Volume 19, Issue 1, January 2026
ISSN 1791-3691 (Print)
ISSN 2732-656X (OnLine)

Hellenic Plant Protection Journal

A semiannual scientific publication of the
BENAKI PHYTOPATHOLOGICAL INSTITUTE

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Hellenic Plant Protection Journal **19:** 1-4, 2026
DOI 10.2478/hppj-2026-0001

SHORT COMMUNICATION

First record of *Theridula aelleni* (Araneae: Theridiidae) in Crete, Greece

T. Angelioudakis[1*], A. Nikolakakis[2], K. Varikou[2], G. Koubouris[2], R. Tarifa[3,4] and P.J. Rey[4,5]

Summary *Theridula aelleni* (Hubert, 1970) (Araneae: Theridiidae) is a Mediterranean species with a limited known distribution, so far reported only in Tunisia, Madeira, and Spain. Here, we present the first record of *T. aelleni* in Greece. Two adult males were collected in an olive grove in the area of Asterousia in the Messara valley of Heraklion, Crete during pitfall trapping in 2022 and 2025. Identification was confirmed through comparison with diagnostic characters provided in the original description and subsequent taxonomic treatments. This finding significantly extends the known geographical range of the species eastwards within the Mediterranean basin and contributes to expanding our knowledge regarding the spider fauna of Greece.

Additional keywords: Mediterranean fauna, olive groves, species distribution, spider

Theridiidae Sundevall, 1833 is one of the most diverse spider families globally, comprising more than 2,500 described species in over 120 genera (World Spider Catalog 2025). Theridiids exhibit substantial morphological and ecological variation but are generally characterized by small to medium body size, three tarsal claws, the absence of paracymbium in males, and the presence of serrated bristles forming a comb on tarsus IV (Jocqué and Dippenaar-Schoeman, 2006). Members of the family typically construct irregular space webs with threads radiating in multiple directions, and prey is subdued using a characteristic wrap-bite attack involving sticky silk (Jocqué and Dippenaar-Schoeman, 2006). In the Mediterranean region their diversity remains incompletely documented, partly due to their small size and inconspicuous morphology, which can reduce detection during general surveys. In Greece, the family is represented by approximately 100 species, with around 50 species recorded from Crete (World Spider Catalog 2025), reflecting a particularly rich theridiid fauna on the island.

Globally, the genus *Theridula* Emerton, 1882 comprises only 16 described species, reflecting a relatively small group within Theridiidae. Of these, three species occur in Europe — although some authors consider *Theridula opulenta* (Walckenaer, 1841) a synonym of *Theridula gonygaster* (Simon, 1873), which would reduce the number to two. Despite the presence of these species in neighboring Mediterranean countries, no member of the genus has previously been recorded from Greece (World Spider Catalog 2025). Species of the genus are small-bodied and characterized by a distinct set of morphological features: the colulus and its setae are absent, the cheliceral promargin bears one or two teeth, the male palp lacks

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conductor, median apophysis and theridiid tegular apophysis, and the cymbium has no distal process; additionally, leg chaetotaxy includes two dorsal bristles on tibiae I, II and IV, one dorsal bristle on tibia III, and a trichobothrium present on metatarsus III but absent on metatarsus IV (Le Peru, 2011).

In this paper, we report the first record of *T. aelleni* from Greece, based on two male specimens collected in olive groves in Crete, thereby extending the known range of the species and contributing to the documentation of Greek Theridiidae.

Sampling was conducted as a part of the regular (monthly) monitoring program for epigeal arthropods in olive groves under the LIFE project "Olivares Vivos +". Sampling was carried out using pitfall traps placed both in the olive fields and in adjacent seminatural habitats (12 and 8 pitfalls, per farm, respectively; see Rey *et al.*, 2019, for more details on sampling procedure). In particular, the collected specimens of *T. aelleni* come from an intensive olive-growing region of Messara in southern Crete, within the Heraklion regional unit, Greece, between the villages of Stavies and Stoloi. One adult male was collected on 19 May 2022 (35° 1'47.43" N, 25° 1'49.55" E) and a second adult male on 19 July 2025 (35° 1'50.47" N, 25° 1'26.12" E).

Identification was carried out through comparison with diagnostic characters and images available on the online database Araneae Spiders of Europe (Nentwig *et al.*, 2025). The specimen has been preserved in 70% ethanol and deposited in the Laboratory of Agricultural Zoology and Entomology of Agricultural University of Athens. Photographs were taken using a Leica S9i Digital Stereo Microscope imaging system (Benaki Phytopathological Institute).

Theridula aelleni was originally described by Hubert (1970) under the genus *Theridion*, and at that time it was considered closely related to, and potentially conspecific with *Theridion spinitarse* O. Pickard-Cambridge, 1876. Subsequent taxonomic revisions clarified the diagnostic differences between the two species and led to the transfer of *T. aelleni* to the genus *Theridula*, where it is currently placed (Knoflach *et al.*, 2009).

Material examined. 2♂, Greece, Crete, Heraklion, olive grove, pitfall trap, 1♂ 19.05.2022 (Coordinates: 35° 1'47.43" N, 25° 1'49.55" E); 1♂ 19.07.2025 (Coordinates: 35° 1'50.47" N, 25° 1'26.12" E).

Habitus. The male specimen possesses a uniform light brown prosoma, with eight eyes arranged in two rows. The chelicerae and pedipalps are similarly pale. The legs are slender and pale yellow. The abdomen is longer than wide, displaying a dorsal pattern of black markings that contrast with the brown background coloration (Fig. 1).

Male palp. The palp (Figs 2, 3) is characterized by a globular bulb and a narrow, elongated cymbium bearing long dorsal setae. The embolus is elongated and corkscrew shaped. This combination of morphological characteristics is consistent with published descriptions and illustrations of *T. aelleni* (Hubert, 1970; Knoflach *et al.*, 2009; Le Peru, 2011), supporting the identification of the specimen.

Habitat. Males of *T. aelleni* were trapped at the margins of an olive orchard adjacent to a river (first sampling site), where species such as *Arundo donax* L. (Poaceae), *Nerium oleander* L. (Apocynaceae), and *Rubus fruticosus* L. (Rosaceae) abound, and in an abandoned vineyard (second sampling site). The study

Figure 1. *Theridula aelleni* Hubert 1970, male, habitus dorsal view.

Figure 2. *Theridula aelleni* Hubert 1970, male, palp lateral view.

area of Messara (southern Crete) is characterized by semi-arid conditions, with high summer temperatures reaching up to 40°C and a mean annual rainfall of approximately 300–450 mm. The Messara plain extends south–southwest of the Heraklion Prefecture, south of Mount Psiloritis and north of the Asterousia Mountains, and is bordered by the Libyan Sea. The landscape is dominated by a continuous olive grove comprising of at least 150,000 trees.

The detection of the species on two separate sampling occasions (2022 and 2025) suggests that *T. aelleni* is established at the site rather than representing a sporadic dispersal or accidental introduction. The new locality fills part of the biogeographical gap between western Mediterranean, North Africa and the eastern Mediterranean, suggesting that the species may be more widespread than currently documented. Its detection in an olive grove also highlights the role of Mediterranean agroecosystems as reservoirs of understudied spider diversity.

Additional fieldwork will be essential to assess the true range of *T. aelleni* in Greece and the broader eastern Mediterranean. Such data will contribute to a more complete understanding of the diversity and distribution of Theridiidae in the region.

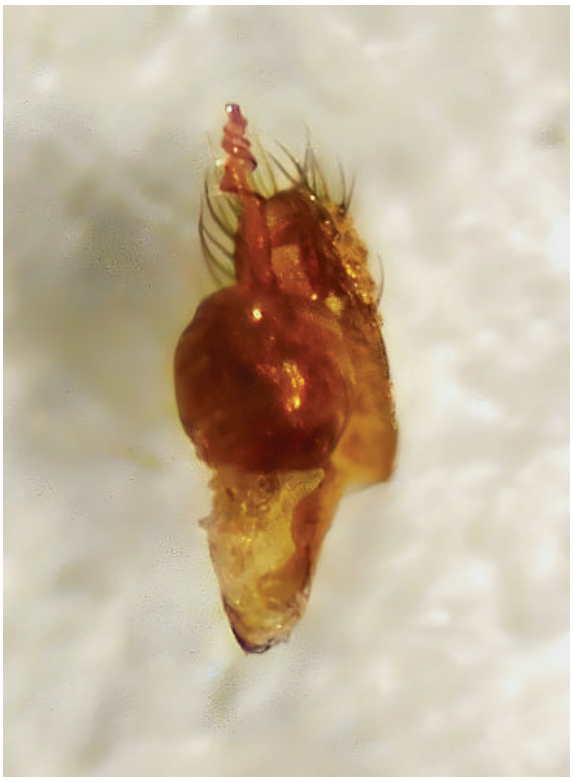

Figure 3. *Theridula aelleni* Hubert 1970, male, palp ventral view.

The research was conducted in olive groves of the Agricultural Cooperative Famelia, with the assistance of Manolis Iatrakis and Spyros Fanourgakis. The authors wish to express their sincere gratitude to D.P. Papachristos (Benaki Phytopathological Institute) for granting access to the laboratory facilities and equipment used for photographic documentation, and to I.Ch. Lytra for her valuable assistance with the operation of the equipment.

Author contribution

P.J.R. and R.T. conceived the ideas and designed the methodology for the olive grove epigeal arthropod monitoring; T.A. analyzed the data and led the writing of the manuscript; K.V. and A.N. collected the spider samples and T.A. identified the samples. All authors, including G.K. contributed critically to the final version of the manuscript and gave final approval for its publication.

Funding

This study was funded by the project 'Olivares Vivos+' (LIFE Olivares Vivos + Increasing the

impact of Olivares Vivos in the EU, Reference: LIFE20 NAT/ES/001487) of the Life Program of the European Commission.

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Received: 18 December 2025; Accepted: 30 December 2025

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του είδους *Theridula aelleni* (Araneae: Theridiidae) στην Κρήτη

Θ. Αγγελιουδάκης, Α. Νικολακάκης, Κ. Βαρίκου, Γ. Κουμπούρης, R. Tarifa και P.J. Rey

Περίληψη Το είδος *Theridula aelleni* (Hubert, 1970) (Araneae: Theridiidae) είναι Μεσογειακό είδος με περιορισμένη εξάπλωση, καθώς μέχρι σήμερα έχει αναφερθεί μόνο στην Τυνησία, τη Μαδέρα και την Ισπανία. Στην παρούσα εργασία παρουσιάζεται η πρώτη καταγραφή του *T. aelleni* στην Ελλάδα. Δύο ενήλικα αρσενικά άτομα συλλέχθηκαν σε ελαιώνα στην περιοχή Αστερούσια Μεσσαράς του Νομού Ηρακλείου Κρήτης, μέσω παγίδων παρεμβολής (pitfall traps), κατά τα έτη 2022 και 2025. Η ταυτοποίηση επιβεβαιώθηκε με σύγκριση των διαγνωστικών μορφολογικών χαρακτήρων που περιγράφονται στην αρχική περιγραφή του είδους καθώς και σε μεταγενέστερες ταξινομικές μελέτες. Το εύρημα αυτό επεκτείνει σημαντικά την γνωστή γεωγραφική κατανομή του είδους προς την ανατολική Μεσόγειο και συμβάλλει στη διεύρυνση της γνώσης για την αραχνο-πανίδα της Ελλάδας.

Hellenic Plant Protection Journal **19:** 1-4, 2026

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Hellenic Plant Protection Journal **19:** 5-14, 2026
DOI 10.2478/hppj-2026-0002

First record of Dermestid and Ptinid beetles as insect pests of stored silkworm products in Egypt – Description of morphological features

M.M.M. Bedewy[1], A.H. Elsaffany[1] and H.A. Gad[1*]

Summary *Attagenus fasciatus* (Thunberg, 1795) and *Anthrenus coloratus* Reitter 1881 (both Coleoptera: Dermestidae), and the spider beetle *Gibbium psylloides* (Czempinski, 1778) (Coleoptera: Ptinidae), were recorded for the first time in stored silkworm products such as cocoons and feaces in Egypt. Notes on the detailed morphological descriptions for all species are presented. The impact on national sericulture industry should be considered. *Attagenus fasciatus* and *A. coloratus* are serious pests of silkworm cocoons which are damaged by their larvae boring into them to feed on pupae. *Gibbium psylloides* is a minor pest of the silkworm industry feeding on dry debris plant, and feaces.

Additional keywords: Anthrenus coloratus, Attagenus fasciatus, Dermestidae, *Gibbium psylloides,* Ptinidae, Sericulture

Introduction

One of the most significant industries in many parts of the world, sericulture, contributes significantly to the national economies of several countries (Elsaffany *et al.,* 2019; Fathy and Gad, 2022; Saha *et al.,* 2022). The mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) provides several commercial industrial products, including degummed silk and feaces, used for the textile industry or some incipient biomedical applications. In Asia, a number of potentially useful medical products include silk proteins (fibroin and sericin) and powder from dehydrated silkworm pupae (Ryu, 2014; Huang *et al*., 2017; Aznar-Cervantes *et al*., 2021).

The silkworm industry has to contend with a number of insect pests destroying silkworm cocoons, raw silk, and stocked silk textiles in stores, amongst these the dermestid beetles which are of particular importance in Egypt, India and other countries. Dermestids cause harm to living cocoons retained for formation of next moths' generation, silkworm eggs, and live larvae in grain age (silkworm culture) (Veer and Rao, 1995; Lawrence and Ślipiński, 2013). The main source of the loss is due to the larvae's feeding activity, which drill the silken cocoons to feed on pupae, rendering cocoons unusable for reeling (Thiagarajan and Govindaiah, 1987; Veer *et al.,* 1996; Taha *et al.,* 2017; Elsaffany *et al.,* 2024; Fouad *et al.,* 2025). Furthermore, larvae of spider beetles of the family Ptinidae feed on dry plant material for silkworms and their feaces (Alfieri, 1976; El Sawaf and El-Sayes, 2009; Abied, 2011; Elsaffany *et al.,* 2024).

This study focuses on three Coleoptera recorded for the first time as pest of silkworm products in Egypt: *Attagenus fasciatus* (Thunberg, 1795) (Coleoptera: Dermestidae) (Löbl and Smetana, 2007) on silkworm cocoons; *Anthrenus* (*Anthrenops*) *coloratus* Reitter 1881 (Coleoptera: Dermestidae) (Nardi and Háva, 2019) on silkworm pupae within cocoons; spider beetle *Gibbium psylloides* (Czempinski, 1778) (Coleoptera: Ptinidae) on silkworm feaces and waste. The morphological and ecology notes on three insect species were studied and damage effects of these pests on silkworm products were recorded.

Materials and Methods

Insect collection

The tested insect samples were collect-

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ed from silkworm material in the Sericulture Research Unit, Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, as follows: *A. fasciatus* was collected from stored cocoons of the silkworm for one year which was found infested at larval and adult stage. *A. coloratus* was collected from silkworm pupae within stored cocoons; *G. psylloides* was collected from silkworm wastes (dry plant materials and feaces). The samples were preserved in Eppendorf containing 70% Ethyl alcohol and deposited at -4ºC until used in the morphological investigation.

Identification and morphological studies

Identification of the species was based on the study of morphological characters, which was carried out within Alfieri collection, Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. The morphological features included antennae, abdomen and male genitalia (the most valuable and preferable character, usually accepted as being the final criteria (Wilson, 1927; Dobzhansky, 1931).

The whole specimens were boiled for 5-15 minutes in 10% KOH, and placed in distilled water to rinse and then immediately to ethyl alcohol for dehydration. All structures were transferred to glycerin mounts. Morphological structures were examined with Olympus SZ61 steromicroscope. All drawings (male genital structure and other parts) were made by a camera lucida attached with Olympus SZ61 steromicroscope. The ocular micrometer was used in making measurements (presented in millimeters) as follows: BL- body length (from anterior margin of the head to apex of the elytra); SL - sternites length (from the anterior margin to the apex of posterior margin). The specimens were photographed by Tucsen USB2.0 H series attached with Olympus SZ61 (Olympus Corp., Tokyo, Japan). The terminology used in species descriptions, nomenclature and systematic adopted are according to Háva (2004); Löbl and Smetana (2007) and Lawrence and Ślipiński (2013).

Results and Discussion

Attagenus fasciatus (Thunberg, 1795)

Family Dermestidae; Subfamily Attageninae Laporte, 1840; Tribe Attagenini Laporte, 1840; Genus *Attagenus* Latreille,1802. Adult species of the genus *Attagenus* are distinguished from other genera of Dermestidae by free mouthparts; antennae with 3-jointed clubbed; hypomeron without distinct antennal cavity, and the first segment of metatarsi at most, as long as the second.

Synonyms: *Anthrenus gloriosae* Fabricius, 1798:76; *A. annulifer* Laporte, 1840b:36; *A. cinnamomeus* Roth, 1851:122; *A. unifasciatus* Fairmaire, 1860:168 *Trogoderma subfasciatum* Chevrolat, 1864:617; *A. plebeius* Sharp, 1885b:147.

