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REVIEW ARTICLE

Scale insect species (Hemiptera: Coccomorpha) and their natural enemies, recorded on agricultural, ornamental and forest plant species in the wider area of Messenian Province (Peloponnese, Greece), 2000 – 2020

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Summary The scale insects (Hemiptera: Coccomorpha) recorded on agricultural, ornamental and forest plant species in the wider area of Messenian Province (Peloponnese, Greece) during the years 2000 – 2020 are reviewed. Twenty species were recorded, which belong to four families: Diaspididae: *Aonidiella aurantii* (Maskell), *Chrysomphalus aonidum* (L.), *Diaspis echinocacti* (Bouché), *Dynaspidiotus abieticola* (Koroneos), *D. abietis* (Schrank), *Lepidosaphes beckii* (Newman), *L. gloverii* (Packard), *Lineaspis striata* (Newstead), *Targionia vitis* (Signoret); Coccidae: *Ceroplastes rusci* (L.), *Eulecanium sericeum* (Lindinger), *Nemolecanium graniformis* (Wünn), *Parthenolecanium corni* (Bouché), *P. persicae* (Fabricius), *Physokermes hemicryphus* (Dalman), *P. inopinatus* Danzig and Kozár, *Protopulvinaria pyriformis* (Cockerell); Pseudococcidae: *Phenacoccus madeirensis* Green, *Planococcus vovae* (Nasonov) and Kermesidae: *Kermes echinatus* Balachowsky. The biology, phenology and natural enemies in Messenia are discussed for fifteen of these scale species.

Additional keywords: Coccidae, Diaspididae, Kermecidae, Messenia, natural enemies, Pseudococcidae

Introduction

Information about the scale insects (Hemiptera: Coccomorpha) of Greece has been published in several articles of entomological journals, monographies and websites (Argyriou *et al.,* 1976; Argyriou, 1983; DeBach, 1964; García Morales *et al.,* 2016; Katsoyannos, 1996; Koroneos, 1934; Kozár *et al.,* 1991; Milonas and Kozár, 2008; Paloukis, 1979; Pellizzari *et al.,* 2011).

The present review contributes to this knowledge with a collective reference for twenty scale insect species found in Messenian Province, Peloponnese, Greece (Fig. 1) on agricultural, ornamental and forest plants species during the last twenty years. Messenia is a dynamic productive area in which economically important crops are cultivated, such as olives, citrus, vines, figs, potatoes, vegetables, etc. The knowledge of entomofauna in the wider region of Messenia and the record of the existing complex of the natural enemies of harmful insects, could be considered important to design plant protection programs in agriculture. The existence of dispersed small colonies of scale insects near the cultivated areas, could constitute a potential reservoir of parasitoids and predators for the control of prospective infestations of cultivations by these scale insect pests.

In fifteen out of the twenty recorded species, which were found in adequate population, biology, phenology and ecology data are available from studies conducted in the Laboratory of Biological Control of the Benaki Phytopathological Institute (years 2000 - 2003) and the Department of Agriculture of the University of the Peloponnese (former Technological Educational In-

