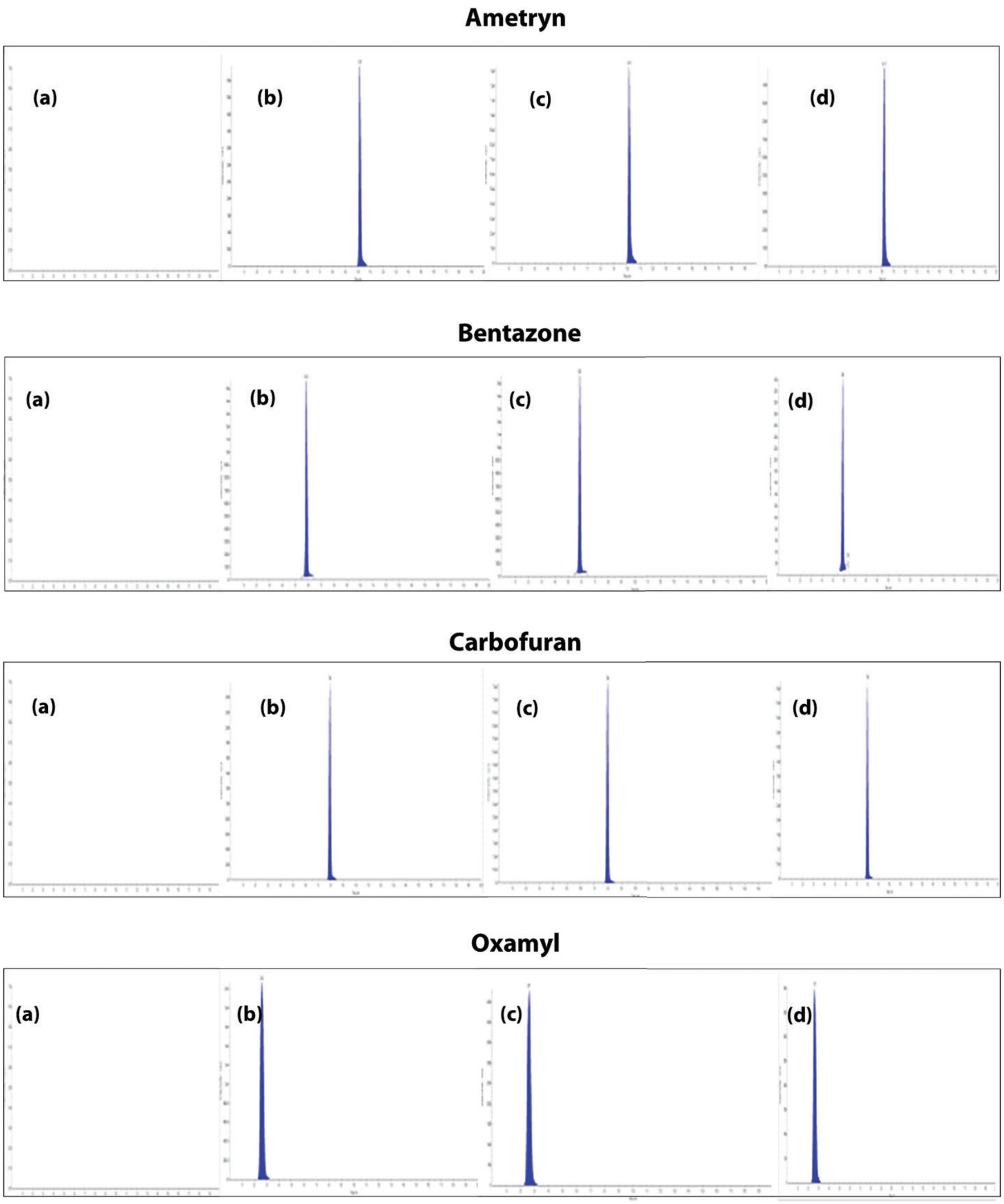


Figure 2. LC–MS/MS chromatograms of the pesticides ametryn, bentazone, carbofuran, oxamyl, in different media (a) blank soil, (b) standard in solvent (10 μg·kg^{-1}) (c) standard in soil matrix (10 μg·kg^{-1}), (d) spiked soil sample (10 μg·kg^{-1}).