Diagnosis of adult stage: Body from 4-5.5 mm in length, elongate oval, dark brown to brownish yellow or black with white to whitish yellow, densely semi-recumbent hairs, and a sinuate fascia at both elytra; head withdrawn into pronotum, anterior clypeal margin slightly straight, eye finely faceted with sparsely short setae; antennae 11-segments, with 3-segmented club-shaped, brownish yellow, segments of antennal club is broader in male than in female; pronotum strongly inclined, convex with rounded anterior margin, postero-lateral margins making right angles, lateral margin with distinct ridge, posterior margin as long as or slightly longer than elytral base; elytra with one fascia or band of whitish hairs at basal third of elytra, and distinct lateral margin, and indistinct elytral suture, elytral apex rounded, scutellum distinctly triangular; legs stout, reddish brown. Abdomen with five visible sternites, with densely recumbent short hairs (Figs 1, 2 and 3 a).

Male genitalia (Figs 3 b, c, and d): aedeagus with paramere distinctly longer than phallobase or penis with slightly and densely subequal setae at apical half, slightly pointed apically, and distinctly curved inward. Phallobase or penis is broader at basal third,

Figure 1. *Attagenus fasciatus*: a) adult and b) larva (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

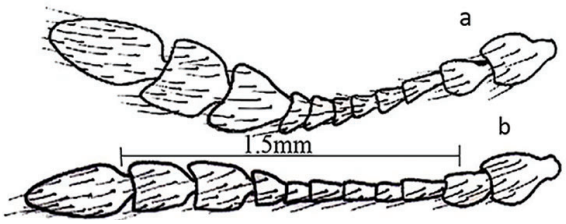

Figure 2. *Attagenus fasciatus:* Antennae of a) male and b) female (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

cone-shaped, bifurcated at base.

Diagnosis of larval stage: body length in late instar larvae nearly 5.5-6.5 mm; body elongated, carrot-shaped, cylindrical and strongly sclerotized, black to dark brown, with densely recumbent black and white backward hairs and long setae; median longitudinal line indistinct; ventral side whitish with greyish densely hairs; abdomen with long caudal brush (Fig. 1).

Damage effects: Damage by *A. fasciatus* larvae was recorded on silken cocoons (Fig. 4). *Attagenus fasciatus* is a well-known pest of silkworm cocoons (Rajashekhargouda and Devaiah, 1986; Ansari and Basalingappa, 1989; Veer *et al.,* 1991, 1996). Nevertheless, *A. fasciatus* is a pest of stored products such as grains, cotton and leather goods, dried milk, flour and many other animal and plant products (Armes, 1990; Ali, 1992; Ali *et al.,* 2011).

***Anthrenus (Anthrenops) coloratus* Reitter, 1881**

Discretion (Figs 5-8)

Family Dermestidae; Subfamily Megatominae Leach, 1815; Tribe Anthrenini Casey, 1900; Genus *Anthrenus* includes nine subgenera, between of these is *Anthrenops* which distinguished by the following characters: antennae 9-segments with terminal antennomere of male is longer than the penultimate one, whereas the terminal antennomere in female as long as the penultimate one.

Synonyms: *A.* (*Anthrenops*) *coloratus* Reitter, 1881; *A. rufescens* Pic, 1923a.

Diagnosis of adult stage: Body length 2-2.7 mm elongate oval, brown to dark brown with whitish to yellowish scales on head, pronotum, and elytra; female much longer

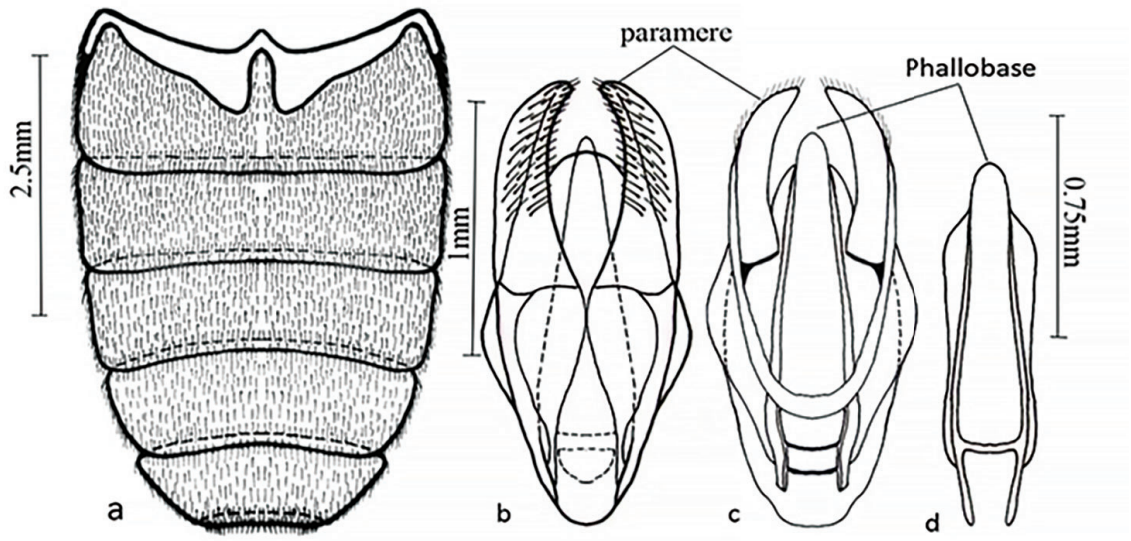

Figure 3. *Attagenus fasciatus*: a) abdominal sternites; b) aedaegus of male dorsal; c) ventral and d) phallobase or penis (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

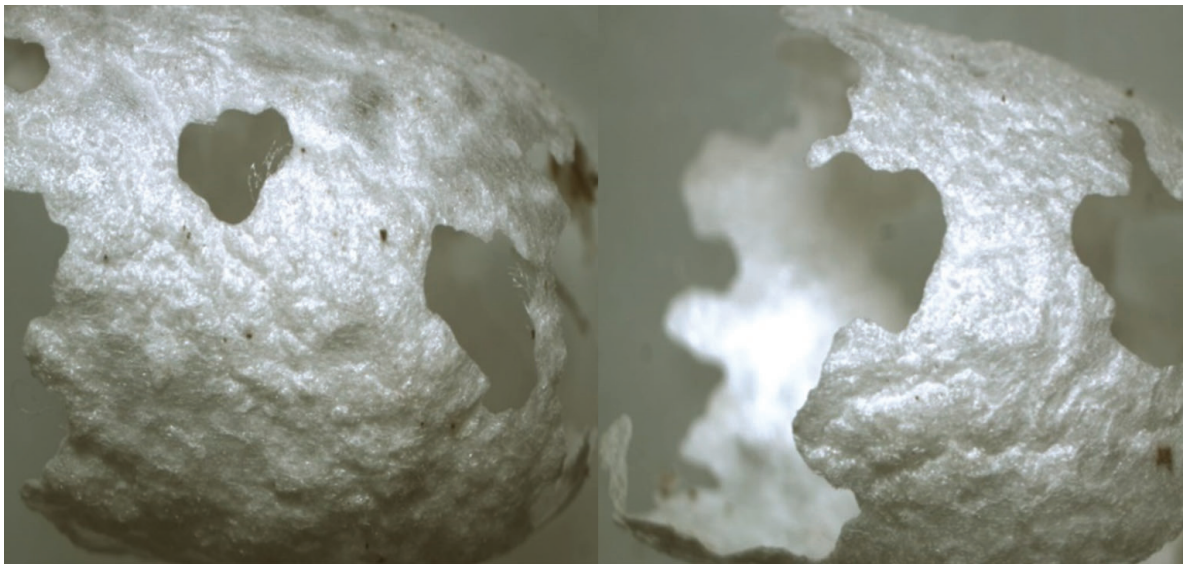

Figure 4. Damage of silkworm cocoons caused by larvae of *Attagenus fasciatus* (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

and broader than male (Fig. 5).

Antennae: 9-segments covered by short hairs, with three segmented club-shaped, the terminal antennomere in female as long as the seventh segment and penultimate ones, while in male the terminal antennomere is much broader at apical portion, and distinctly longer than the seventh and penultimate ones (Fig. 6).

Abdomen: five segments with densely short hairs, the first sternite is the longest (Fig. 7 a).

Male genitalia: aedeagus with parameres wider at basal half to nearly apex, slightly rounded terminally, distinctly longer than phallobase with densely short setae viewed from ventral and dorsal aspects. Phallobase or penis broader at basal half tapered toward apex upturned inward (Figs 7 b and c).

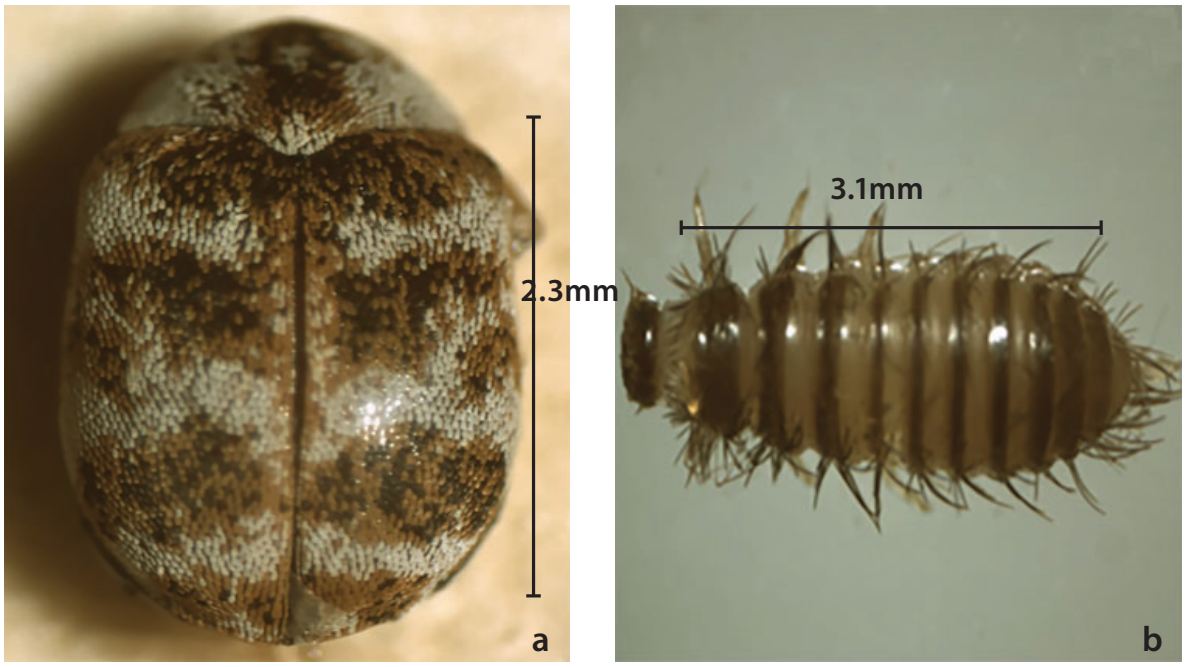

Figure 5. *Anthrenus* (*Anthrenops*) *coloratus*: a) adult and b) larva (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

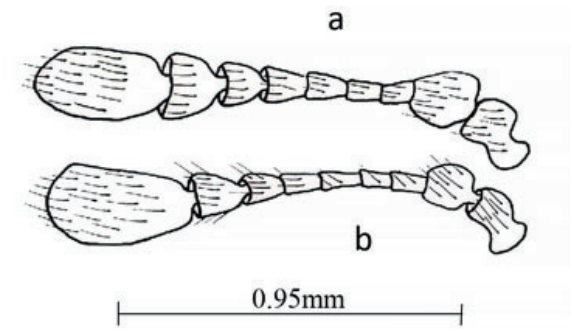

Figure 6. *Anthrenus* (*Anthrenops*) *coloratus*: Antennae of a) female and b) male (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

Diagnosis of larval stage: body length in late instar larvae nearly 3-4 mm; body cylindrical, slightly sclerotized, brown to beige in color in dorsal view, with ring of disperse long and slightly longer brown setae; head brown with golden recumbent hairs; median longitudinal line distinct particularly on the abdominal tergites; end of abdomen with short caudal brush; ventral side yellowish white dispersal setae and slightly denser hairs especially on abdominal sternites (Fig. 5).

Damage effects: Individuals collected from April to August caused serious damage to preserved insects and silkworm pupae (Fig. 8), primarily infested by the black carpet beetle *A. fasciatus*. Since May and June, infestation could be detected at preserved insects in wooden boxes, as exuviae of larvae, larvae and adult stages. Our observation is consistent with several reports indicating that *A. coloratus* has a very wide distribution and is recorded from the Mediterranean subregion, Africa, Asia and North America (Halstead, 1974). It is reported as a pest of insect collections in museums in Sudan (Hinton, 1945) and stuffed animals in India, e.g., leopard, civet cat, loris and bat (Ansari and Basalingappa, 1986), birds and mammals in zoological museums (Ansari, 2021). The total time required to complete its life cycle varies depending on the type of material it infests. It takes 140-159 days on dried silkworm pupae, 145-163 days on dried silk moths, 145-175 days on feathers, and 159-192 days on fur (Ansari, 2021). It is frequently collected from stores and houses, on wool clothes, blankets, brushes, etc (Veer *et al.,* 1991).

Gibbium psylloides (Czenpinski) Discretion (Figs 9-11)

Family Ptinidae Latreille, 1802; Subfamily

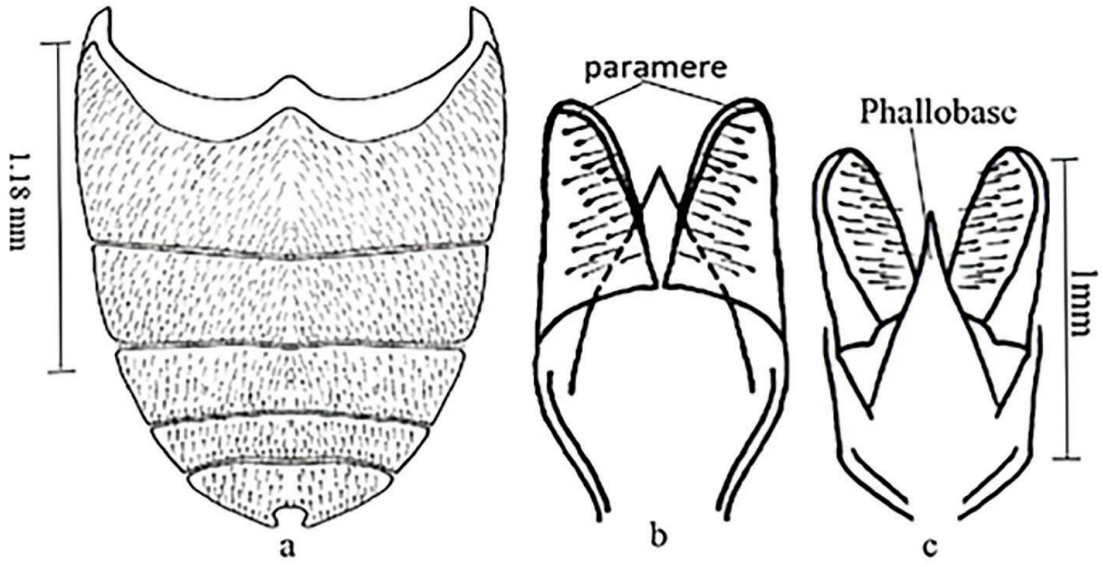

Figure 7. Abdominal sternites (a); dorsal (b), and ventral aspects (c) of aedaegus, of *Anthrenus* (*Anthrenops*) *coloratus* (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

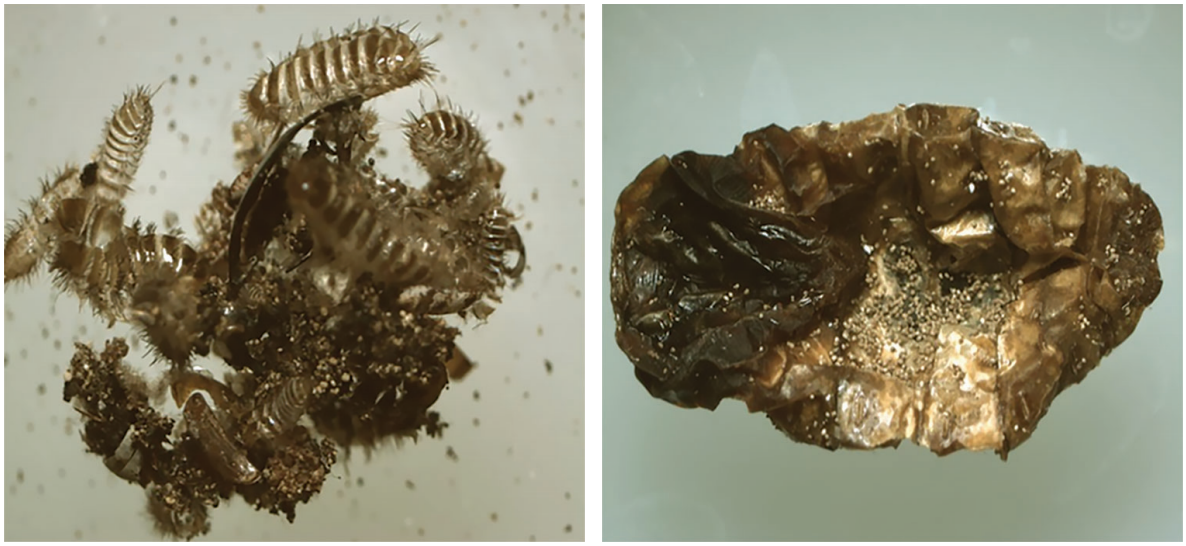

Figure 8. Damage of silkworm pupae within cocoons caused by larvae of *Anthrenus* (*Anthrenops*) *coloratus* (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

Gibbiinae Mulsant and Rey, 1868; Tribe Gibbiini Mulsant and Rey, 1868; Genus *Gibbium* Scopoli, 1777; *Gibbium psylloides* (Czenpinski), 1778 or shiny spider beetle is closely related to *G. aequinoctiale* Boieldieu (Bellés, 1985; Bellés and Halstead, 1985).