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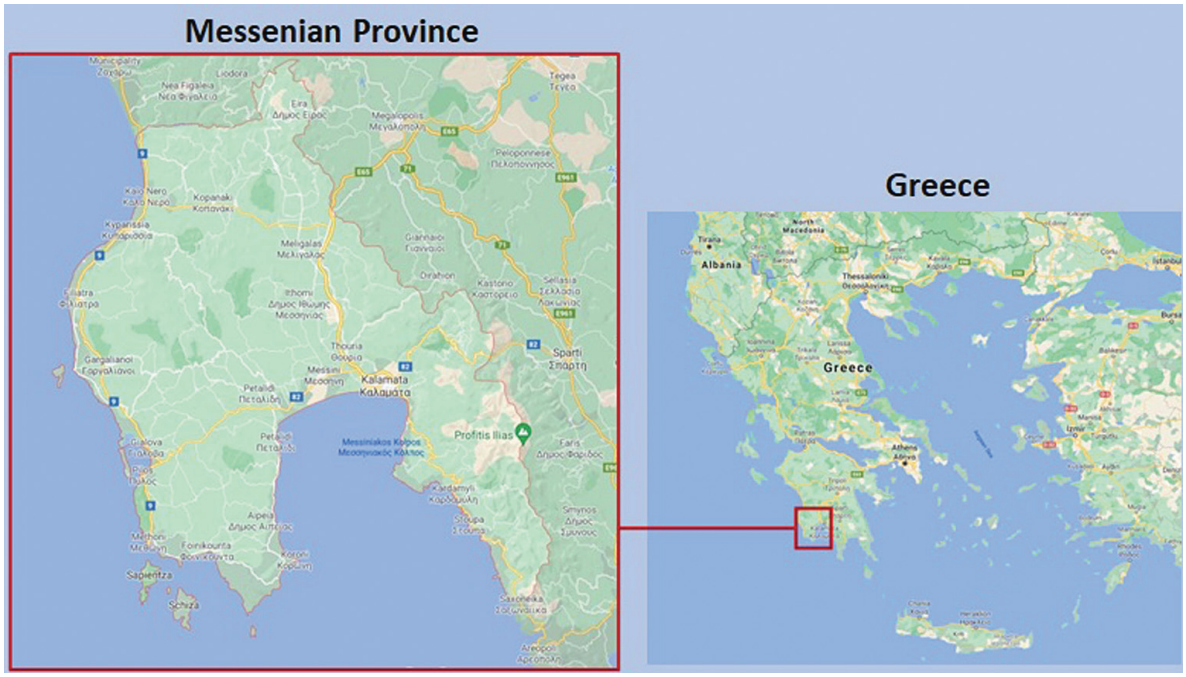

Figure 1. Messenian Province in Peloponnese, Southwestern Greece (Google map 1; Google map 2).

stitute of Peloponnese) (years 2004 - 2020). Information about the identification of the species, locations and materials and methods used for the biology/ecology studies is described in the articles of the cited bibliography. However, some information on materials and methods may be briefly presented when considered necessary.

Some scale insects recorded in this article were first records for Greece or first records on new host plant species of the scales, as it is referred in the cited references. Additionally, some natural enemies of the reviewed scale insects were first records for Greece or for Europe.

Figure 2. Orange fruit infested by *Aonidiella aurantii* in Kalamata, Greece (Photo by George Stathas).

DIASPIDIDAE

Aonidiella aurantii (Maskell)

Aonidiella autantii is a cosmopolitan species distributed in 89 countries, infesting plants of 178 genera belonging to 84 families (García Morales *et al.*, 2016).

It was recorded in Kalamata infesting *Citrus sinensis* (L.) Osbeck (Rutaceae) (Fig. 2). Field observations and laboratory examinations of infested leaves during March 2004 - April 2005 showed that *A. aurantii* completes three overlapping generations in Kalamata (Stathas *et al.*, 2005). It overwintered under all developmental stages, but the majority of the population during November 2004 – March 2005 consisted of preovipositing and ovipositing female adults. During the rest months, scales of all developmental stages were recorded. Three picks of numbers of crawlers were observed: the first during the third ten days of April to the beginning of May, the second during the third ten days of June to the beginning of July and a third smaller increase observed from the end of August to the end of September.

Natural enemies of *A. aurantii* found in Kalamata were the ectoparasite *Aphytis chrysomphali* (Mercet) (Aphelinidae) and the predator *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinelidae). These natural enemies are reported also in other studies in Greece (Argyriou *et al.,* 1976; Katsoyannos, 1996).

Chrysomphalus aonidum (L.)

Chrysomphalus aonidum originates from Asia but has become distributed in subtropical countries and worldwide in 87 countries, infesting plants of 181 genera belonging to 74 families (García Morales *et al.*, 2016). It could be considered a serious threat for many European countries as it has been recorded to infest more and more several plants in Spain (Garcia Mari *et al.*, 2000), Italy, (Pellizzari and Vacante, 2007), Hungary (Reiderne and Kozàr, 1994), France (Germain and Matile Ferrero, 2005) and the Netherlands (Jansen, 2004).

The first record of *C. aonidum* in Greece was reported by Koroneos (1934), as a pest of imported *Citrus* sp., which was not acclimatized in the country. Later, Argyriou and Mourikis (1981) reported that the scale was accidentally introduced in Greece during 1962-1965, but it had been under complete control. In April of the year 2000, *C. aonidum* was found on *Dracaena* sp. in Athens. The colony of this pest on *Dracaena* sp., was used to infest artificially pumpkins *Cucurbita maxima* Duchense (Cucurbitaceae) and potato tubes *Solanum tuberosum* L. (Solanaceae) in the laboratory and was the first record of the scale on plant species of these families (Stathas *et al.,* 2002).