using a calibration curve. The correlation coefficient (R^2) for all pesticides analyzed was greater than 0.999, indicating a strong level of linearity in the findings. Additionally, the impact of the matrix was evaluated by comparing the pesticide standards in a solvent (acetonitrile) with standards specific to the matrix (soil) over six repetitions at a concentration of 30 μg·kg^{-1}. The results indicated no interference from natural peaks, and the retention time (RT) of the substances in the spiked soil sample matched that of the standard samples, as shown in Table 2. The average recovery values ranged from 91% to 98%, with a relative standard deviation (RSD) between 3.8% and 12.5% for all pesticides tested. The study assessed measurement precision using two methods: repeatability and reproducibility. Repeatability (RSD_r) was established through three analyses conducted on the same day. In contrast, reproducibility (RSD_R) was evaluated via a single analysis performed over three days, focusing on a fortification level of 10 μg·kg^{-1}, as detailed in Table 3. The RSD_r and RSD_R values for all tested pesticides did not exceed 15% (Table 4). During the method validation process, the limit of quantification (LOQ) for ametryn, bentazone, carbofuran, and oxamyl in soil was established at ten μg·kg^{-1} based on validation and precision data obtained from recovery assessments. The satisfactory recovery and precision indicate that the analytical method is suitable for detecting ametryn, bentazone, carbofuran, and oxamyl in soil.

Dissipation of pesticides in soil

Pesticides are routinely employed to safeguard crop plants against weeds, diseases, insect damage, and nematodes. These chemicals typically interact with the soil, undergoing a series of transformations, resulting in a complex array of metabolites (Carpio *et al.*, 2021). Figure 3 presents the dissipation curves for ametryn, bentazone, carbofuran, and oxamyl at two varying concentrations in clay and sandy soils over a 60-day incubation period at 26°C. Overall, the dissipation graphs for the pesticides examined show a steady decline over time for all the soil treatments and concentration levels (Figure 3). The initial concentrations two h after pesticide treatments at low concentrations ranged from 22.52±1.44 to 24.89±1.90 mg·kg^{-1} for sandy soil and from 23.70±1.71 to 25.56±0.07 mg·kg^{-1} for clay soil. Also, high concentrations ranged between 81.70±2.14 and 99.01±1.92 for sandy soil and 97.60±1.70 and 108.68±2.92 for clay soil. After 30 days of incubation, ametryn residues at low concentrations in sandy and clay soil reduced to 10.20±0.07 and 15.30±0.04 mg·kg^{-1} (58% and 40% of the initial dose), while at high concentrations decreased to 40.20±0.07 and 55.10±0.01 mg·kg^{-1} (49%, and 51% of initial dose), respectively. Also, the residue of bentazone at low concentrations in sandy and clay soils decreased to 1.8±0.90 and 9.1±0.03 mg·kg^{-1} (92% and 62% of the initial dose), while at high concentrations decreased to 23.3±0.05 and 62.1±0.10 mg·kg^{-1} (76%, and 41% of initial dose), respectively. In addition, on the 30th day, the carbofuran residue in sandy and clay soil decreased to 5.4±0.11 and 9.8±0.06 mg·kg^{-1} (78% and 61% of the initial dose) at low concentration while decreased to 35.07±0.091 and 46.3±

Table 3. Recovery % and relative standard deviation (RSD%) of the pesticides ametryn, bentazone, carbofuran, oxamyl in soil (n = 6).

Compound	Spiked level (ng·mL^{-1})	Recovery (%)	RSD (%)
Ametryn	10	98	3.80
	30	92	5.23
	60	95	6.74
Bentazone	10	94	4.26
	30	91	8.43
	60	97	11.79
Carbofuran	10	96	6.54
	30	93	10.23
	60	91	12.50
Oxamyl	10	94	6.71
	30	92	9.44
	60	98	4.74

Table 4. Recovery%, RSD_r%, and RSD_R% values were acquired from the analysis of samples fortified with the pesticides ametryn, bentazone, carbofuran, oxamyl in soil at 60 ng·mL^{-1} (n = 6).