Synonyms: *Scotias psylloides* Czenpinski, 1778:51; *Ptinus scotias* Fabricius, 1781:74; *Ptinus seminulum* Schrank, 1781a:36; *Gibbium longicorne* Reitter, 1884h:296; *Bruchus apterus* Geoffroy, 1785:57.

Diagnosis of adult stage: Adult pear-shaped to globular, pronotum and elytra strongly convex, smooth, reddish to orangish in color, slightly darker than ventral surface; body from 1.8-2.75 mm in length; antennae 11-segmented filiform, with densely short recumbent hairs, the terminal antennomere

is longer than penultimate one, distinctly tapered apically; antennal fossa strongly produced laterally at posterior margin, lateral third sloping to middle, median confluence of margins forming an acute angles; ridges beneath more prominent, eyes are slightly closer to the posterior margin of antennal fossae; legs and ventral surface clothed with densely, golden- yellowish pubescence making an obscure surface, femorae stout apically, tibiae of all legs with two apical spurs, tarsi 5-segmented. Elytra sealed concealing scutellum, strongly convex. Abdomen with four segments, distinctly narrower than elytra when viewed from ventral side, with an obscured pubescence, the first two segments are equal in length, the third segment is slightly shorter than the second one, the fourth segment as long as or slightly shorter than the first three segments (Figs 9, 10 and 11 a).

Male genitalia: aedeagus with paramere slightly longer than phallobase or penis provided with sparsely short setae at apical third, apical portion of penis not chitinized, the sclerotized portion with narrow and long dorsal carina (Figs 11 b, c, and d).

Diagnosis of larval stage: body length in the late instar 4 mm or slightly longer, scrab-like, stout, slightly chitinized, with densely semi-erect whitish yellow setae; head brown, with distinct chewing mouthparts (Fig. 9).

Damage effects: This species is present all over Egypt, all year round. Many individuals were obtained from an alabaster jar, hermetically closed in Tut Ank Amoun tomb 3500 years old, present on silkworm wastes (dry plant materials and feaces). Our observation is consistent with several reports indicating that *G. psylloides* is a small flightless detritus feeder, sometimes found in damp, mouldy grain residues, but also in vegetable leather and other animal products. The larvae bore holes in the host to pupate and cause damage to packaging or the commodities them-

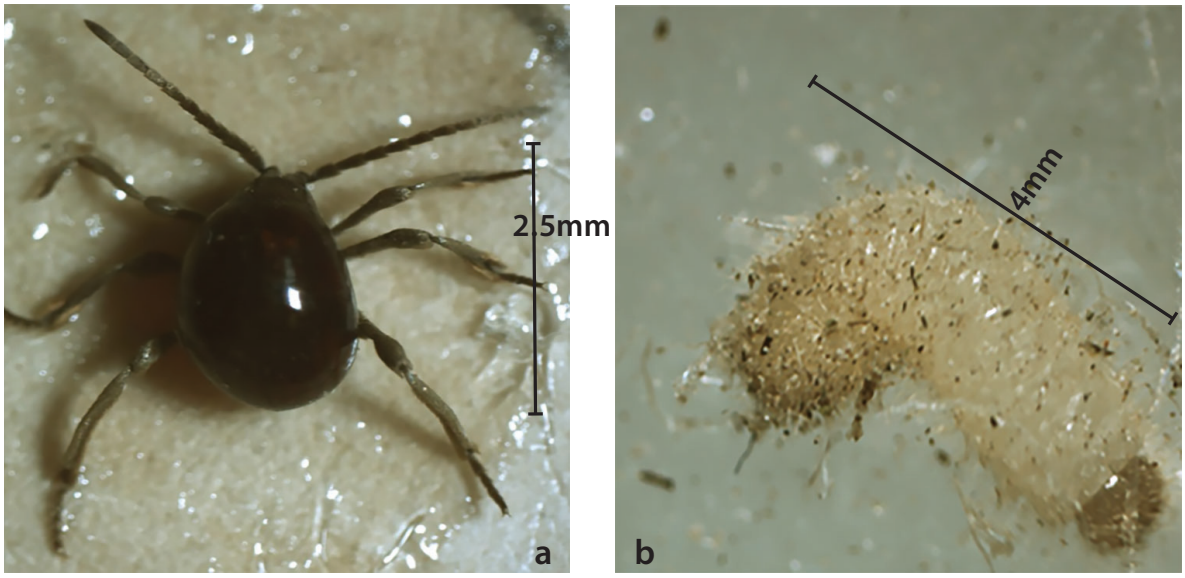

Figure 9. a) Adult and b) larva of *Gibbium psylloides* (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A)

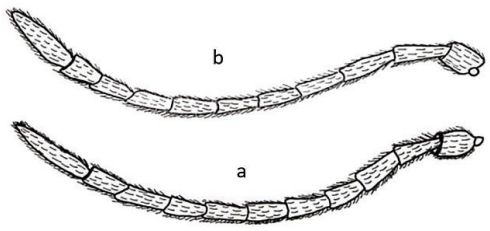

Figure 10. Antennae of *Gibbium psylloides*: a) ventral and b) dorsal view (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

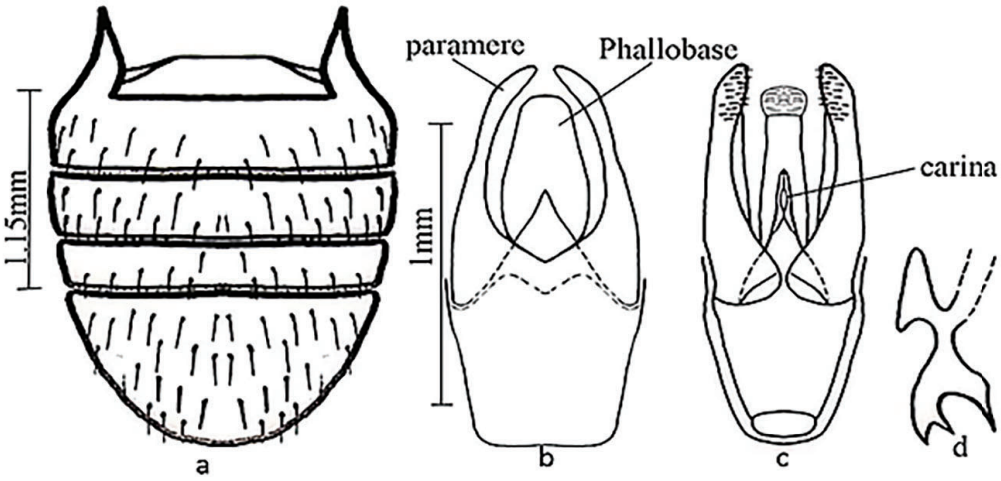

Figure 11. *Gibbium psylloides*: a) abdominal sternites, aedeagus; b), c) dorsal ventral aspect; d) chitinized carina (by Bedewy, M.M.M., Elsaffany, A.H., Gad, H.A.).

selves. In addition to furniture and dried plants in herbaria collections, larvae of *G. psylloides* can harm fabrics, mummies, animal mounts and silkworm wastes (dry plant materials and feaces) (Querner, 2015; Elsaffany *et al.,* 2024).

Conclusion

Based on our results, *A. fasciatus*, *A. coloratus* and *G. psylloides* are insect pests on stored silkworm products such as cocoons, pupae and feaces in Egypt, causing damage which could affect the national sericulture industry.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Received: 3 April 2025; Accepted: 3 January 2026

Πρώτη καταγραφή σκαθαριών Dermestidae και Ptinidae ως εχθρών αποθηκευμένων προϊόντων μεταξοσκώληκα στην Αίγυπτο – Περιγραφή μορφολογικών χαρακτηριστικών

M.M.M. Bedewy, A.H. Elsaffany και H.A. Gad

Περίληψη Η παρουσία των εντόμων *Attagenus fasciatus* (Thunberg, 1795), *Anthrenus coloratus* Reitter 1881 (και τα δύο Coleoptera: Dermestidae), και *Gibbium psylloides* (Czempinski, 1778) (Coleoptera: Ptinidae), καταγράφηκε για πρώτη φορά σε αποθηκευμένα προϊόντα μεταξοσκώληκα, όπως κουκούλια και περιττώματα, στην Αίγυπτο. Παρουσιάζονται λεπτομερείς μορφολογικές περιγραφές για όλα τα είδη. Θα πρέπει να γίνει εκτίμηση για τις πιθανές επιπτώσεις στην εθνική βιομηχανία σηροτροφίας. Τα είδη *A. fasciatus* και *A. coloratus* είναι σοβαροί εχθροί των κουκουλιών του μεταξοσκώληκα, τα οποία καταστρέφονται από τις προνύμφες τους που εισχωρούν σε αυτά για να τραφούν στις νύμφες. Το *G. psylloides* είναι ένας ήσσονος σημασίας εχθρός στη βιομηχανία της σηροτροφίας, το οποίο τρέφεται με ξηρά υπολείμματα φυτών και περιττώματα.

Hellenic Plant Protection Journal **19:** 5-14, 2026

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Hellenic Plant Protection Journal **19:** 15-25, 2026
DOI 10.2478/hppj-2026-0003

Effectiveness of microwave radiation on life stages of *Sitotroga cerealella* and its impact on chemical composition of wheat grains

H.A. Gad[1*], H.A. Mohamed[1], M.M. Abd El-Ghaffar[1] and I.L. Ibrahim[1]

Summary The efficacy of microwave power (MP) (110, 220, and 440 W) for five exposure times against different stages of *Sitotroga cerealella* was assessed. Mortality percentage of life stages, adult emergence, and chemical components in wheat grains were evaluated. Complete egg mortality (100%) was achieved with 440 W and an exposure time of 270 sec. Larvae and pupae were affected by increasing MP, resulting in a reduction in adult emergence. Furthermore, full mortality of adults increased with longer exposure periods to MP, reaching 100% 3 days after treatment with 110 W of MP and exposure times of 150, 210, and 270 sec. Complete adult mortality was achieved 2 days after treatment with 220 W and exposure times of 210 and 270 sec, and 1 day after treatment with 440 W and exposure times of 150, 210, and 270 sec. Moreover, MP caused a slight reduction in the chemical components of wheat grains except for fibre content. These findings suggest that MP may be a useful method for the management of *S. cerealella.*

Additional keywords: Adult mortality, angoumois grain moth, chemical components, heat grains, microwave

Introduction

Twenty percent of the world's protein and forty percent of its calories come from wheat (Kumar *et al.,* 2015). Furthermore, 35% of the world's population primarily consumes wheat as a food source. Consequently, wheat is cultivated more extensively than maize on a global scale (Breitkreuz *et al.,* 2020; Miedaner *et al.,* 2020). According to Bouchelos (2018), 17% of the food produced globally is believed to be damaged during storage due to insect infestations. Stored grains can be infested by several destructive insect pests, such as *Trogoderma granarium* Everts, *Sitophilus* spp., angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), and other insects (Cogburn and Bollich, 1980; França-Silva *et al.,* 2020; Stathas *et al.,* 2023).

Sitotoroga cerealella larvae infest various stored cereals, leading to significant damage to the grains in terms of weight and quality loss. If populations of this insect pest become substantial, they could potentially decimate grain stocks (Shafique *et al.,* 2006; Borzoui *et al.,* 2017). Management of this insect pest typically involves the use of chemical fumigants, but the widespread use of chemical insecticides poses serious risks to the ecosystem and to animal and human health (Hagstrum and Phillips, 2017). In accordance with insect management strategies, scientists in this field advocate for a significant reduction in the use of synthetic insecticides and the adoption of more environmentally friendly methods (Phillips and Throne, 2010; Lucchi and Benelli, 2018).

Microwaves are electromagnetic waves that fall within the electromagnetic spectrum between radio waves and infrared radiation. Their frequency ranges from 300 MHz to 300 GHz, which means their wavelength is between 1 and 1000 mm (Šuhajda, 2006). The transformation of electrical energy to microwave energy occurs within a generator which is composed of high-voltage tubes that produce microwave radiation. A portion of this radiation is reflected, some is absorbed, and some penetrates through. Heat is then generated from the absorbed microwave radiation (Novotny *et*

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al., 2013). The dielectric heating effect produced by grains, which are typically poor electrical conductors, forms the basis for using microwaves to kill insects. There is potential for selective heating of insects in grain due to the dependence of this heating on the material's electrical properties. The primary advantage of using microwave energy as a green control tool is that food does not contain any chemical residues, meaning there are no negative effects on humans or the environment (Taheri *et al.,* 2022).

Several reports have illustrated how microwave energy, when applied to stored products effectively combats a range of insect pests (El-Naggar and Mikhaiel, 2011; Das *et al.,* 2013; Manickavasagan *et al.,* 2013; Pandir and Guven, 2014; Sadeghi *et al.,* 2018; Patil *et al.,* 2020; Abed *et al.,* 2023). Furthermore, few reports studied effect of microwave on *S. cerealella* larvae such as García-Mosqueda *et al.* (2019). However, the effect of microwave powers on the mortality of life stages of *S. cerealella* and the biological aspects such as adult emergence from treated stages have not been studied before. Therefore, the current study focuses on examining the susceptibility of various *S. cerealella* stages to microwave powers (MP) as well as their latent effects on adult emergence from treated stages and their impact on the chemical components of stored wheat**.**

Materials and Methods

Rearing of *S. cerealella*

The culture of *S. cerealella* was initiated from eggs of a laboratory strain provided by the Plant Protection Institute of the Ministry of Agriculture in Giza, Egypt. The individuals of *S. cerealella* used in the study were obtained from the laboratory culture that had been raised in glass jars for several generations at a consistent temperature and relative humidity of 26°C and 65% r.h. Fresh egg masses were collected from the jars, and the neonate larvae were placed in glass jars containing sterilized wheat grain. The larvae reached adulthood, and the life cycle of *S. cerealella* was maintained on wheat grains as described by Akter *et al.* (2013). Different stages were used in this study, including newly laid eggs and one-day-old adults. Larvae and pupae were treated by exposing wheat grains to microwave powers (MP) for 21-23 days and 27-29 days.

Microwave energy treatment equipment

A Sharp model R-340 resistivity microwave oven was used, operating at 230-240V and 50Hz, with an input of 1.60 kilowatts and 7.2A output power of 1100 watts, a capacity of 33 liters, and cavity dimensions of 375 mm (width) x 226 mm (height) x 387 mm (diameter). The operating frequency of the oven was 2450 megahertz. The various life stages of wheat grains under investigation were exposed to different microwave powers of 110, 220, and 440 watts (10%, 20%, and 40% of the output power of 1100 watts).

Exposure of *S. cerealella* life stages to microwave powers (MP)

One day old *S. cerealella* eggs (100 eggs), fourth instar larvae (10 g of wheat grains containing larvae 21-23 days), 5 days old pupae (10 g of wheat grains containing pupae 27-29 days) and one day old adults (50 adults) were exposed to three MP levels, 110, 220, and 440 W for 30, 90, 150, 210, and 270 seconds (sec). The experiments were conducted in four replicates, with treated insects compared to untreated insects. Subsequently, treated immature stages (eggs, larvae, and pupae) were placed in glass jars with muslin cloth coverings (5 cm in diameter and 5 cm in depth) for incubation under the same conditions as in rearing. To determine the percentage of mortality after treatment, the jars were examined daily until a percentage of adult emergence was noted (since it can be difficult to find larvae or pupae inside the grains) (Abd El-Ghaffar *et al.,* 2016; Gad *et al.,* 2025a, b).

Major chemical components of wheat treated with microwave powers (MP)

The impact of MP on the chemical components of wheat was investigated using

standard analytical methods. The MP treatment was in the 750–2500 nm wavelength range using a near-infrared (NIR) spectroscope (model DA1650 – FOSS Corporation – Denmark) (Gad *et al.,* 2021). The analyses were conducted on 20 grams of wheat grains, assessing the percentages of protein, fat, carbohydrates, moisture, fibre, and ash in both treated and untreated wheat.

Statistical analysis

The mortality data were analyzed using probit analysis (Finney, 1971) to determine the LT_{50} and LT_{90} values. Subsequently, the data underwent ANOVA analysis. Mean separations were computed using Tukey's HSD test at a significance threshold of less than 0.05. The statistical analysis was performed using SPSS 21.0 software (SPSS, Chicago, IL, USA).