In January 2007, *C. aonidum* was found on heavily infested fruits of *Citrus limon* and *C. sinensis* (L.) (Rutaceae) and leaves of *Ficus benjamina* L. (Moraceae) and *Ligustrum japonicum* Thunb. (Oleaceae) in Kalamata (Stathas and Kozár, 2008) and later in the same year on *Nerium oleander* L. (Apocynaceae) (Fig. 3). Infestation on *F. benjamina* (Moraceae) was the first record of the scale on this new host. It is a biparental and oviparous species. The population of the scale found on all the host plants in Kalamata from January to April 2007 consisted mainly of young female adults.

Regarding the natural enemies of the scale, the biology of the predator *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) was studied under controlled conditions in the laboratory (Stathas *et al.*, 2002).

Diaspis echinocacti (Bouché)

Diaspis echinocacti (Bouché) is recorded in 74 countries of the world, infesting 58 plant species belonging to the family Cactaceae and 9 plant species belonging to other families (García Morales *et al.,* 2016).

In Kalamata it was recorded on *Opuntia ficus-indica* (L.) Mill. (Cactaceae) (Fig. 4) and examination on its populations were made in nature and in laboratory, from February to June 2009 (Japoshvili *et al.*, 2010). *Diaspis echinocacti* was found to be biparental and oviparous. The populations of the scale in February 2009 consisted of all development stages except crawlers, which hatched during the first half of April. Until June, all development stages of the scale were present on *O. ficus-indica*.

Natural enemies of *D. echinocacti* recorded in Kalamata include the ectoparasitoid *Aphytis debachi* Azim (Hymenoptera: Aphelinidae), the endoparasitoid *Plagiomerus diaspidis* Crawford (Hymenoptera: Encyrtidae), the predator *Cybocephalus fodori* Endrödy-Youga (Coleoptera: Cybocephalidae) and small numbers of individuals of an unidentified predatory mite of the family Bdellidae (Prostigmata) (Japoshvili *et al.*, 2010). *Aphytis debachi* parasitized second instar nymphs and preovipositing females of the scale. Its parasitization rate reached 9.3% in March 2009. Both sexes of the parasitoid were recorded, which support the biparental status of the species (Japoshvili *et al.*, 2010) whereas only female adults were obtained in Hong Kong (Rosen and DeBach, 1979). *Plagiomerus diaspidis* parasitized second instar nymphs. Its parasitization rate reached 86% in March 2009. The endoparasite *P. diaspidis* has also been reported as a natural enemy of *D. echinocacti* in other studies (Gordh and Lacey, 1976; Panis and Pinet, 1999). The predator *C. fodori* was found to be very ac-

Figure 3. Infestation of *Chrysomphalus aonidum* on several host plants in Kalamata, Greece: (a): *Citrus sinensis,* (b): *Citrus limon,* (c): *Ficus benjamina*, (d): *Nerium oleander,* (e): rearing of the scale on *Cucurbita maxima* in laboratory for experimental reasons (Photo by George Stathas).

tive as the predated scales reached 90% of the population in June 2009 in Kalamata. This is the first record of *A. debachi* in Europe and the first record of *P. diaspidilis* in Greece (Japoshvili *et al*., 2010).

Dynaspidiotus abieticola (Koroneos)

Dynaspidiotus abieticola is a Paleartctic species, recorded in Greece, Iran, Lebanon and Turkey, on the following plant species of the family Pinaceae: *Abies bornmuelleriana, A. cephalonica, A. concolor, Cedrus libani* and *Picea pungens* (García Morales *et al.,* 2016). The first record of the scale in Greece was made by Koroneos (1934) on *A. cephalonica*, in the area of Ano Lekhonia and in the surrounding region of mount Pelion (Thessaly).

In the area of Messenia, it was recorded on Taygetus mountain at an altitude of 760m (Fig. 5). The study of phenology of the

scale was made by examinations of branches of the infested trees in the laboratory from February 2013 to January 2014 (Stathas, 2015). It was recorded as an oviparous biparental species which completed one generation per year. It was settled on fir trees causing chlorosis (Fig. 5). On heavily infested fir trees, needles got dry. It overwintered as a mated adult female from the beginning of October to the end of April. Egg-laying and hatching of crawlers occurred from early May to the end of June. Settled first instar nymphs were present from early June until early September. Second instar nymphs occurred from the first days of August until late September, the male nymphs in September and the first adult females at the beginning of October.