Compound	Analysis day	Recovery (%)	RSD_r (%)	RSD_R (%)
Ametryn	1	97	12.70	13.41
	2	100	11.80	
	3	95	9.80	
Bentazone	1	94	7.60	14.26
	2	92	3.14	
	3	96	4.56	
Carbofuran	1	98	12.21	9.40
	2	100	10.24	
	3	92	8.80	
Oxamyl	1	95	7.14	11.07
	2	93	6.36	
	3	99	8.79	

0.03 mg·kg^{-1} (63%, and 53% of initial dose) at high concentration, respectively. Moreover, the residue of oxamyl at low concentrations decreased to 6.5±0.081 and 9.1±0.077 mg·kg^{-1} (72% and 68% of the initial dose), while at high concentrations decreased to 32.07±0.03 and 25.1±0.05 mg·kg^{-1} (72%, and 64% of initial dose), in sandy and clay soil, respectively.

The observed degradation of the tested pesticides exhibited a modest response to variations in concentration levels. Pesticides can undergo various processes in soil, including hydrolysis, photolysis, oxidation, or reduction (Singh *et al*., 2021). Various factors, including microorganisms in the soil influence the breakdown rate of pesticides (Masutti and Mermut, 2007; Magalhães *et al*., 2018). The elevated levels of pesticides can negatively impact soil microorganisms, which are essential for breaking down pollutants, potentially hindering their functions. After the incubation period, degradation levels in sandy soil reached an impressive percentage >97%, with pesticide residue percentages recorded as follows: 80–86% for ametryn, 89–80% for bentazone, 87–88% for carbofuran, and 87–85% for oxamyl after 60 days, for both low and high concentration levels. In contrast, the initial dissipation of the tested pesticides in clay soil was slow (less than 8% for all pesticides) across both concentration treatment levels; however, the dissipation rate subsequently demonstrated a slight increase, as illustrated in Figure 3. These results indicate that pesticide dissipation occurred marginally faster in sandy soil than in clay soil. The significantly enhanced persistence of the tested pesticides in clay soil, as opposed to sandy soil, is likely attributable to the higher organic matter content and clay content, which result in a greater adsorption capacity for pesticides in clay soil (Badawy *et al*., 2017; Fouad *et al*., 2023; El-Aswad *et al*., 2024;). Besides, there is a higher activation energy threshold for degradation/volatilization (Carpio *et al*., 2021). Therefore, pesticides are less bioavailable to be degraded *(*James *et al*., 2019*;* Vischetti *et al*., 2020). Within the first month of incubation, the pesticide quantities were strongly reduced, followed by a gradual decrease in the second month.

Half-lives of pesticides in soil

The dissipation rate constants for all examined pesticides in sandy soil, at both low and high concentration levels, ranged between 0.0485 and 0.0819 d^{-1}, corresponding