Results

Treatment of eggs by MP and their latent effects

Our results show that as exposure time or microwave power (MP) increased, the percentage of treated eggs of *S. cerealella* that were killed also increased compared to the control group. Strong egg mortality rates of 93.6% and 99.7% were achieved with an exposure time of 270 sec and MP levels of 110 and 220 W, as shown in Figure 1. The LT_{50} values were 0.62 and 131 sec, while the LT_{90} values were 212.5 and 88.9 sec, respectively (Table 1). Complete egg mortality of 100% was observed at 440 W after an exposure time of 270 sec (Fig. 1). When considering the reduced emergence of *S. cerealella* adults from treated eggs, the percentage of reduction increased sequentially with lon-

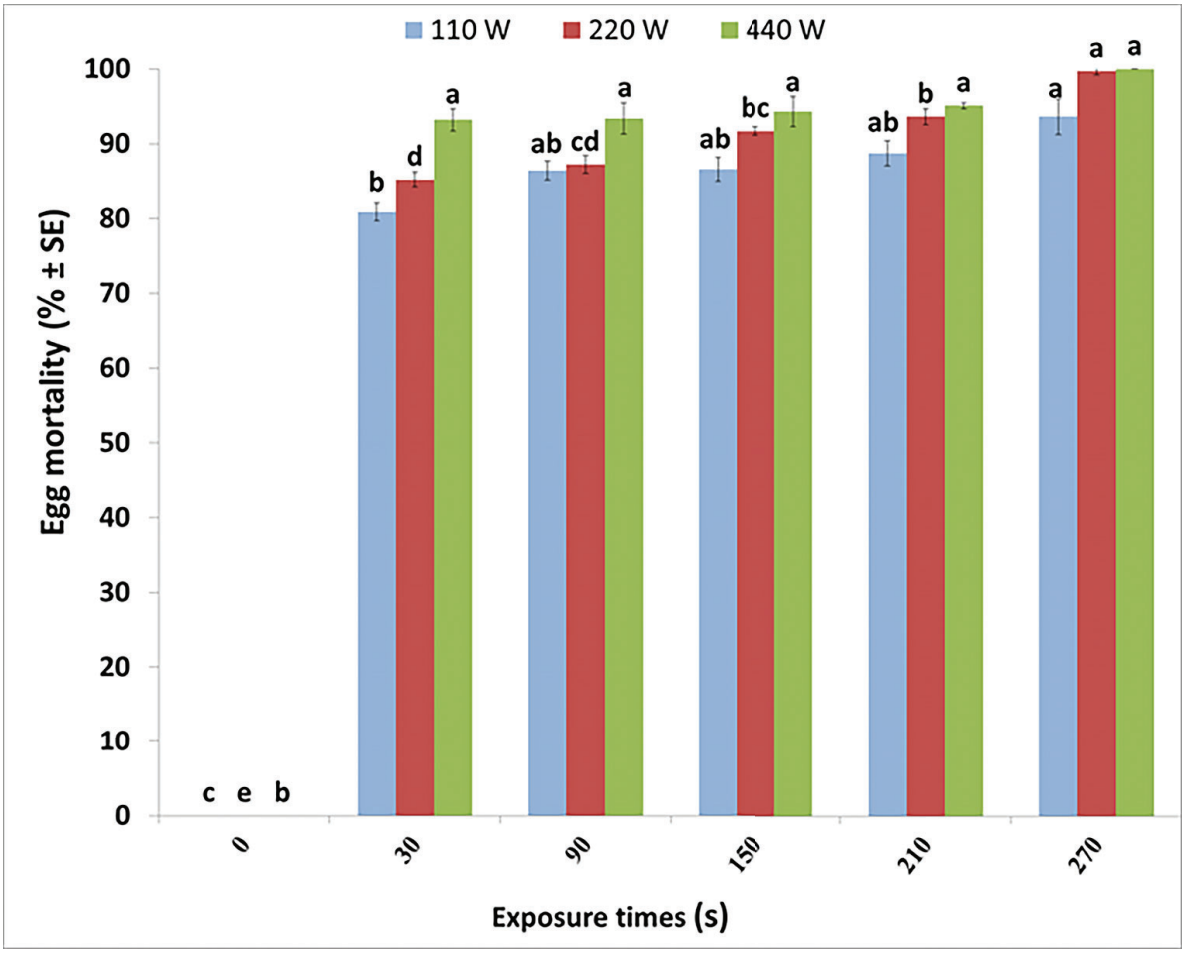

Figure 1. Mean mortality of *Sitotroga cerealella* eggs exposed to different microwave powers (W) for various exposure times (seconds-s). Different letters on top of the bars indicate statistically significant differences in all cases (df =5, 18, $p < 0.05$).

Table 1. LT_{50} and LT_{90} values for eggs of *Sitotroga cerealella* exposed to different microwave powers (W).

Microwave powers (W)	LT_{50} (sec)	LT_{90} (sec)	Confidence limits (sec) of LT_{50}		Confidence limits (sec) of LT_{90}		Slope ± SE	(χ^2)	*P*
			Lower	Upper	Lower	Upper			
110	0.62	212.5	0.16	1.40	114.5	506.4	0.505±0.061	1.48	0.915
220	1.31	88.9	0.002	7.12	42.47	146.01	0.700±0.210	3.05	0.384

ger exposure periods or higher MP levels. With MP levels of 220 and 440 W, the reduction in adult emergence reached 100% after 210 and 270 sec, respectively (Table 2).

Treatment of larvae and pupae by MP and their latent effects

Table 2 indicates the reduction in adult emergence from fourth instar larvae and 5-day-old pupae of *S. cerealella* under different exposure periods. The reduction in adult emergence of *S. cerealella* from treated larvae or pupae increased gradually with an increase in either microwave power (MP) or exposure period. The highest percentage reduction in adult emergence resulted from treated larvae at 99.8% (Table 2) with LT_{50} and LT_{90} values of 0.96 and 123.4 sec, respectively (Table 3), and from pupae at 100% (Table 2) with LT_{50} and LT_{90} values of 110.1 and 517.9 sec, respectively (Table 3) 270 sec after treatment under 220 W of MP. Additionally, complete reduction in adult emergence was observed in larvae and pupae at exposure times of 150, 210, and 270 sec under 440 W of MP.

Treatment of adults by MP

The mortality percentage of *S. cerealella* adults increased with longer exposure periods to MP (Table 4). Full adult mortality reached 100% 3 days after treatment at 110 W of MP with exposure times of 150, 210, and 270 sec. Complete adult mortality was achieved 2 days after treatment at 220 W with exposure times of 210 and 270 sec, and after 1 day at 440 W with exposure times of 150, 210, and 270 sec.

The major chemical components of wheat grains treated with MP

Table 5 displays the main chemical components of untreated and treated wheat grains at two MP (220 W for 270 sec and 440 W for 90 sec). The treated wheat with MP had slightly lower levels of all chemical components except for fibre. The treated wheat grains with two MP, showed a slight reduction in the percentage of relative content of protein (0.05 and 0.47%), fat (1.24 and 1.43%), carbohydrates (1.18 and 2.08%), moisture (4.29 and 3.61%) and ash (0.03 and 0.08%). The highest increases in fibre (0.56 and 0.09%) were noted in the treated wheat compared to the untreated wheat grains, respectively.

Discussion

Numerous safer techniques have been proposed as substitutes for methyl bromide since it was added to the list of substances that deplete the ozone layer (UNEP, 1995). One of these techniques is microwave irradiation which has shown potential in managing postharvest insects (Manickavasagan *et al*., 2013; Sadeghi *et al*., 2018). Our results indicate that as exposure time or microwave power (MP) increased, the percentage of treated *S. cerealella* eggs that were killed also increased, leading to a suppression of adult emergence compared to the control group. These findings are in accordance with Baysal *et al.* (1998) who found that MP of 90 sec was sufficient to control *Ephestia cautella* (Walker) on figs and destroy the insect's eggs. Vadivambal *et al.* (2010a) revealed that samples with *Tribolium castaneum* eggs were most vulnerable when exposed to 200, 300, 400, or 500 W for 28 or 56 sec. Abo-El-Saad *et al.* (2011) demonstrated that complete inhibition of egg

Table 2. Reduction percentages of adult emergence from *S. cerealella* immature stages (eggs, larvae and pupae) exposed to different microwave powers (W) for various exposure times.

Exposure times (sec)	Reduction percentages of adult emergence (% ± SE)								
	110 W			220 W			440 W		
	Eggs	Larvae	Pupae	Eggs	Larvae	Pupae	Eggs	Larvae	Pupae
Control	0.0±0.0c	0.0±0.0e	0.0±0.0e	0.0±0.0c	0.0±0.0d	0.0±0.0e	0.0±0.0c	0.0±0.0d	0.0±0.0d
30	86.2±1.3b	74.8±1.0d	10.5±1.8d	88.1±1.8b	82.7±1.4c	20.9±1.7d	90.3±1.2b	87.5±2.8c	52.2±3.1c
90	87.8±1.3b	79.0±1.6cd	17.7±1.7cd	93.1±1.4b	83.0±1.0c	28.5±2.5d	94.9±2.1b	96.2±1.6b	94.7±1.8b
150	90.3±1.2b	83.3±1.2bc	23.3±1.6bc	94.4±1.7b	86.2±1.8c	52.9±1.6c	97.0±1.9b	100.0±0.0a	100.0±0.0a
210	92.2±1.0ab	86.4±2.5ab	32.2±1.4b	100.0±0.0a	93.9±1.8b	84.4±1.7b	100.0±0.0a	100.0±0.0a	100.0±0.0a
270	96.1±1.1a	92.1±0.8a	50.5±1.0a	100.0±0.0a	99.8±0.2a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a
F	259.5	619.3	65.2	361.7	575.3	216	354.2	464.2	311.1
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Values within a column sharing the same letter are not significantly different (df =5, 18, $p < 0.05$).

Table 3. LT_{50} and LT_{90} values for larvae and pupae of *Sitotroga cerealella* exposed to different microwave powers (W).

Microwave powers (W)	Insect stage	LT_{50} (sec)	LT_{90} (sec)	Confidence limits (sec) of LT_{50}		Confidence limits (sec) of LT_{90}		Slope ± SE	(χ^2)	*P*
				Lower	Upper	Lower	Upper			
110	**Larvae**	3.38	351.8	0.02	12.77	193.4	2313.8	0.635±0.191	2.29	0.514
	Pupae	405.9	4220.9	292.4	731.6	1771.9	23297.8	1.26±0.213	7.52	0.057
220	**Larvae**	0.96	123.4	0.09	1.96	57.14	609.6	0.609±0.065	13.19	0.022
	Pupae	110.1	517.9	73.9	166.6	487.3	1799.4	1.90±0.175	24.50	0.002

Table 4. Mean mortality (% ± SE) of *Sitotroga cerealella* adults exposed to different microwave powers (W) for five exposure times after 1, 2 and 3 days of treatment.

Exposure times (sec)	Adult mortality (% ± SE)								
	110 W			220 W			440 W		
	1 day	2 days	3 days	1 day	2 days	3 days	1 day	2 days	3 days
Control	0.0±0.0d	0.0±0.0c	0.0±0.0d	0.0±0.0d	0.0±0.0d	0.0±0.0c	0.0±0.0d	0.0±0.0c	0.0±0.0
30	9.5±3.4c	27.8±3.9b	61.7±1.2c	41.1±3.4c	50.0±5.8c	66.6±1.7b	80.9±2.1c	88.9±3.9b	100.0±0.0
90	27.8±1.9b	38.9±2.0b	83.3±3.1b	54.7±1.8b	61.1±3.9b	100.0±0.0a	94.4±2.7b	100.0±0.0a	100.0±0.0
150	34.5±2.1b	60.0±1.2a	100.0±0.0a	62.2±1.7b	66.7±1.2bc	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0
210	62.5±3.1a	66.6±1.4a	100.0±0.0a	83.3±3.1a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0
270	66.7±3.2a	66.7±4.7a	100.0±0.0a	86.7±2.5a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0
F	70.6	123.4	619.3	224.9	221.0	814.9	357.0	375.2	
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	

Values within a column sharing the same letter are not significantly different (df =5, 18, $p < 0.05$).

Table 5. Major chemical components of wheat grains treated with two microwave powers (220 W for 270 sec and 440 W for 90 sec).

Component (%)	Chemical components of wheat grains				
	220 W (270 sec)	440 W (90 sec)	Untreated	F	P
Protein	13.65a (–0.05)*	13.23a (–0.47)	13.7a	0.118	0.891
Fat	1.56a (–1.24)	1.37a (–1.43)	2.80a	2.374	0.174
Carbohydrates	53.92a (–1.18)	53.02a (–2.08)	55.1a	0.237	0.796
Moisture	9.01b (–4.29)	9.69b (–3.61)	13.3a	9.42	0.014
Fibre	3.46a (+0.56)	2.99a (+ 0.09)	2.90a	0.363	0.709
Ash	1.67a (–0.03)	1.62a (–0.08)	1.70a	0.251	0.786

* Percentage of decrease (–) and increase (+)
Values within a raw sharing the same letter are not significantly different (df =2, 6, $p < 0.05$).

hatchability in *E. cautella* was achieved with 100% of 2450 MHz after 10 and 15 sec. Azizoglu *et al.* (2011) showed that full mortality of *Ephestia kuehniella* (Zeller) eggs occurred at 150 and 600 W after 300 and 10 sec, respectively. Darwish *et al.* (2014) observed that egg mortality in *E. kuehniella* reached 100% after 300 and 120 sec of exposure to 330 and 550 W, respectively. Our results indicate that full egg mortality in *S. cerealella* was achieved with the highest MP (440 W) and 270 sec of exposure. This rapid mortality may be attributed to the detrimental effects of microwave radiation on insects, particularly on embryonic cells which divide and expand quickly compared to other cells in immature stages (Zhao *et al.,* 2007).

Additionally, the current investigation demonstrated that *S. cerealella* larvae and pupae were susceptible to MP similar to the egg stage. These two stages were impacted by short exposure times of MP, resulting in a decrease in adult emergence from treated larvae and pupae of *S. cerealella*, and complete suppression of adult emergence at higher MP levels. García-Mosqueda *et al.* (2019) revealed that using microwave powers of 293, 390, and 475 W for exposure times of 56, 40, and 37 sec led to complete larval mortality of *S. cerealella*. The ability of microwaves to penetrate dielectric materials deeply and produce full-volume heating makes them more effective against *S. cerealella* larvae and pupae. These findings could be crucial in eliminating insects hidden within stored grains (Qu *et al.,* 2017; Gillani *et al.,* 2022).

Moreover, our results are in agreement with results on microwave treatments on other insect pests. Vadivambal *et al.* (2006), who stated that 100% mortality was observed at 500 W for pupae of *T. castaneum*. Al-Azab and Abo-El-aad (2007) observed that treating infested dates with MP for 20 sec was highly effective in killing *E. cautella* pupae. Vadivambal *et al.* (2010b) also showed that full mortality of *Sitophilus zeamais* (Motschulsky) and *T. castaneum* larvae was achieved at 600 W for 14 sec and 500 W for 28 sec, while for *Plodia interpunctella* (Hübner) larvae, 100% mortality was attained at 500 W for 14 sec and 400 W for 28 sec. Manickavasagan *et al.* (2013) discovered that using two microwave powers (600 and 800 W) for 40 and 30 sec resulted in complete larval mortality of *T. castaneum*. Darwish *et al.* (2014) also found that larval and pupal mortality of *E. kuehniella* reached 100% after 300 and 120 sec of exposure to 330 and 550 W for larvae, and 240 sec at 550 W for pupae. Pandir and Guven (2014) demonstrated that complete mortality of *E. kuehniella* larvae was achieved with 70 W for 50 sec. Naher *et al.* (2015) observed strong larval mortality of *E. cautella* (90%, 96.7%, and 100%) with microwave powers of 600, 800, and 1000 W at 10 sec of exposure, respectively. Full pupal mortality (100%) was achieved with 800 W and 18 sec of exposure. Tayeb *et al.* (2018) showed that mortality of

treated larvae and pupae of *T. castaneum* was 100% with a power of 800 W and exposure time of 20 sec.

The current results demonstrate that the majority of *S. cerealella* moths had higher sensitivity to MP compared to immature stages, reaching almost complete mortality at most tested MP levels with shorter exposure times. Our findings are consistent with El-Disouky (2002) demonstrated that MP treatment of adult *Sitophilus oryzae* (L.) for 25 sec was highly effective, resulting in 100% mortality. Trandeel (2003) found that 19 sec of exposure to MP (2450 MHz) was necessary for *T. castaneum* to achieve 50% mortality. Abo-El-Saad *et al.* (2011) reported that adult mortality of *E. cautella* reached 100% at 60% power of 2450 MHz after 15 sec of exposure. Manickavasagan *et al.* (2013) demonstrated that exposure to 800 W of MP for 30 sec resulted in full mortality of adult *T. castaneum*, while 40 sec of exposure caused full mortality of *Oryzaephilus surinamensis* (L.). Abed *et al.* (2023) showed that 700 W of MP for 30 sec caused complete adult mortality of *S. oryzae*.

Our results indicate that two microwave powers (220 and 440 W) applied for 270 and 90 sec, respectively, cause changes in the chemical components of wheat grains. These changes include a slight reduction in protein, carbohydrates, fat, moisture, and ash contents, as well as a slight increase in fibre content. Our results are in accordance with several studies showing that microwave power induces slight changes in the chemical components of treated stored products (Oomah *et al.,* 2014; Zhong *et al.,* 2019; Peng *et al.,* 2021; Feng *et al.,* 2023).