Figure 4. *Diaspis echinocacti* on *Opuntia ficus-indica* in Kalamata, Greece (Photo by George Stathas).

Figure 5. *Dynaspidiotus abieticola* on *Abies cephalonica* on Taygetus mountain, Messenia, Greece (Photo by George Stathas).

Dynaspidiotus abietis (Schrank)

Dynaspidiotus abietis is a species of Nearctic and Palaearctic region, recorded in 26 countries, infesting plants of the families Cupressaceae, Pinaceae, Rosaceae and Sapindaceae (García Morales *et al.,* 2016).

Its first record for Greece was made by Koroneos (1934), who referred to the scale as *Aspidiotus abietis* (Schr.) Loew, on *A. cephalonica* on the mountains Parnitha (Attica) and Oeta (central Greece).

In Messenia, *D. abietis* was recorded on *A. cephalonica* on northwestern part of the mountain Taygetus (near to the County Dyrachi). Its morphology, biology, phenology and natural enemies were studied during June 2004 – August 2006, on infested fir trees and on samples of infested branches examined in the laboratory (Stathas, 2008). The scale was found to infest only needles of the fir trees, in low infestation levels (Fig. 6). It is biparental and oviparous; it developed one generation per year and it overwintered as mated pre-ovipositing female adult. Ovi-

Figure 6. Female adults of *Dynaspidiotus abietis* settled on needles of *Abies cephalonica* on Taygetus mountain, Messenia, Greece (Photo by George Stathas).

positing females were recorded from the second week of May to the beginning of July. Crawlers appeared from the end of May to the beginning of July. Settled first instar nymphs were recorded during July and second instar nymphs from August to October.

Predated individuals of the scale on the infested trees were attributed to the activity of the larvae and adults of the predator *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae), which were recorded between the months May to August (Stathas, 2008). Activity of *C. bipustulatus* against *D. abietis* is also reported in other European countries by Kozstarab and Kozár (1988).

Lepidosaphes beckii (Newman)

Lepidosaphes beckii is a widely distributed, cosmopolitan, tropical and subtropical species. It infests citrus in all northern Mediterranean countries and is especially harmful in littoral areas (Katsoyannos, 1996). It is distributed to 120 countries and its host plant species belong to 60 genera of 42 families, but most of its hosts belong to the family Rutaceae (García Morales *et al.,* 2016). The presence of *L. beckii* in Greece was reported by Hall (1922), DeBach (1964), Argyriou (1976) and Katsoyannos (1996).

The ecology of the scale was studied on infested *Citrus sinensis* var. *navelina* in Kalamata during 2009-2011 (Fig. 7) (Stathas *et al.*, 2015a). Its phenology was studied on samples of infested leaves, which were transferred in the laboratory. The numbers of caught males of *L. beckii*, the parasitoids and predators of the scale were monitored by yellow sticky traps on the infested trees. *Lepidosaphes beckii* mainly infested the leaves and fruits and to a lesser extent the shoots and stems. The fluctuations of the population of crawlers were recorded using sticky transparent band traps placed on shoots of the infested trees. All developmental stages of the scale were observed during all the period of the study. Three peaks of crawlers were recorded in June, August and October in both years of the study.

The natural enemies of *L. beckii* recorded in Kalamata were the parasitoids *Aphytis lepidosaphes* Compère and *Encarsia* sp. (Hymenoptera: Aphelinidae) which reached a parasitization rate 32%, and the predators *Chilocorus bipustulatus* (L.) and *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) (Stathas *et al*., 2015a). García Morales *et al.* (2016) reported that the recorded natural enemies of *L. beckii* belong to 35 genera of 17 insect families, in which the above-mentioned natural enemies of the scale recorded in Kalamata are included.

Lepidosaphes gloverii (Packard)

Lepidosaphes gloverii is distributed to 81 countries of Central and North America, Asia and Europe, recorded on host plants of 40 genera in 28 families. Its main host is citrus (García Morales *et al.,* 2016).