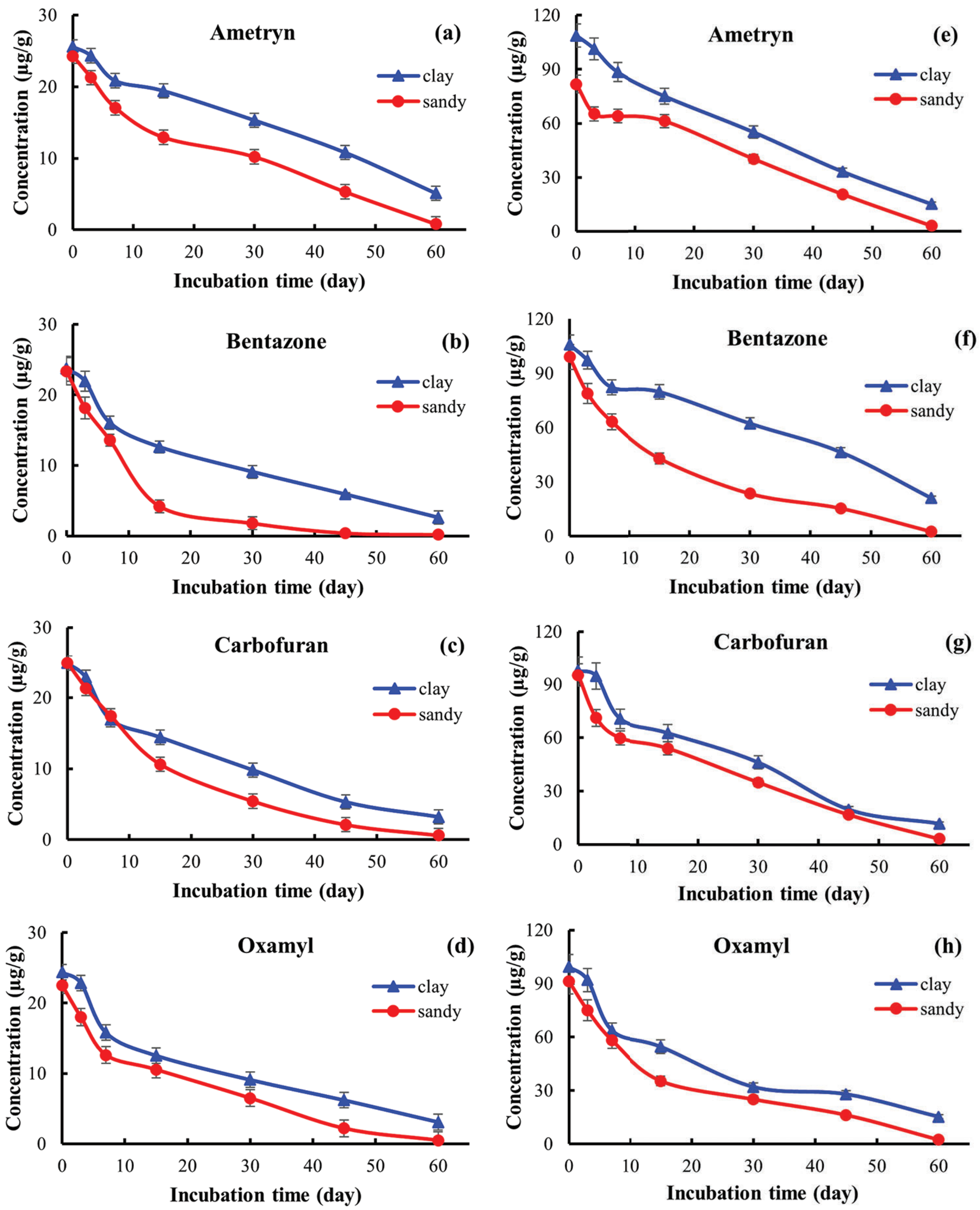


Figure 3. Dissipation pattern of the pesticides ametryn, bentazone, carbofuran, oxamyl, in sandy and clay soils spiked with low concentrations (a, b, c, d) and high concentrations level (e, f, g, h).

to half-lives spanning 14.3 to 8.5 days. In addition, the rates for low and high concentration levels were measured between 0.0466 and 0.0550 d^{-1}, translating to half-lives ranging from 14.8 to 12.6 days, respectively (see Table 5). Conversely, in clay soil, the dissipation rate constants for all tested pesticides at low and high concentration levels varied between 0.0242 and 0.0340 d^{-1}, with corresponding half-lives from 28.6 to 20.4 days and between 0.0237 and 0.0348 d^{-1}, correlating to half-lives between 29.2 and 19.9

Table 5. Regression equation, correlation coefficient and half-life (DT_{50}) of the pesticides ametryn, bentazone, carbofuran, oxamyl in soil.