Conclusion

The tested MP 110, 220, and 440 W had a lethal impact on all life stages of *S. cerealella*. All stages were sensitive to the MP resulting in reduced adult emergence from immature stages and a slight decrease in the chemical components of wheat grains. Our research indicates that MP could be a beneficial alternative for managing *S. cerealella* moderating potential issues associated with the widespread use of chemical insecticides such as environmental pollution and the development of resistance. However, further research is needed to determine the potential long-term effects of MP on the chemical composition of stored grains.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Received: 12 October 2024; Accepted: 3 January2026

Αποτελεσματικότητα της ακτινοβολίας μικροκυμάτων στα βιολογικά στάδια του *Sitotroga cerealella* και η επίδρασή της στη χημική σύνθεση των σπόρων σιταριού

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Περίληψη Η αποτελεσματικότητα ακτινοβολίας μικροκυμάτων διαφορετικής ισχύος (110, 220 και 440 W) αξιολογήθηκε για πέντε χρόνους έκθεσης εναντίον διαφορετικών βιολογικών σταδίων του εντόμου αποθηκών *Sitotroga cerealella*. Εκτιμήθηκε το ποσοστό θνησιμότητας των βιολογικών σταδίων, η έξο-

δος των ενηλίκων και η επίδραση στη χημική σύσταση των σπόρων σιταριού. Η πλήρης θνησιμότητα των ωών (100%) επιτεύχθηκε στην ισχύ των 440 W και σε χρόνο έκθεσης 270 δευτερολέπτων. Η αύξηση της ισχύος των μικροκυμάτων επηρέασε τη δράση τους στις προνύμφες και νύμφες του εντόμου, με αποτέλεσμα τη μείωση της εμφάνισης ενηλίκων. Επιπλέον, η πλήρης θνησιμότητα των ενηλίκων αυξήθηκε με μεγαλύτερες περιόδους έκθεσης στην ακτινοβολία μικροκυμάτων, φτάνοντας το 100%, τρεις ημέρες μετά την έκθεση στην ισχύ των 110 W για 150, 210 και 270 δευτερόλεπτα. Πλήρης θνησιμότητα των ενηλίκων επιτεύχθηκε δύο ημέρες μετά την έκθεση σε ακτινοβολία μικροκυμάτων ισχύος 220 W για 210 και 270 δευτερόλεπτα, και μία ημέρα μετά την έκθεση στην ισχύ των 440 W για 150, 210 και 270 δευτερόλεπτα. Επιπλέον, η ακτινοβολία μικροκυμάτων προκάλεσε μικρή μείωση στα χημικά συστατικά των σπόρων σιταριού, εκτός από την περιεκτικότητα σε φυτικές ίνες. Από τα αποτελέσματα φαίνεται ότι η ακτινοβολία μικροκυμάτων μπορεί να αποτελέσει μια χρήσιμη μέθοδο για την αντιμετώπιση του *S. cerealella*.

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Hellenic Plant Protection Journal **19:** 26-32, 2026
DOI 10.2478/hppj-2026-0004

SHORT COMMUNICATION

New Records of Tetranychidae (Prostigmata) in avocado crops in Crete, Greece

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Summary In April 2024, infestation was recorded on leaves of avocado trees, bearing brown necrotic spots on the upper surface of the leaves and/or whitish, almost circular spots on the underside, in Zounaki, Crete, Greece. In September of the same year, infestation of different symptomatology was observed, consisting of a bronze tone at the upper surface of the leaves, in an avocado orchard in Ayia village, Crete. A microscopic examination of infested leaves revealed the presence of mites which were identified as *Oligonychus* (*Olygonychus*) *perseae* Tuttle, Baker and Abbatiello and *Oligonychus* (*Olygonychus*) *punicae* (Hirst) (Prostigmata: Tetranychidae) which are both first recorded in Greece. By autumn of 2024, the persea mite rapidly spread across nearly all regions where avocados are cultivated in the western and coastal part of Crete. Up to date, nearly one year after the first detection of these mite species, infestations of *O.* (*O.*) *perseae* remains below economic thresholds, whereas *O.* (*O.*) *punicae* is found in a few orchards and in limited populations (end of 2025).

Additional keywords: avocado pests, economic pest, invasion, Tetranychidae

Introduction

Avocado (*Persea americana* Mill.) is one of the most socioeconomically important tree crops in tropical and sub-tropical regions, demonstrating good adaptation to these climatic conditions (Bhore *et al*., 2021).

In Greece, commercial avocado production is concentrated in the western part of Crete, where the climate and soil conditions are particularly suitable. Over the past decade, many traditional crops, such as citrus and olive trees in Chania prefecture, Crete, have become less profitable and have been increasingly replaced with avocado which is well suited to local climatic conditions. Increase of public awareness (farmers and consumers) of its nutritional value has contributed to the increase of the production levels of avocado over the last decade in Crete (Kourgialas and Dokou, 2021). In 2018, avocado fruit production in Greece reached 6,633 tons per year, with approximately 90% of this output originating from the prefecture of Chania. During the same period, the total cultivated avocado area in Chania was 550 ha, producing 6,300 tons annually, representing a 4.7% increase in fruit production compared with 2017–2018 (Antonopoulou, 2022). Recently, the estimated cultivated area of avocado was 1.096.2 ha with an annual production of 9.573 tons (8.2.25, INCOFRUIT HELLAS based on ELSTAT). The most common cultivated avocado varieties in the region are Hass, Fuerte, and Zutano (Tzatzani *et al.,* 2023).

The only tetranychid mite preciously recorded from Greece in avocado, is *Tetranychus urticae* Koch, without causing serious economic damage to the crop (Papaioannou-Souliotis *et al*., 1994). Therefore, the aim of this study was to conduct a brief survey

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to determine tetranychid mites infesting avocado trees in Greece.

Materials and Methods

Avocado trees with infested leaves were observed in orchards located in Zounaki and Ayia village, Crete, Greece, in April and September of 2024, respectively. For the identification of the pest, a sample of at least 50 leaves were collected from various avocado trees of each orchard. The examination of the samples was conducted under a dissecting microscope at the magnification of (×20). Mites were collected directly from the infested leaves using a fine brush and were stored in 70% ethanol in Eppendorf®'s tubes. Identification was carried out at the Laboratory of Acarology and Agricultural Zoology of Benaki Phytopathological Institute (BPI). Permanent mounts were made of all collected specimens using Hoyer's medium, after cleansing with lactic acid for 48 hours in 50^{0}C. Photos of the mounded mites were taken with an Euromex Camera model CMEX 5.0 adapted to the microscope. For the identification of the mites the original description and available redescriptions were used for each species.

Results

Oligonychus (*Olygonychus*) *persea* Tuttle, Baker and Abbatiello, 1976

In April 2024, infested leaves of avocado were observed during inspections in a mixed orchard of orange and avocado trees, in Zounaki of Chania, Crete (35° 28′ 58. 64′′ N–23° 49′ 41. 63′′ E-84m). The infested avocado trees were about 10-15 years old (cv. Hass and Fuerte) while avocado plantations of certified trees imported from Spain and subsequently purchased from a local nursery, were established in the broader area since 2022.

The recorded symptoms of the infested avocado leaves displayed circular necrotic spots on the underside surface and chlorotic yellow on the upper leaf surface (Figs 1, 2,3). The symptomatology looked like the typical infestation of the persea mite, which forms circular colonies beneath protective dense webbing on the underside surface of leaves, by its feeding. These colonies are formed along midribs and veins and produce characteristic circular necrotic spots. All life stages are mainly inhabited in the nests, which serve as a site for feeding, mating, reproduction and development.

All adult individuals were identified as *Oligonychus* (*Olygonychus*) *perseae* Tuttle, Baker and Abbatiello, based on their mor-

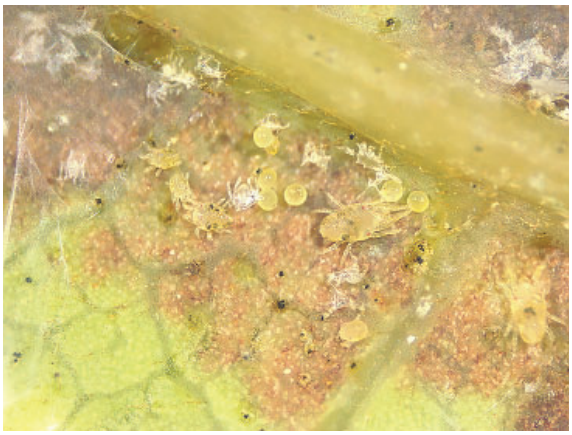

Figure 1. Colony of *Oligonychus (O.) perseae* on the lower surface of the leaf showing eggs and various mobile stages (larval and nymphal stages).

Figure 2. Characteristic necrotic spots on the lower surface of avocado leaf.

Figure 3. Avocado tree infested by *Oligonychus (O.) perseae.*

Figure 4. *Oligonychus (O.) perseae* female.

phological characteristics (Figs 4, 5), using the original description of the species by Tuttle *et al*., 1976.

Following the initial detection of the persea mite in Zounaki in April 2024, the infestation expanded rapidly to other avocado orchards in the region within the same growing season. By autumn 2024, the species was widely spread and established in orchards of western and coastal Crete, including the areas of Agia, Mournies, Tavronitis, Nerokourou, and Alikianos, where the majority of avocado cultivation is concentrated. In contrast, no infestations were documented in Apokoronas or Rethymno (until October 2025), where avocado cultivation occurs only in small, isolated orchards, up to date (Fig. 6).

Oligonychus (*Oligonychus*) *punicae* (Hirst, 1926)

During inspection of an avocado orchard in Agia village (35° 29′ 00. 36′′ N–23° 56′ 57. 63′′ E-53m), a different symptom was observed, described as a bronze discoloration on the upper surface of the leaves (Fig. 7).

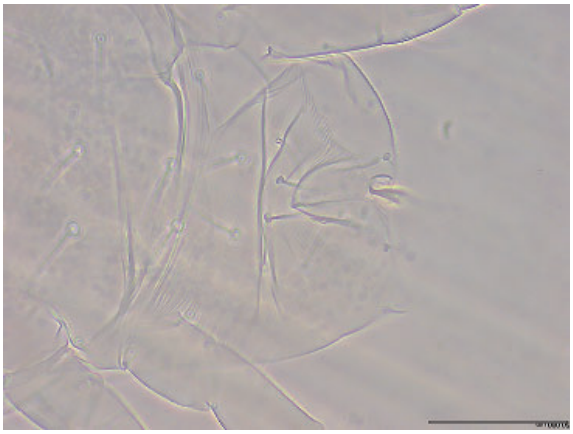

Figure 5. *Oligonychus (O.) perseae* male aedeagus.

After stereoscope examination, another species of Tetranychidae was detected, forming a distinctly different community structure from *O.* (*O.*) *perseae*. In this case, numerous adult individuals were distributed across the leaf surface, intermingled with orange-brown eggs, immature stages, secretions, exuviae, feeding damage (circular leaf lesions), and webbing. Based on the observed symptoms, it was suspected that the mite was the tetranychoid mite known as "avoca-

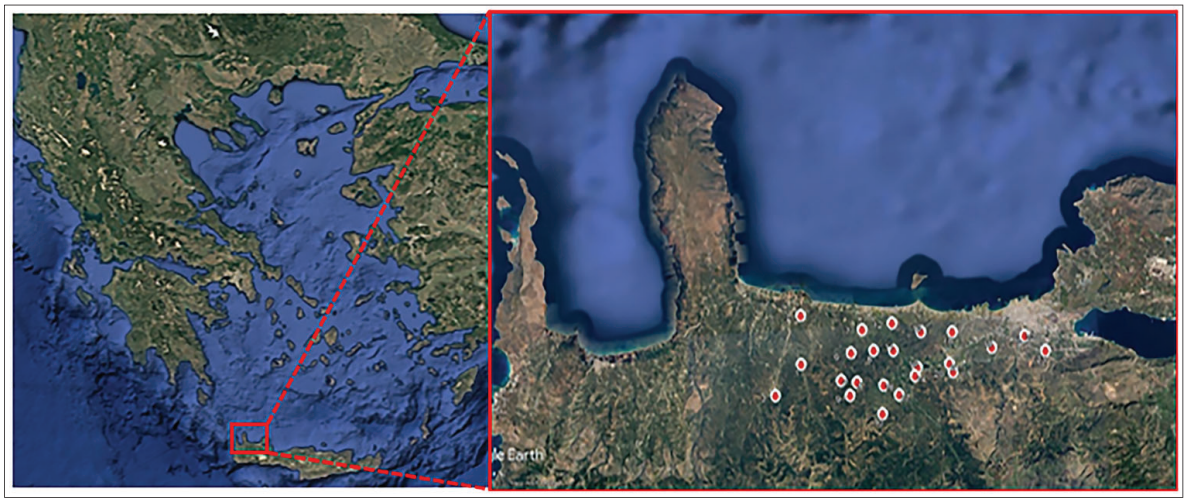

Figure 6. Avocado cultivation areas infested by the persea mite in western region of Crete Island, Chania prefecture, Greece (Source: Google Earth). The left panel shows the map of Greece, indicating the infested avocado area within the red square.

Figure 7. Characteristic bronze discoloration on the upper surface of avocado leaf caused by *Oligonychus (O.) punicae.*

do brown mite". Individuals of all specimens were identified as *Oligonychus* (*Olygonychus*) *punicae* (Hirst), based on their morphological characteristics (Figs 8, 9), using the original description (Hirst, 1926) and available redescriptions (Mushtaq *et al*., 2023).

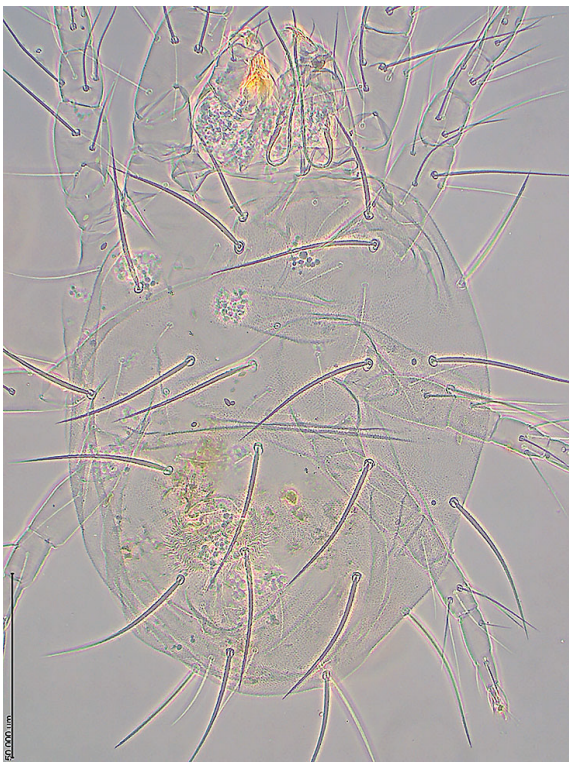

Figure 8. *Oligonychus (O.) punicae* female.

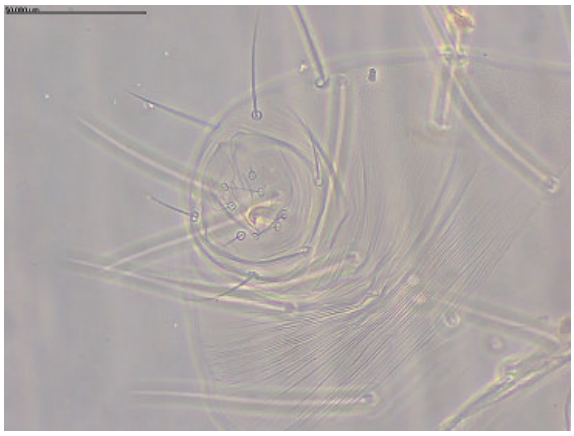

Figure 9. *Oligonychus (O.) punicae* male aedeagus.

Discussion

Persea mite and avocado brown mite are considered important avocado pests (Torres *et al*., 2023; González-Hernández *et al*., 2024). Although both mite species inhabit avocado leaves, they occupy distinct microhabitats and exhibit different ecological behaviors thereby minimizing direct interspecific competition. *Oligonychus* (*O.*) *perseae* resides on the underside of leaves constructing dense webbing that provides significant protection from natural enemies while *Oligonychus* (*O.*) *punicae* colonizes the upper leaf surface, without dense webbing, making it more vulnerable to predation by beneficial arthropods (Fleschner *et al*., 1956). This spatial and structural segregation likely facilitates coexistence among the species by reducing overlap in resource use and exposure to natural enemies.

Management recommendations provided to avocado growers were limited to pruning to increase light penetration within the tree canopy, application of pollen spot solutions to attract beneficial arthropods (Montserrat *et al*., 2012) and the release of *Neoseiulus californicus* (McGregor) as biocontrol agent (Hoddle *et al*., 1999, 2000). In addition, *Euseius* (=*Iphiseius*) *degenerans* (Berlese) was frequently recorded, particularly in mite-infested avocado orchards located near citrus or well-pruned avocado trees in sites with wild vegetation beneath the canopy. It is important to note that, as of 2025, the only acaricides approved in Greece for avocado cultivation are potassium salts of fatty acids and paraffin oil, since prior to the appearance of *O.* (*O.*) *perseae* the crop was totally free from arthropod pests; the only insects observed on the canopy were non-feeding visitors (resting individuals) or a few small-sized immature stages of e.g. white flies trying to establish. At present, nearly one year after the first detection of mite infestations, injury levels in orchards affected by *O.* (*O.*) *perseae* remain below economic thresholds while *O.* (*O.*) *punicae* is found in a few orchards and in limited populations. However, a few growers reported localized leaf drop and a reduction in avocado fruit size.

Both mites were likely introduced in Chania Crete through the import of certified avocado plants from Spain, where the mites were already present (Alcázar *et al.* 2005, Boyero *et al*., 2014; Torres *et al*., 2023) due to inadequate spraying of the imported material. This is attributed to the fact that in all cases of the avocado orchards infested by the persea mite, the growers have purchased certified avocado trees from Spain through local nurseries. To prevent further spread and introduction of other economically pests, it is essential the regulations governing certified avocado plants to be revised in Greece, and the imported material to be treated by pesticides. In the case of mites, it is proposed to apply acaricide to the imported material before the trees are given to the growers, with a second application by the grower before planting.