In Greece, it was recorded in southern Peloponnese in the area Gastouni on *Citrus sinensis* var. *navelina* (Stathas, 2004a). Its biology, phenology and ecology were studied during June 2001 – August 2003, with examination of the infested trees and on samples of infested leaves in the laboratory. The scale infests mainly the upper leave surface and the fruits and less the lower leaf surface and the shoots of the trees. Although all developmental stages were recorded during the winter period, the scale was found to overwinter mainly as preovipositing and ovipositing female adult. It developed 3 overlapping generations per year. Three peaks of the population of crawlers were recorded in June, August and October. The number

Figure 7. Male and female nymphs and adults of *Lepidosaphes beckii* settled on upper surface of orange tree leaf in Kalamata, Greece (Photo by George Stathas).

of eggs recorded under the ovipositing female adults in June, ranged between 32 and 57 eggs, with a mean number of 37.7 eggs per female.

Concerning its control, the combination of mineral oil applications in September 2001, February and September 2002 with mass releasing of adults of the predators *Chilocorus bipustulatus* (L.) and *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) in July 2002, reduced significantly the infestation density of the scale (Stathas, 2004a). The coccinellid predators *C. bipustulatus* and *R. lophnathae* are also cited as natural enemies of *L. gloverii* in other studies (Belguendouz *et al.*, 2017; Herting and Simmonds, 1972).

Lineaspis striata (Newstead)

Lineaspis striata is recorded in 16 countries infesting plants of 9 genera belonging to the families Cupressaceae, Iridaceae, Santalaceae and Taxaceae (García Morales *et al.,* 2016).

It was recorded and described in Greece by Koroneos (1934) as *Chionaspis striata* Newstead found on the species of the family Cupresaceae: *Thuja orientalis* L. in Volos, *Cupresus sempervirens* L. in Peloponnese, on *Juniperus oxycedrus* L. in Pelion Mountain (Tsangarada and Milies), on *Juniperus macrocarpa* (Sibth. and Sm.) in Attica (Voula) and on *Juniperus phoemicae* L. in Attica (Vouliagmeni). It is also recorded in Crete by Panis (1981) and by Pellizzari *et al.* (2011) on *C. sempervirens* in Agios Nikolaos and on *Cupresus* sp. in Heraklion. In Messenia, *L. striata* was found on *Juniperus oxycedrus* L. (Cupressaceae) on Taygetus Mountain in January 2007 (Stathas *et al.,* 2011).

The phenology and the natural enemies of the scale were studied by Katsoyannos (1993) on different species of the family Cupresaceae during the years 1991-1992 in Attica. As it is referred in the above study, the scale is oviparous biparental species. It developed three generations per year and it overwintered as pre-ovipositing female adult. The average fecundity was 50 ± 12 eggs per female. The endoparasitoid *Physcus testaceus* Masi (Hymenoptera: Aphelinidae) was found to parasitize female adults of *L. striata* and the parasitism rate reached 44.8% in May 1991. Parasitism of a single female adult *L. striata* by an ectoparasite *Aphytis* sp. was recorded in April 1991 (Katsoyannos, 1993).

Pseudaulacaspis pentagona (Targioni Tozzetti)

Pseudaulacaspis pentagona is distributed in 113 countries, was recorded on host plants of 251 genera, belonging to 89 families (García Morales *et al.,* 2016). Its presence in Greece is referred by Balachowsky (1954), Paloukis (1967), Argyriou *et al.* (1976). Its phenology and ecology have been studied in the past in northern Greece (Paloukis and Mentzelos, 1971; Kyparissoudas, 1992).

In southern Greece *P. pentagona* was recorded in Kalamata infesting *Actinidia deliciosa* (Actinidiaceae), *Prunus persica* (Rosaceae) *Morus alba* (Moraceae) (Fig. 8) (Stathas *et al.*, 2020). In Kalamata the biology and ecology of *P. pentagona* on *M. alba* was studied during the years 2016 – 2018. It is a biparental and oviparous species. It overwintered as mated female adult. The fecundity of females on September 2017 fluctuated between 97 to 133 eggs, with a mean 118.5 ± 25.7 eggs per female. The scale completed 3 generations per year. Three peaks of crawlers were recorded in April, July and September. The parasitism rate by an unidentified ectoparasite reached 18 and 21% in 2016 and 2017, respectively. The main natural enemies of the scale were the coccinellid predators *Chilocorus bipustulatus* and *Rhyzobius lophanthae* (Stathas *et al.*, 2020). These predators are referred as natural enemies of *P. pentagona* in other countries (García Mo-

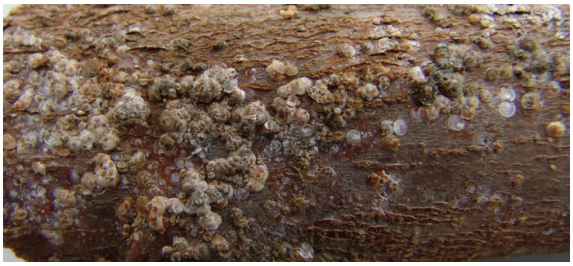

Figure 8. *Pseudaulacaspis pentagona* on branch of *Morus alba* in Kalamata, Greece (Photo by George Stathas).

rales *et al.,* 2016) and in northern Greece (Argyriou *et al.,* 1976).