Low concentration						
Compound	Regression equation		Correlation coefficient (R^2)		DT_{50} (days)	
	Sandy	Clay	Sandy	Clay	Sandy	Clay
Ametryn	-0.0485x + 3.3124	-0.0242x + 3.2913	0.8889	0.9417	14.3	28.6
Bentazone	-0.0819x + 3.0370	-0.0340x + 3.1336	0.9848	0.9775	8.5	20.4
Carbofuran	-0.0599x + 3.2837	-0.0333x + 3.1831	0.9903	0.9919	11.6	20.8
Oxamyl	-0.0578x + 3.1591	-0.0319x + 3.1261	0.9579	0.9772	11.9	21.7
High concentration						
Compound	Regression equation		Correlation coefficient (R^2)		DT_{50} (days)	
	Sandy	Clay	Sandy	Clay	Sandy	Clay
Ametryn	-0.0466x + 4.5931	-0.0307x + 4.749	0.8551	0.9691	14.8	22.6
Bentazone	-0.0550x + 4.6242	-0.0237x + 4.6874	0.9391	0.9270	12.6	29.2
Carbofuran	-0.0485x + 4.6052	-0.0348x + 4.6271	0.9175	0.9761	14.3	19.9
Oxamyl	-0.0523x + 4.5278	-0.0293x + 4.4977	0.9169	0.9670	13.3	23.6

days, respectively (see Table 5). Variations in rate constants and half-lives were noted based on the soil type.

A strong linear relationship was established between the logarithmic concentrations of ametryn, bentazone, carbofuran, and oxamyl residues over time, indicating first-order kinetic rates of pesticide dissipation, as evidenced by a correlation coefficient R^2 values were generally greater than 0.92, except for ametryn in sandy soil. Several studies addressing pesticide dissipation have described the dissipation process utilizing first-order kinetics (Huan *et al*., 2013; Wang *et al*., 2014; Hou *et al*., 2016; Badawy *et al*., 2017; Ge *et al*., 2017; Fouad *et al*., 2023; El-Aswad *et al*., 2024). The half-lives of all tested pesticides were longer in the case of clay soil than in sandy soil. This result agrees with those obtained for different pesticides (Badawy *et al*., 2017; Fouad *et al*., 2023; El-Aswad *et al*., 2024). It can be observed that among the pesticides studied, ametryn possesses the longest half-life. In comparison, bentazone exhibits the shortest half-life in sandy soil, recorded as 14.3 and 8.5 days, and in clay soil, measured at 28.6 and 20.4 days, at the lower concentration levels of 14.8 and 12.6 days in sandy soil, and at the higher concentration (Table 5).

Our results revealed that ametryn was more persistent than bentazone in sandy and clay soils, whereas carbofuran was less persistent in these soils. This result can be attributed to the adsorption process, which is correlated with water solubility. The water solubility of ametryn (200 mg/L) is lower, while that of bentazone (7112 mg/L) is higher than that of carbofuran (351 mg/L). Other studies supported this result (El-Aswad *et al*., 2024).

Furthermore, the molecular structure of the pesticides determined the breakdown mechanism; a significant and minor mechanism could occur, or some mechanisms could occur together or consequently (Kah *et al*., 2007; Juretic *et al*., 2014; Ruomeng *et al*., 2023). The chemical structure of bentazone, which had a shorter half-life than carbofuran, includes S=O and C=O bonds that can chemically break down quickly. Additionally, the molecule contains a few methyl groups that may be easily demethylated. Meanwhile, ametryn, which has a longer half-life than carbofuran, only has methyl groups that can be demethylated. Both bonds of C=O and methyl groups are included in the chemical structure of carbofuran. The breakdown of these compounds could be attributed to the chemical breakdown of these bonds and the demethylation process.