The research was conducted in avocado orchards of the Agricultural Cooperative of Producers of Organic Products of Chania Cretan Avos, with the assistance of Rodanthi Tontou and Vaggelis Koutoupis. The authors also wish to express their sincere gratitude to Maria Nomikou and Pavlos Skenteridis (BIOINSECTA IKE) as well as to Marta Montserrat Larrosa (IHSM "La Mayora" - UMA – CSIC) for their valuable scientific guidance on the biological control of the persea mite.

Funding

This work was supported by the European Regional Development Fund of the European Union and Greek national funds through the Rural Development Program (RDP/ΠΑΑ) 2014 – 2020, under the call "Cooperation for Environmental Projects, Environmental Practices and Actions for Climate Change" (grant no. M16ΣΥΝ2-00158 with the title 'Management of Pest and Disease of Existed and Invasive species in Avocado').

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Received: 23 December 2025; Accepted: 9 January 2026

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Νέες αναφορές ειδών της οικογένειας Tetranychidae σε αβοκάντο, στο Νομό Χανίων

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Περίληψη Τον Απρίλιο του 2024, παρατηρήθηκαν σε δένδρα αβοκάντο στην περιοχή Ζουνάκι (Χανιά, Κρήτη) καστανές νεκρωτικές κηλίδες στην άνω επιφάνεια των φύλλων ή/και υπόλευκες, σχεδόν κυκλικές κηλίδες στην κάτω επιφάνεια. Τον Σεπτέμβριο του ίδιου έτους, στην περιοχή Αγυά, καταγράφηκε ένα διαφορετικό σύμπτωμα, το οποίο συνίστατο σε μπρούτζινο μεταχρωματισμό στην άνω επιφάνεια των φύλλων του αβοκάντο. Η εργαστηριακή εξέταση των συλλεχθέντων προσβεβλημένων φύλλων αποκάλυψε την παρουσία ακάρεων, τα οποία ταυτοποιήθηκαν ως *Oligonychus* (*Olygonychus*) *perseae* Tuttle, Baker and Abbatiello και *Oligonychus* (*Olygonychus*) *punicae* (Hirst) (Prostigmata: Tetranychidae), και αναφέρονται για πρώτη φορά στην Ελλάδα. Μέχρι το φθινόπωρο του ίδιου έτους, το άκαρι *O.* (*O.*) *perseae* εξαπλώθηκε ταχύτατα σε σχεδόν όλες τις περιοχές όπου καλλιεργείται το αβοκάντο στο δυτικό και παράκτιο τμήμα της Κρήτης. Μέχρι σήμερα, σχεδόν ένα έτος μετά την πρώτη ανίχνευση των ειδών αυτών, οι προσβολές του *O.* (*O.*) *perseae* παραμένουν κάτω από τα οικονομικά όρια ζημιάς, ενώ το *O.* (*O.*) *punicae* εντοπίζεται σε λίγους οπωρώνες και σε περιορισμένους πληθυσμούς (τέλος 2025).

Hellenic Plant Protection Journal **19:** 26-32, 2026

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Hellenic Plant Protection Journal **19:** 33-47, 2026
DOI 10.2478/hppj-2026-0005

Residue dynamics of fluopyram and trifloxystrobin in grapes: Consumer safety insights from Saudi agriculture

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Summary This study examines the dissipation and residue behavior of fluopyram, trifloxystrobin, and their metabolites (fluopyram-benzamide and trifloxystrobin acid, CGA 321113) in grapes grown in Al-Qassim, Saudi Arabia. The fungicide Luna Sensation® 500 SC (250 g/L fluopyram, 250 g/L trifloxystrobin) was applied at the recommended rate (100–150 g a.i./ha). A multi-residue LC-MS/MS method was developed and validated according to SANTE 11312/2021 guidelines, demonstrating high linearity ($R^2 > 0.999$), low detection limits (0.00021–0.00094 mg/kg), and recoveries of 84.8–94.8% with RSDs < 20%. Dissipation studies revealed first-order kinetics, with fluopyram exhibiting faster degradation ($t_{1/2}$ = 2.23–3.02 days) than trifloxystrobin ($t_{1/2}$ = 3.46–4.04 days). CGA 321113 dissipated to below detectable levels within 3 days, while fluopyram-benzamide remained undetected. Terminal residues were below the maximum residue limits (MRLs) of 3 mg/kg (trifloxystrobin) and 2 mg/kg (fluopyram), with pre-harvest intervals of 6.29 and 2.13 days, respectively. Chronic dietary risk assessments confirmed negligible health risks and provided valuable insights for effective pest management and the safe use of pesticides in Saudi grape cultivation.

Additional keywords: consumer risk assessment, dissipation, field trial, LC–MS/MS, residue definition, risk assessment, terminal residues

Introduction

Grapevine (*Vitis vinifera* L.) is widely cultivated for the sweet, juicy flavor and diverse applications of its fruit. Beyond their culinary appeal, they offer significant health benefits, rich in vitamins C, B1, and B6, as well as antioxidants and phytonutrients protecting against diseases such as cancer, heart disease, and diabetes (Zhou *et al.,* 2022). In 2021, Saudi Arabia produced over 106,400 tons of grapes from approximately 3,925 hectares, making it one of the largest grape producers in the Middle East (Taylor, 2005).

However, due to their high carbohydrate content, grapes are highly susceptible to pests and insects (Conde *et al.,* 2007). Pesticides play a crucial role in protecting crops from diseases and minimizing economic losses (Heshmati *et al.,* 2020), with approximately 7% of agricultural pesticides used in viticulture (Zengin and Karaca, 2018). Several factors, including dosage, formulation, application method, frequency, climate conditions, grape variety, growth dilution, and degradation processes such as photochemical breakdown, influence pesticide dissipation. While pesticides offer essential benefits in agriculture and viticulture, their potential risks must also be taken into consideration (Fantke *et al*., 2012).

Fluopyram (ISO common name; IUPAC: *N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl] ethyl}-α,α,α-trifluoro-o-toluamide*; Fig. 1a) is a pyridylethylamide fungicide with broad-spectrum activity (EFSA 2023). It effectively controls pathogens such as *Botrytis* spp., *Sclerotinia* spp., and *Monilia* spp., as well as powdery mildew and leaf spot diseases in various crops (Kandel *et al.,* 2018). Fluopyram functions by inhibiting succinate dehydrogenase (SDH), also known as complex II in the mitochondrial respiratory chain, a key

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component of the tricarboxylic acid cycle (Abad-Fuentes *et al.,* 2015).

Trifloxystrobin (ISO common name; IUPAC: *methyl (2E)-(methoxyimino)(2-{[({(1E)-1-[3-(trifluoromethyl)phenyl]ethylidene}amino)oxy]methyl}phenyl)acetate;* Fig. 1b) is another broad-spectrum fungicide belonging to a class of fluorine-containing compounds (EFSA, 2025). Derived synthetically from naturally occurring strobilurin found in wood-decaying fungi, trifloxystrobin is widely used to combat diseases such as powdery mildew, downy mildew, and anthracnose in fruits, vegetables, and cereals (Ziegler *et al.,* 2003). It disrupts mitochondrial respiration by inhibiting electron transfer in the electron transport chain (Bartlett *et al.,* 2002).

Assessing the metabolism of these fungicides is critical when evaluating their residues. Fluopyram-benzamide (Fig. 1c) and trifloxystrobin acid (CGA 321113) (Fig. 1d) are the primary metabolites of fluopyram and trifloxystrobin, respectively. Research suggests that these metabolites may pose greater environmental and health risks than their parent compounds due to their mobility and persistence (Bartlett *et al.,* 2002; Wei *et al.*, 2016). Therefore, it is essential to quantify fluopyram, trifloxystrobin, and their metabolites to ensure consumer safety (Sharma *et al.*, 2022; Cui *et al.,* 2023).

Luna Sensation® 500 SC is a suspension concentrate containing 250 g/L trifloxystrobin and 250 g/L fluopyram. This systemic, preventive, and curative fungicide controls a range of pathogenic fungi, including powdery mildew and botrytis rot in fruits, as well as sclerotinia and monilia diseases in various vegetables and crops (Bayer Crop Science 2026).

Extensive research has employed liquid and gas chromatography to analyze the dissipation behavior of trifloxystrobin and fluopyram residues in various crops, both individually and in combination with other analytes (Jyot *et al.,* 2010; Mohapatra *et al.,* 2010; Sahoo *et al.,* 2012; Patyal *et al.,* 2013; Dong and Hu 2014; Paramasivam *et al.,* 2017; Katna *et al.,* 2018; Sharma *et al.,* 2019; Ren *et al.,* 2023). Investigating the dissipation pattern of a ready-to-use trifloxystrobin and fluopyram formulation in grapevines holds significant practical value. However, data on their behavior in grapevines remains limited, despite similar studies on onions (Sharma *et al.,* 2022) and chili peppers (Mandal *et*

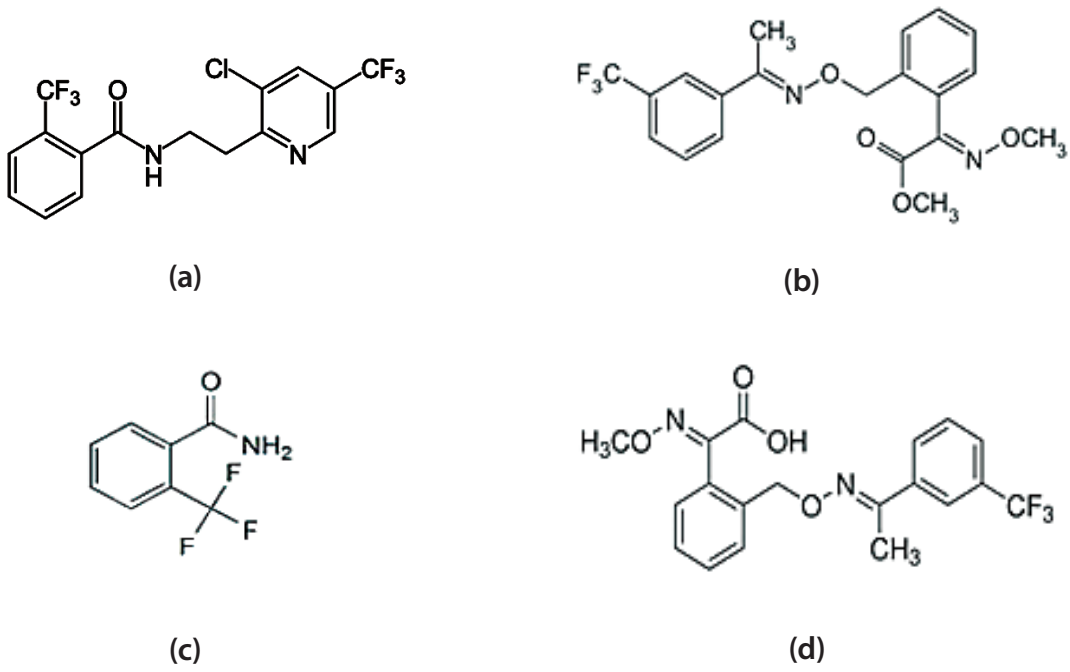

Figure 1. Chemical structure of a) fluopyram, b) trifloxystrobin, c) fluopyram-benzamide and d) CGA 321113 (trifloxystrobin acid).

al., 2023; Parmar *et al.,* 2023).

In this study, the persistence, dissipation kinetics, terminal residues, and consumer risk estimation of a trifloxystrobin and fluopyram formulation (Luna Sensation® 500 SC) under Saudi Arabian climatic and cultivation conditions were assessed. Although Luna Sensation® 500 SC is a registered product and has undergone regulatory evaluation as part of the authorization process, residue behavior can vary with crop, climate, and agricultural practices. Therefore, generating residue and dissipation data under local Saudi field conditions supports evidence-based risk management and helps confirm compliance with relevant maximum residue limits (MRLs) and pre-harvest intervals.

Materials and Methods

Chemicals and Reagents

Certified reference standard of trifloxystrobin (99.4% purity) was obtained from Chem Service Inc. (West Chester, PA, USA). Certified reference standard of fluopyram (98.7% purity), fluopyram-benzamide (99.6% purity), and CGA 321113 (98.68% purity) were sourced from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC-grade methanol, acetonitrile, LC-MS-grade formic acid, and ammonium formate were obtained from Fisher Scientific Ltd. (Loughborough, UK). Sodium chloride, trisodium citrate, disodium hydrogen citrate sesquihydrate and anhydrous magnesium sulfate were purchased from Chem-Lab NV (Zedelgem, Belgium). A ceramic homogenizer was purchased from Chrom Tech, Inc. (Copure®, Apple Valley, MN, USA). Bulk sorbents, including primary secondary amine (PSA) and Octadecylsilane (C18), were obtained from Macherey-Nagel (Chromabond, Düren, Germany). Ultrapure water was produced using an Evoqua Ultra Clear system (Evoqua Water Technologies LLC, Günzburg, Germany). The commercial formulation Luna Sensation® 500 SC (Bayer Crop Science Ltd.) was acquired locally and contains 25% fluopyram and 25% trifloxystrobin.

Standard solution preparation

Individual pesticide standard stock solution of each of the four tested analytes (1000 mg/L each) were prepared in pure acetonitrile. An intermediate standard mixture of acetonitrile was prepared at 100 mg/L by mixing the individual stock solutions. The working standard mixture of the four analytes (10 mg/L) was prepared from intermediate solutions at serial concentrations of 0.001-0.5 mg/L for the construction of solvent-based or matrix-matched calibration curves, respectively, by diluting with acetonitrile or the final sample extracts. All standard solutions were stored in a freezer at -20°C.

Field Experiments

The open-field trials were conducted in Buraidah City, Al-Qassim Region, Saudi Arabia, in August 2024. The cultivated table grape area was divided into 12 plots: four plots were treated with Luna Sensation® 50 SC at the low recommended application rate of 400 mL/ha (equivalent to 100 g a.i./ha of fluopyram and 100 g a.i./ha of trifloxystrobin), and four plots were treated at the high recommended application rate of 600 mL/ha (equivalent to 150 g a.i./ha of each active ingredient). The remaining four plots were maintained as an untreated buffer zone between treated areas to minimize spray drift. The formulation was diluted with tap water to a spray volume of 1000 L/ha and applied using a knapsack sprayer calibrated before application. Spraying was conducted by walking along the vine rows and applying the spray uniformly to the canopy and grape clusters in each plot to achieve consistent coverage.

Approximately 2 kg of grapes were collected from each treated plot at 0 (2 h), 1, 3, 7, 10, 14, and 20 days after application to assess dissipation and to determine half-lives ($t_1/_2$) and pre-harvest intervals (PHIs).

Terminal residues

Terminal residues were assessed in a

separate set of plots (independent of the dissipation study). For the terminal residue study, each application rate (low and high) was applied twice, with a 7-day interval between the first and second applications. Grape samples (~2 kg per plot) were collected at 3 and 7 days after the second (final) application to determine terminal residues under a repeated-use pattern. Applications and sampling were conducted at a late production stage close to commercial maturity to reflect residue-relevant pre-harvest conditions.

During the field trial, temperatures ranged from 29 to 45°C, with a mean relative humidity of 13–18%. Samples were transported under cold conditions, frozen overnight, homogenized, thoroughly mixed, divided into sub-samples, and stored at –20°C until analysis.

LC-MS/MS

The LC-MS/MS analysis was conducted using a Dionex Ultimate 3000 RS UHPLC system (Thermo Fisher Scientific, Austin, TX, USA) coupled with a TSQ Altis triple quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA). Chromatographic separation was achieved on an Accucore RP-MS C18 column (100 x 2.1 mm, 2.6 µm film thickness) (Thermo Fisher Scientific, Ireland), maintained at 40°C. The mobile phase comprised (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v). The gradient elution program began with 10% B, held for 1 minute, and increased to 90% B over 5 minutes, where it was held for 2 minutes. At 8.1 minutes, the mobile phase returned to 10% B and was maintained for 6 minutes, resulting in a total run time of 14 minutes.

The analytes were detected in multiple reaction monitoring (MRM) mode (Table 1), optimized with positive electrospray ionization (ESI+) at a capillary voltage of 3.8 kV. The sheath and auxiliary gas were set to 40 and 10 Arb, respectively, with the vaporizer and ion transfer tube temperatures set to 350°C and 275°C, respectively. High-purity nitrogen was used as a nebulizer gas. Data acquisition and processing were carried out using Trace Finder software (version 4.1).

Extraction and cleanup

Extraction was based on the citrate-buffered QuEChERS approach, followed by dispersive-SPE cleanup using $MgSO_4$, PSA, and C18 to reduce co-extractives from the grape matrix. A 10-g sample of frozen, homogenized berries was weighed into a 50-mL centrifuge tube. For extraction, 10 mL of acetonitrile was added, followed by vigorous vortexing for 2 minutes with a ceramic homogenizer. A salt mixture containing 4 g of magnesium sulfate, 1 g of trisodium citrate, 0.5 g of disodium hydrogen citrate sesquihydrate, and 1 g of sodium chloride was added, then vortexed for 1 minute and centrifuged at 5000 rpm for 5 minutes. Two milliliters of the acetonitrile supernatant were trans-

Table 1. LC-MS / MS Parameters.