Moreover, the persistence of pesticides in soil could be due to the amino groups that bind with soil components (El-Aswad *et al*., 2023). The ametryn molecule has two amino groups, the carbofuran molecule has one amino group, and the bentazone molecule lacks any amino groups. The soil's combination of moisture and temperature fluctuations encourages the growth of different microorganisms (Broznić and Milin, 2012). Consequently, microbial breakdown is among the initial possible routes for the loss of pesticides, as bacteria have adapted to use pesticides as energy sources (Bending *et al*., 2003). It was observed that adding clay to the soil increased adsorption processes, resulting in increased pesticide persistence in the soil (Copaja and Gatica-Jeria, 2021). High concentration increased persistence in a laboratory trial using thiobencarb and butachlor for 90 days (Jitender *et al*., 1993). In this study, the degradation rate of the examined pesticides at elevated concentration levels exceeded 96% of the initial concentration. However, the residual concentration of the tested pesticide in both soil samples was 9% higher than at the lower concentration level. This finding indicates the persistence of pesticides at higher concentration levels.

Conclusion

The dissipation patterns of ametryn, bentazone, carbofuran, and oxamyl in clay and sandy soils were evaluated over a 60-day incubation period at 26°C, using two distinct concentration levels. The dissipation process adhered to first-order kinetics and demonstrated a notable increase in the dissipation rate in sandy soil relative to clay soil. Among the pesticides examined, ametryn exhibited the most extended half-life. In contrast, bentazone exhibited the shortest half-life in sandy soil at both low and high concentration levels, and in clay soil at lower concentrations.

Author contribution
Sara Heikal: conceptualization, methodology, formal analysis, software, data curation, investigation, writing—review and editing. Farag Malhat: conceptualization, investigation, visualization, writing—original draft, writing—review and editing. Anwar El-Sheikh: investigation. Mahmoud Rashwan: investigation. Ahmed F. El-Aswad: conceptualization, supervision, writing—reviewing and editing, project administration.

Data availability
All of the data analyzed and used during the current study will be available from the corresponding author on reasonable request.

Competing interests
The authors declare no competing interests.

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Μοτίβα αποδόμησης και χαρακτηριστικά τεσσάρων φυτοφαρμάκων σε αμμώδες και αργιλώδες έδαφος υπό ελεγχόμενες συνθήκες

S. Heikal, F. Malhat, A. El-Sheikh, M. Rashwan και A.F. El-Aswad

Περίληψη Η αποδόμηση των φυτοφαρμάκων επηρεάζει σημαντικά τη συμπεριφορά τους στο έδαφος, γεγονός κρίσιμο για την αξιολόγηση της σταθερότητας και ασφάλειάς τους. Η παρούσα μελέτη εξέτασε τα μοτίβα αποδόμησης και το χρόνο ημίσειας ζωής τεσσάρων φυτοφαρμάκων—ametryn, bentazone, carbofuran και oxamyl—σε αμμώδη και αργιλώδη εδάφη, σε δύο επίπεδα συγκέντρωσης (25 mg/kg και 100 mg/kg). Το πείραμα πραγματοποιήθηκε στους 26°C με 60% ικανότητα κατακράτησης ύδατος, με την κινητική πρώτης τάξης ($R^2 > 0.92$) να περιγράφει αποτελεσματικά την εν λόγω αποδόμηση. Μετά από 60 ημέρες, η αποδόμηση των φυτοφαρμάκων ξεπέρασε το 97% στο αμμώδες έδαφος, ενώ τα εναπομείναντα υπολείμματα ήταν 80–86% για το ametryn, 80–89% για το bentazone και 85–88% για τα carbofuran και oxamyl. Στο αργιλώδες έδαφος, η αποδόμηση ήταν αρχικά πιο αργή (<8% για όλα τα φυτοφάρμακα), αλλά επιταχύνθηκε στη συνέχεια. Η ποσότητα των φυτοφαρμάκων μειώθηκε απότομα τον πρώτο μήνα και ακολούθησε σταδιακή μείωση τον δεύτερο μήνα. Το ametryn παρουσίασε τον μεγαλύτερο χρόνο ημίσειας ζωής, ενώ το bentazone τον μικρότερο. Συνολικά, η μείωση των φυτοφαρμάκων συσχετίστηκε με τη μείωση της συγκέντρωσης και της περιεκτικότητας σε οργανική ουσία.

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