Pesticide	t_R (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (v)	RF lens (v)
Trifloxystrobin	8.61	409.1	145	44	57
			<u>186</u>	18	57
CGA 321113	8.04	395.1	<u>145</u>	41	50
			186	18	50
Fluopyram	7.68	396.9	<u>208</u>	22	75
			173	29	75
Fluopyram-benzamide	5.76	190.1	<u>170</u>	12	30
			130	22	30

The underlined ions used as quantifier ion.

ferred to a 15 mL tube containing 300 mg of magnesium sulfate, 50 mg of PSA, and 50 mg of C18, vortexed for 30 seconds, and centrifuged again at 5000 rpm for 5 minutes. Finally, 1 mL of the cleaned extract was filtered through a 0.22 µm nylon syringe filter (Whatman, USA) into an LC-MS/MS vial for analysis.

Method validation

The method was validated according to SANTE guidelines (European Commission, 2021). Selectivity was evaluated by analyzing six replicates of blank grape berry samples alongside six replicates of grape berry samples spiked with the target analytes at the method's limit of quantification (LOQ). Chromatographic data were examined to confirm the absence of peaks at the retention times of the target analytes in the blank samples, ensuring no interference from matrix components. Linearity was evaluated using matrix-matched calibration curves prepared at eight concentration levels covering 0.001–0.2 mg/L in the final cleaned extract. The matrix effect (ME) was assessed by comparing the slope of the matrix-matched calibration curve with that of the calibration curve constructed in pure acetonitrile. Detection sensitivity was evaluated using the limit of detection (LOD), defined as the lowest concentration detectable at a signal-to-noise ratio (S/N) of 3. The LOQ was defined as the lowest concentration that could be quantified with acceptable accuracy (70%–120%) and precision (RSD < 20%). Recovery experiments were performed at 0.01–3 mg/kg to assess accuracy across a broad range, while LOQs were confirmed separately at 0.0025 mg/kg (trifloxystrobin, fluopyram) and 0.005 mg/kg (CGA 321113, fluopyram-benzamide) based on S/N ≥ 10 and acceptable accuracy/precision. Accuracy and precision were assessed by fortifying blank grape samples at 0.01, 0.1, 1, and 3 mg/kg (n = 6 per level). Precision at the LOQ was evaluated as intra-day repeatability (n = 6, RSD_r) and inter-day reproducibility (n = 18 across three days, RSD_R). All final residue concentrations are reported as mg/kg (fresh weight) of grape berries.

Calculations

Residual concentrations: The residual concentrations were calculated according to the residue definition for risk assessment, in particular total trifloxystrobin and total fluopyram were expressed as the sum of the parent compound and its specific metabolite, using equations 1 and 2, where a conversion factor to parent compound of 1.04 and 2.1 for trifloxystrobin and fluopyram respectively was used by dividing the molecular weight of the parent compound by the molecular weight of its metabolite:

Total residue of trifloxystrobin = Trifloxystrobin residue + [1.04 × CGA 321113 residue] (Eq. 1)

Total residue of fluopyram= Fluopyram residue + [2.1 × Fluopyram-benzamide residue] (Eq. 2)

Dissipation rates: The dissipation rates of trifloxystrobin and fluopyram were calculated using the first-order kinetics equation (Eq. 3). The half-lives ($t_{1/2}$) were calculated using equation 4 (Hoskins, 1961). The PHI or the safe waiting period was calculated using equation 5 (Hingmire *et al.,* 2015; Abdallah *et al.,* 2023).

$$C_t = C_0 e^{-kt} \quad \text{(Eq. 3)}$$

$$t_{1/2} = \frac{ln2}{k} \quad \text{(Eq. 4)}$$

$$PHI = \frac{lnc_0 - lnMRL}{k} \quad \text{(Eq. 5)}$$

where C_t is the concentration of total trifloxystrobin and fluopyram residues at time t, C_0 is the initial concentration, and *k* is the dissipation rate ($days^{-1}$), *kt* denotes the product of *k* and time *t* (days) in the exponential term of the first-order dissipation equation.

Chronic dietary risk assessment: The long-term dietary risk of total trifloxystrobin and fluopyram was evaluated by determining

the hazard quotient (HQ) using equations 6 and 7 (EFSA 2018; Malhat and Abdallah, 2019) as follows:

$$NEDI = \sum (STMR_i \times F_i) \qquad \text{(Eq. 6)}$$

$$\%HQ_c = \frac{NEDI}{ADI \times bw} \times 100 \qquad \text{(Eq. 7)}$$

where NEDI (mg/kg bw/day) represents the national estimated daily intake, STMR (mg/kg) is the median total residue of the supervised trials, and F_i (kg/day) is the mean daily consumption of fresh table grapes used for chronic exposure assessment (F_i = 0.0168 kg/day) (FAO/WHO, 2014). bw (kg) represents the mean adult body weight (60 kg), and ADI is expressed as mg/kg bw/day. The calculation used STMR values consistent with the residue definition for risk assessment (i.e., total trifloxystrobin and total fluopyram, where total residues represent the parent compound plus relevant metabolites using the conversion factors described in Eqs 1–2). The chronic dietary risk assessment was conducted for adult consumers, and the referenced consumption value was used as an input to evaluate consumer safety under Saudi conditions.

Results and discussion

Method Validation

Selectivity and linearity: The selectivity assessment confirmed the absence of interfering peaks at the retention times of the target analytes in the blank grape berry samples. Spiked samples showed clear, well-resolved peaks corresponding to the analytes, demonstrating the method's ability to accurately differentiate and quantify the target compounds in the grape berry matrix.

The linearity of the LC-MS/MS method was assessed by constructing a matrix-matched calibration curve for each analyte in the final cleaned extract, as described in the proposed procedure. The curves were constructed using eight concentration levels, ranging from 0.001 to 0.2 mg/kg (equivalent to mg/L). Satisfactory linearity was obtained for trifloxystrobin and fluopyram in the range of 0.001-0.2 mg/kg with coefficients of determination (R^2) of higher than 0.9996, and 0.0025-0.2 mg/kg and 0.005-0.2 mg/kg for CGA 321113 and fluopyram-benzamide with coefficients of determination (R^2) of 0.9994 and 0.9995, respectively (Table 2). Samples containing concentrations above the upper limit of the calibration curve are diluted with a blank extract to bring them within the calibration curve's range.

Matrix effects: The signal enhancement or suppression percentage was investigated using the formula ME% = Sm/Ss × 100, where Sm is the slope of the matrix-matched calibration curve, and Ss is the slope of the in-solvent calibration curve. To minimize instrument drift, solvent- and matrix-matched calibration standards were injected in the

Table 2. Validation results.

	Trifloxystrobin	CGA 321113	Fluopyram	Fluopyram-benzamide
Linearity (mg/kg)	0.001-0.2	0.0025-0.2	0.001-0.2	0.005-0.2
Correlation coefficient (r)	0.9996	0.9994	0.9997	0.9995
LOD (mg/kg)	0.00021	0.00055	0.00027	0.00094
LOQ (mg/kg)	0.0025	0.005	0.0025	0.005
RSD_r[a]	10.71	11.33	8.94	11.58
RSD_R[b]	15.42	16.81	14.24	16.25
Matrix effect (%)	-8.16	-6.25	-4.73	+11.14

[a] RSD_r: the relative standard deviation (intra-day repeatability, n=6).
[b] RSD_R: the relative standard deviation (inter-days repeatability, n=18).

same batch using a bracketing sequence and QC checks; therefore, slope differences were attributed to matrix effects rather than signal instability. For all test analytes, a weak matrix effect was observed, with signal suppression noted in trifloxystrobin (-8.16%), CGA 321113 (-6.25%), and fluopyram (-4.73%). In contrast, the fluopyram-benzamide signal was enhanced by 11.14% (Table 2). Ferrer *et al*. (2011) reported a weak matrix effect when values were within ±20% (Ferrer *et al.,* 2011). To achieve more accurate, precise results, matrix-matched calibration curves were used to quantify trifloxystrobin, fluopyram, and their metabolites in the tested samples, thereby compensating for matrix effects (MEs).

LODs and LOQs: The confirmed limits of quantification (LOQs) were 0.0025 mg/kg for trifloxystrobin and fluopyram, with recovery rates of 81.3 ± 9% and 79.8 ± 6%, respectively, and 0.005 mg/kg for CGA 321113 and fluopyram-benzamide, with recovery rates of 83.2 ± 7% and 78.5 ± 7%, respectively. The limits of detection (LODs) were 0.00021 mg/kg for trifloxystrobin and 0.00055 mg/kg for CGA 321113, while fluopyram and fluopyram-benzamide had LODs of 0.00027 mg/kg and 0.00094 mg/kg, respectively. These LOQs were well below the established MRLs of 2 mg/kg for total trifloxystrobin and 3 mg/kg for total fluopyram, highlighting the high sensitivity of the analytical method described. Representative chromatograms for all tested analytes at these LOD and LOQ levels are provided in Figures S1 and S2.

Recovery and precision: The recovery study was conducted using four spiking levels: 0.01, 0.1, 1 and 3 mg/kg. These levels included concentrations lower than, equal to, or higher than the MRL of the tested analytes. For each spiking level, six replicates were analyzed. The recoveries ranged from 90.8 to 94.5% and 84.8-89.7% for trifloxystrobin and its metabolite CGA 321113, respectively, and from 90.7 to 94.8% and 83.3-86.2% for fluopyram and its metabolite fluopyram-benzamide, respectively (Table 3). The relative standard deviations (RSDs) ranged from 2.6% to 8.4%, which satisfied the requirements of the SANTE guideline of 70-120% and RSD of <20%. Method precision was estimated at the LOQ level for each analyte, relative standard deviation (RSD) in terms of intra-day repeatability (RSD_r), which were 10.71, 11.33, 8.94, and 11.58%, and inter-day repeatability (RSD_R) that were 15.42, 16.81, 14.24, and 16.25%, for trifloxystrobin, CGA 321113, fluopyram, and fluopyram-benzamide, respectively (Table 2), which are within the satisfactory limit of < 20%, indicating consistency of the proposed method.

The present study's validation criteria data satisfied the SANTE method validation guideline. It is considered appropriate for quantifying the residue of trifloxystrobin and fluopyram, as well as their metabolites, in grape berries.

Dissipation patterns of trifloxystrobin and fluopyram in grape berries

Dissipation of trifloxystrobin and its metabolite CGA 321113: The dissipation patterns of trifloxystrobin and its metabolite CGA 321113 in grapes are summarized in Table 4.

Table 3. Average recoveries ± RSD (%) of the tested analytes in grape berries.

Spiking level (mg/kg)	Average recoveries (%)			
	Trifloxystrobin	CGA 321113	Fluopyram	Fluopyram-benzamide
0.01	91.4±6.2	87.4±8.4	90.7±3.3	84.8±8.1
0.1	94.5±4.8	85.2±6.5	92.4±5.4	86.2±3.7
1	93.3±2.6	89.7±3.1	94.8±7.8	83.3±4.4
3	90.8±4.1	84.8±4.9	93.1±6.8	85.1±7.1

RSD refers to the relative standard deviation of replicate measurements (n = 6).

Initial deposits of trifloxystrobin (including its metabolite CGA 321113) were 0.97 mg/kg and 1.08 mg/kg for the low (100 g a.i./ha) and high (150 g a.i./ha) application rates, respectively. The dissipation followed first-order kinetics, as demonstrated in the semi-logarithmic dissipation curves (Fig. 2).

By the third day after application, trifloxystrobin residues had dissipated by 49% for the low application rate and 46.46% for the high application rate. By the 10th day, dissipation reached 89.96% and 83.25%, respectively, and by the 20th day, residues decreased by 97.72% and 96.50%, resulting in final residues of 0.022 mg/kg and 0.038 mg/kg, respectively. The half-lives ($t_1/_2$) for trifloxystrobin were calculated to be 3.46 days and 4.04 days for the low and high application rate, with dissipation rate constants (k) of 0.2005 and 0.1714 days^{-1} (Table 4).

The dissipation behavior of trifloxystrobin can be attributed to its lower water solubility (0.16 mg/L at 20°C) and relatively low vapor pressure (1.2×10^{-3} mPa), which likely reduces its rate of volatilization and leaching. The observed hydrolysis of trifloxystrobin, leading to the formation of its metabolite CGA 321113, contributed to overall dissipation. The metabolite CGA 321113 exhibited a rapid decline, with its highest concentration observed at one day post-application and subsequently falling below the detection limit (BDL) by the third day. By the 20th day, metabolite levels were below the quantification limit of both application rates (LOQ).

In previous studies, Mohapatra *et al.* (2010) reported longer half-lives for tri-

Table 4. Dissipation kinetics of total trifloxystrobin and total fluopyram in table grape berries.

	Total trifloxystrobin (mg/kg)		Total Fluopyram (mg/kg)	
	100 g a.i/ha^{-1}	150 g a.i/ha	100 g a.i/ha	150 g a.i/ha
Slope (C_0) (mg/kg)	0.8496	0.9985	1032.3	1215.4
Intercept (k)(day^{-1})	0.2005	0.1714	0.3104	0.2295
R^2	0.9692	0.9902	0.9802	0.9871
$t_{0.5}$ (days)	3.46	4.04	2.23	3.02
EU-MRL (mg/kg)	3	3	2	2
PHI (days)	6.29	6.42	2.13	2.17

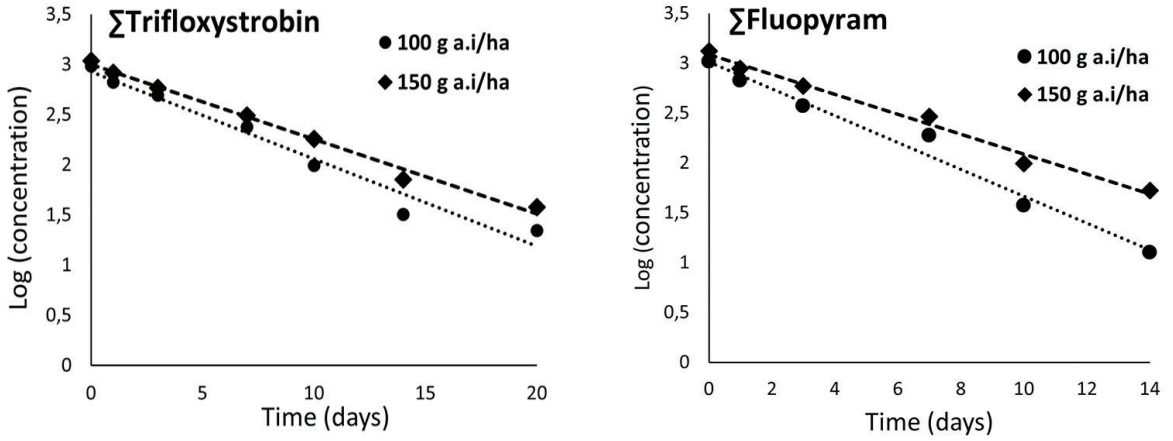

Figure 2. Semi-logarithmic dissipation curves of total trifloxystrobin and fluopyram across high-low doses of 100 and 150 g a.i./ha.

floxystrobin when combined with tebuconazole (Nativo 75 WG), with half-lives of 8.3 to 8.6 days in grapes (Mohapatra *et al.,* 2010). In contrast, shorter half-lives were observed in crops such as chili (1.58–1.81 days) (Sahoo *et al.,* 2012), gherkin (2.9–3.7 days) (Mohapatra, 2015), and tomatoes (1.08–2.13 days) (Sharma *et al.,* 2019). These differences suggest that the dissipation rate of trifloxystrobin depends on crop type and environmental conditions. The primary degradation pathway involves hydrolysis, producing the metabolite CGA 321113 (Banerjee *et al.,* 2006), which has been shown to dissipate rapidly in matrices such as tea (Paramasivam *et al.,* 2017).

Dissipation of fluopyram and its metabolite fluopyram-benzamide: The dissipation kinetics of fluopyram and its metabolite fluopyram-benzamide in grape berries are presented in Table 4. Initial fluopyram deposits were 1.04 mg/kg and 1.32 mg/kg for the low (100 g a.i./ha) and high (150 g a.i./ha) application rates, respectively. The dissipation of fluopyram followed first-order kinetics, as shown by the linear decline in concentration over time on a semi-logarithmic scale (Fig. 2). By the third day, fluopyram residues had dissipated by 64.08% and 55.23% for the low and high application rates, respectively. By the 10th day, dissipation reached 96.38% and 92.55%, with residues falling below detection limits by day 20.

Fluopyram exhibited shorter half-lives than trifloxystrobin, with half-lives of 2.23 and 3.02 days at the low and high application rates, respectively, and dissipation rate constants (k) of 0.3104 and 0.2295 days^{-1}. The faster dissipation of fluopyram can be attributed to its higher water solubility (16 mg/L at 20°C) and greater vapor pressure (2.4×10^{-3} mPa), which enhance volatility and hydrolysis under hot, dry conditions. The absence of detectable levels of fluopyram-benzamide throughout the study suggests that it undergoes rapid degradation or is not formed in grape tissues. Fluopyram's half-life vary across different crops, with values ranging from 4.04 to 5.18 days in pomegranates (Patil *et al.,* 2018), 6.48 to 6.60 days in watermelons (Dong and Hu, 2014), and 3.8 to 4.4 days in peppers, tomatoes, and cucumbers (Wei *et al.,* 2016). Patel *et al.* (2016) reported longer half-lives of 8.85 to 9.12 days in onions (Patel *et al.* 2016), highlighting the influence of crop type and environmental conditions on residue dissipation.

The distinct dissipation rates of trifloxystrobin and fluopyram can be attributed to their physicochemical properties. Trifloxystrobin's lower solubility and vapor pressure result in slower dissipation, whereas fluopyram's higher solubility and vapor pressure enhance its degradation, particularly under high-temperature and low-humidity conditions. This was evident from the study results, which showed that residues of both fungicides declined below the relevant EU MRLs. For trifloxystrobin, the EU MRL for table grapes is 3 mg/kg (EU-MRL database). For fluopyram, EU legislation distinguishes between table grapes (2 mg/kg) and wine grapes (1.5 mg/kg) (EU-MRL database); since the present study was conducted on table grapes, compliance was assessed against the 2 mg/kg MRL. The calculated pre-harvest intervals (PHIs) were 6.29 days (trifloxystrobin) and 2.13 days (fluopyram). The extended ripening period in grapes, combined with the environmental conditions in the Al-Qassim region of Saudi Arabia—high temperatures (29 to 45°C) and low humidity (13% to 18%)—likely contributed to the rapid dissipation observed The findings confirm that both fungicides, when applied at recommended rates, are safe for use in grape cultivation in the Al-Qassim region, as they meet food safety standards well before harvest.

Terminal Residues of Trifloxystrobin and Fluopyram

Terminal residue results are presented for the parent compounds (trifloxystrobin and fluopyram) (Table 5). The metabolites (CGA 321113 and fluopyram-benzamide) were below the LOQ in samples collected; therefore, total residues (parent + metabolites) were effectively equivalent to parent

residues under the conditions of this study. At a lower application rate of 100 g a.i/ha, after two applications with a short 3-day harvest interval, trifloxystrobin residues were 0.663 mg/kg, and fluopyram residues were 0.55 mg/kg. Extending the harvest interval to 7 days resulted in a significant reduction in residues to 0.38 mg/kg for trifloxystrobin and 0.21 mg/kg for fluopyram, demonstrating effective dissipation over time Increasing the application rate to 150 g a.i/ha raised residues up to 0.74 mg/kg for trifloxystrobin and 0.67 mg/kg for fluopyram with the same 3-day interval. However, a longer 7-day interval effectively reduced residues, underscoring the benefit of extended pre-harvest periods in promoting dissipation.

All recorded residue levels were well below the established MRLs of 3 mg/kg for trifloxystrobin and 2 mg/kg for fluopyram, indicating compliance with safety guidelines. The calculated PHIs were approximately 6.29 days for trifloxystrobin, 2.13 days for fluopyram at the lower application rate, and 6.42 days and 2.17 days at the higher application rate. Given the combined use of these fungicides, a 7-day PHI is recommended to ensure compliance and safeguard consumer health.

Chronic dietary risk assessment

The dietary risk analysis, using National Estimated Daily Intake (NEDI) and Hazard Quotient (HQ) metrics (Table 6), confirmed a low consumer risk, even with higher application rates. For the 100 g a.i/ha application rate with two applications and a 3-day harvest interval, NEDI values were 1.40E-04 mg/kg bw for trifloxystrobin and 1.12E-04 mg/kg bw for fluopyram. The corresponding HQ values were 0.14% and 0.93%, which dropped to 0.06% and 0.47% with a 7-day interval.

Table 5. Terminal residues of trifloxystrobin and fluopyram in grapevine.

Dosages (g a.i/ha)	Number of applications	Harvest interval (days)	Mean residues (mg/kg)	
			Trifloxystrobin	Fluopyram
100	2	3	0.6633	0.5517
		7	0.3782	0.2113
150	2	3	0.7426	0.6738
		7	0.4572	0.2324

Mean residues are reported for the parent compounds; metabolites were below the LOQ in terminal samples; therefore, total residues were effectively equivalent to parent residues under these conditions.

Table 6. Supervised trials median residues (STMR), national estimated daily intake (NEDI, mg/kg bw/day), and hazard quotient (HQ, %) values of trifloxystrobin and fluopyram in grape berries.

Dosages (g a.i/ha)	Number of applications	Harvest interval (days)	STMR (mg/kg)		NEDI (mg/kg bw/day)		HQ (%)	
			Trifloxystrobin	Fluopyram	Trifloxystrobin	Fluopyram	Trifloxystrobin	Fluopyram
100	2	3	0.524	0.418	1.40E-04	1.12E-04	0.140	0.934
		7	0.228	0.208	6.13E-05	5.59E-05	0.061	0.466
150	2	3	0.613	0.513	1.65E-04	1.38E-04	0.165	1.147
		7	0.294	0.267	7.89E-05	7.15E-05	0.079	0.596

STMR values correspond to total residues according to the risk-assessment residue definition; metabolites were <LOQ in terminal samples.

At the higher 150 g a.i/ha application rate, NEDI values rose slightly to 1.65E-04 mg/kg bw for trifloxystrobin and 1.38E-04 mg/kg bw for fluopyram after the second application with a 3-day interval. HQ values of 0.165% and 1.147% were observed for trifloxystrobin and fluopyram, respectively. These figures are significantly below the 100% threshold, demonstrating that residues, even with intensive use, remain at safe levels for chronic exposure.

The extended 7-day PHI for the combination of trifloxystrobin and fluopyram ensures that residue levels drop to safe levels, minimizing dietary risk. This adjustment is crucial, particularly in grape cultivation in Saudi Arabia, as it aligns with both national and international MRLs. The findings also support the safe use of the 50% SC formulation (containing 25% each of Trifloxystrobin and Fluopyram) in integrated pest management. The data provide a reliable foundation for establishing national MRLs, which support sustainable agricultural practices and ensure consumer safety.

Conclusion

In the current study the efficient dissipation of fluopyram and trifloxystrobin, along with their primary metabolites, in grape berries grown under the hot, dry conditions of the Al-Qassim region, Saudi Arabia was investigated. The developed and validated LC-MS/MS method proved adequate for the simultaneous determination of fungicides and their metabolites, providing reliable results for routine residue monitoring. High temperatures and low humidity contributed to the rapid dissipation of fluopyram, with rates reaching levels well below established MRLs before harvest.

The study confirmed that the PHIs of 6.29 days for trifloxystrobin and 2.13 days for fluopyram are adequate as to ensure consumer safety.. The results, therefore, highlighted the excellent suitability of the Luna Sensation 500 SC formulation for safe, practical application in integrated pest management in grape cultivation. These studies provide the information needed to establish national MRLs, which will contribute to the development of sustainable agriculture in compliance with food safety regulations in Saudi Arabia.

The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2025).

Authorship contribution

Saleh Alhewairini: Review & editing, Project administration, Funding acquisition. Osama Abdallah and Ayman F. Omar: Conceptualization, Writing – original draft, Validation, Resources, Methodology, Investigation. Khaled Al-Jamhan and Fahad Almulhim: Sample collection, Formal analysis, Data curation, Software.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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Received: 28 May 2025; Accepted: 9 January 2026

Συμπεριφορά υπολειμμάτων φυτοπροστατευτικών ουσιών fluopyram και trifloxystrobin και εκτίμηση διατροφικής επικινδυνότητας σε σταφύλια στη Σαουδική Αραβία

O.I. Abdallah, F.S. Almulhim, A.F. Omar, K.A. Al-Jamhan και S.S. Alhewairini

Περίληψη Η παρούσα μελέτη διερευνά τη συμπεριφορά των υπολειμμάτων των δραστικών ουσιών fluopyram και trifloxystrobin σε σταφύλια που καλλιεργήθηκαν στην περιοχή Al-Qassim της Σαουδικής Αραβίας. Οι δραστικές ουσίες εφαρμόστηκαν με ψεκασμό φυλλώματος, μέσω σκευάσματος τύπου SC, στην εγκεκριμένη συνιστώμενη δόση (100–150 g δ.ο./ha). Για τον προσδιορισμό των υπολειμμάτων αναπτύχθηκε και επικυρώθηκε αναλυτική μέθοδος LC-MS/MS σύμφωνα με τις ευρωπαϊκές κατευθυντήριες οδηγίες, παρουσιάζοντας υψηλή γραμμικότητα ($R^2 > 0,999$), χαμηλά όρια ανίχνευσης (0,00021–0,00094 mg/kg) και ικανοποιητικές ανακτήσεις (84,8–94,8%) με σχετικές τυπικές αποκλίσεις (RSD) < 20%. Οι δοκιμές αποδόμησης έδειξαν κινητική πρώτης τάξης, με το fluopyram να αποδομείται ταχύτερα ($t_{1/2}$ = 2,23–3,02 ημέρες) σε σύγκριση με το trifloxystrobin ($t_{1/2}$= 3,46–4,04 ημέρες). Ο μεταβολίτης CGA 321113 μειώθηκε σε επίπεδα κάτω από το όριο ανίχνευσης εντός 3 ημερών από τον ψεκασμό, ενώ ο fluopyram-benzamide δεν ανιχνεύθηκε. Τα τελικά υπολείμματα βρέθηκαν κάτω από τα ανώτατα επιτρεπτά όρια (MRLs) των 3 mg/kg για το trifloxystrobin και των 2 mg/kg για το fluopyram, με τελευταία επέμβαση πριν την συγκμιδή (PHI) 6,29 και 2,13 ημερών, αντίστοιχα. Τα αποτελέσματα καταδεικνύουν αμελητέο κίνδυνο χρόνιας διατροφικής έκθεσης και τεκμηριώνουν την ασφαλή χρήση των εν λόγω φυτοπροστατευτικών ουσιών στην αμπελοκαλλιέργεια της Σαουδικής Αραβίας.

Hellenic Plant Protection Journal **19:** 33-47, 2026

Supplementary material

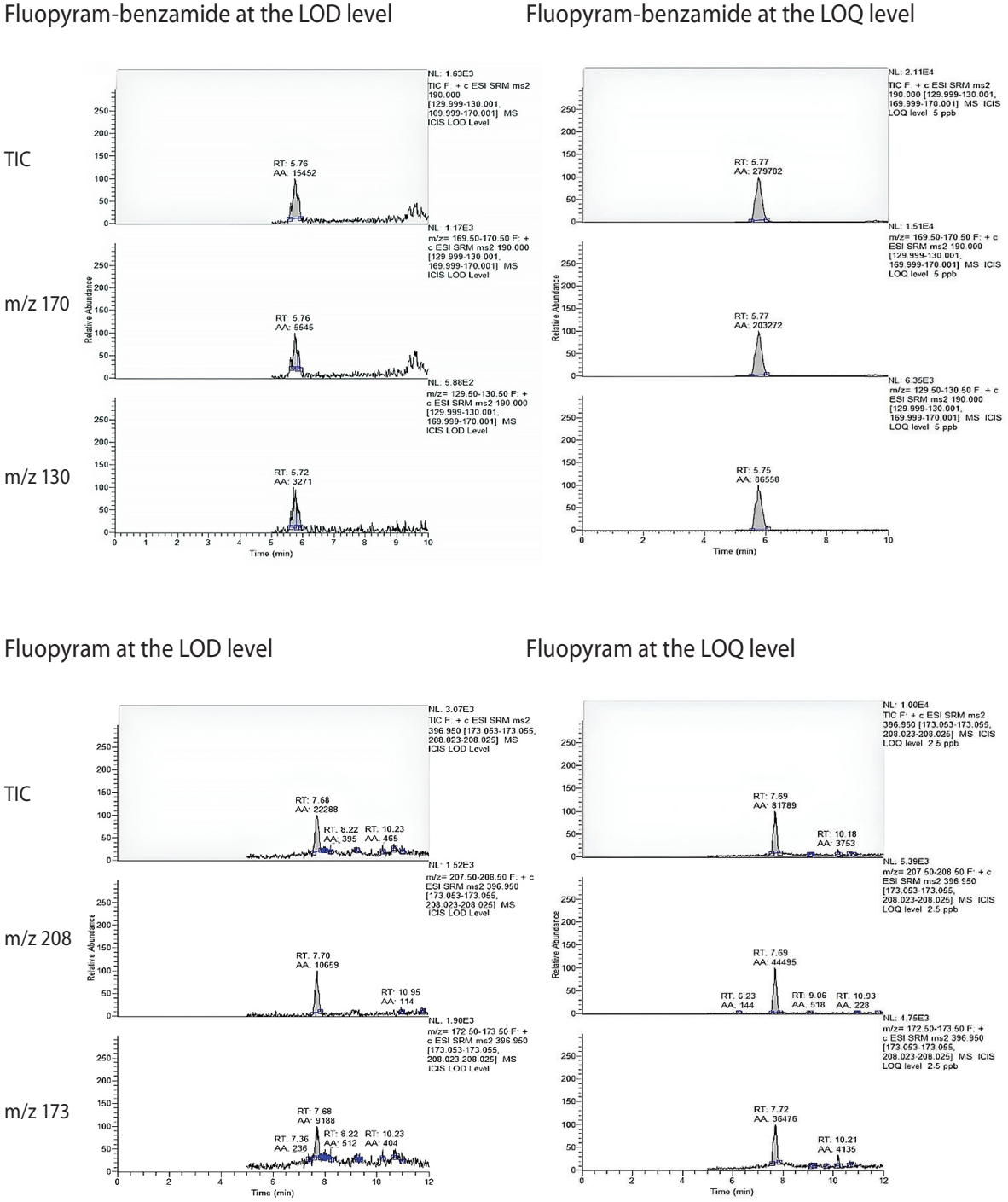

Figure S1. Representative LC-MS/MS chromatograms of Fluopyram and Fluopyram-benzamide at the Limit of Detection (LOD) and Limit of Quantitation (LOQ).

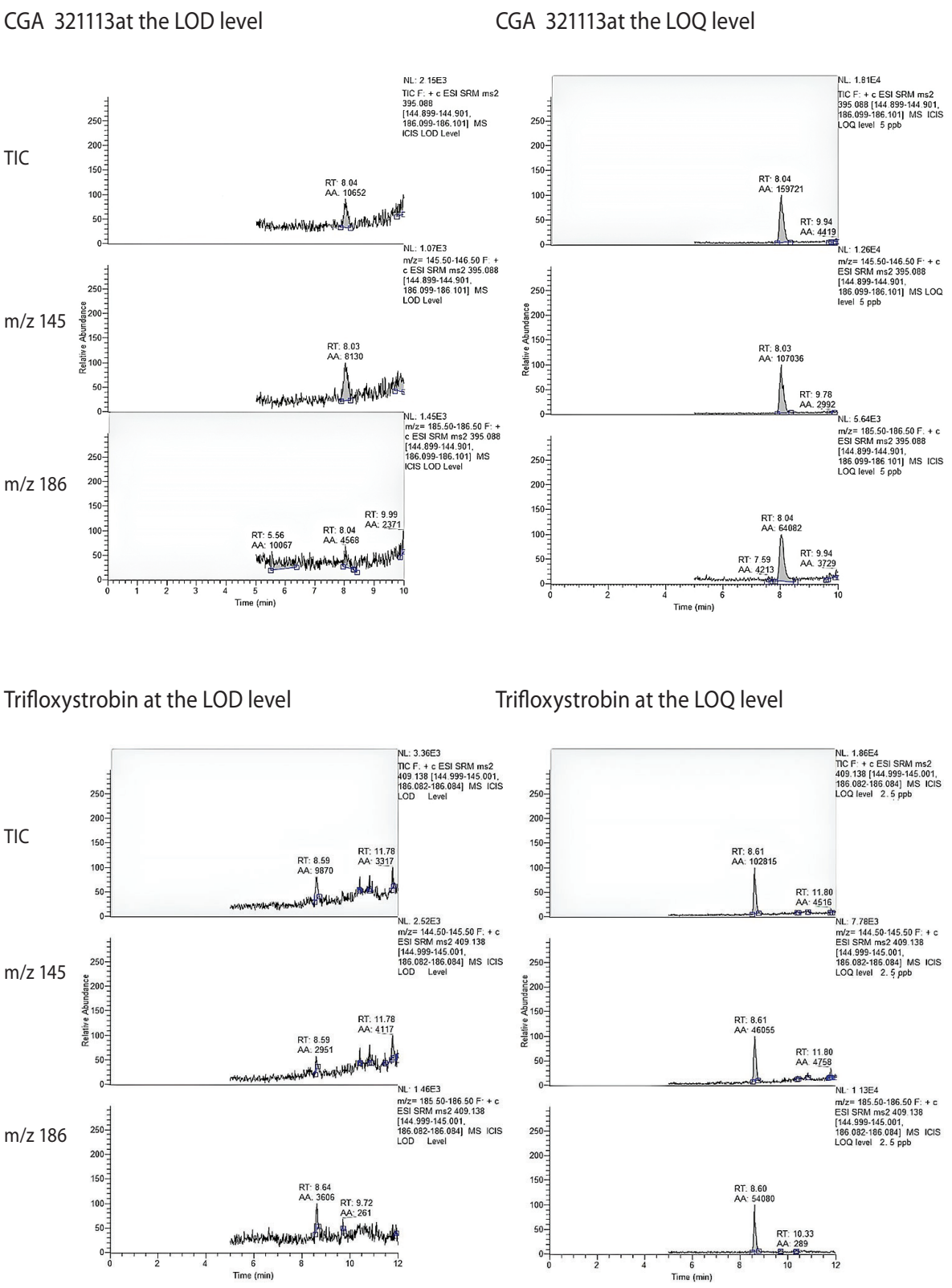

Figure S2. Representative LC-MS/MS chromatograms of CGA 31113 and trifloxystrobin at the Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Τόμος 19, Τεύχος 1, Ιανουάριος 2026
ISSN 1791-3691 (Print)
ISSN 2732-656X (OnLine)

Περιεχόμενα

Volume 19, Issue 1, January 2026
ISSN 1791-3691 (Print)
ISSN 2732-656X (OnLine)

Hellenic Plant Protection Journal

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