Targionia vitis (Signoret)

Targionia vitis is recorded in 25 countries, infesting plants of 9 genera in 6 families, but most of its host plants belong to the family Fagaceae (García Morales *et al.,* 2016). Its presence in Greece was recorded by Koroneos (1934) on *Arbutus unedo* (L.) (Ericaceae), *Quercus* sp. and *Q. coccifera* L. (Fagaceae) and *Platanus orientalis* L. (Platanaceae).

Targionia vitis was recorded and studied in Messenia on *Vitis vinifera* L. (Vitaceae) in a vineyard containing the varieties Black Currents (black raisin), Rodites and Fraoula (Stathas and Kontodimas, 2001). It was recorded as a biparental, viviparous univoltine species, which overwinters as mated female adult. The crawlers hatched in mid – May, while the first and second instar nymphs appeared in June and developed to male and female nymphs by mid-July. Male adults were observed from mid-July until the end of August while by the beginning of September, the whole population of the scale consisted of mated female adults. The fecundity of *T. vitis* ranged between 82 and 105 eggs, with an average of 94.9 $\pm$ 9.37 eggs per female. The natural enemies of *T. vitis,* which were recorded in Messenia, included the ectorarasite *Aphytis abnormis* (Howard) (Hymenoptera Aphelinidae), an unidentified endoparasite and the predator *Cybocephalus fodori* Entrödy-Younga (Coleoptera: Nitidulidae). The activity of the above natural enemies could not reduce the population density of the scale on the infested grapes (Stathas and Kontodimas, 2001).

COCCIDAE

Ceroplastes rusci (L.)

Ceroplastes rusci is distributed to 58 countries, infesting plants of 79 genera belonging to 48 families (García Morales *et al.*, 2016). In Greece it is referred by Argriou (1983) as widely distributed scale insect causing serious damage, especially in southern areas of the country, on *Ficus carica* L. (Moraceae) in Attica and Messenia and on citrus in Aegean islands. Kozár *et al.* (1991) referred *C. russci* in Greece on *Albizia* sp. (Fabaceae) in Athens, on *Pittosporum* sp. (Pittosporaceae) in Iraklion, on *Nerium oleander* L. (Apocynaceae) in Knossos and on *Osyris alba* L. (Santalaceae) in island Hydra. *Ceroplastes rusci* was reported to develop two generations per year on fig trees in Greece (Argyriou and Santorini, 1980).

In Messenia, *C. rusci* is widely spread on fig trees (Fig. 9). Pellizzari *et al.* (2010) recorded *C. rusci* on *F. carica* in a fig cultivation area near Kalamata (province Aristomenes). Description of immature females from this population provided an identification key of the different instars of the scale which is a useful tool for the determination of the appropriate period for effective chemical control applications in fig cultivation.

Eulecanium sericeum (Lindinger)

Eulecanium sericeum is distributed in 14 European countries, infesting 6 species of genus *Alba* (Pinaceae). In Greece, it has been recorded on *A. cephalonica* and *A. borisii-regis* Mattf. (Argyriou, 1983; Santas,1983;1988). Santas (1983) reported that *E. sericeum* appeared in patches on the *Abies* trees, although sometimes a whole tree may be infested. In Messenia, *E. sericeum* was found on *A. cephalonica* on Taygetus Mountain in June 2005 and in June 2007 in small colonies

Figure 9. Immature stages of *Ceroplastes rusci* on fig tree leaf (Photo by George Stathas).

(Stathas *et al.*, 2011). According to Hadzibejli (1967) *E. sericeum* develops one generation per year in Georgia.

Nemolecanium graniformis (Wünn)

Nemolecanium graniformis is recorded only in Europe, in Czech Republic, France, Germany, Greece, Italy and Poland, infesting *Abies alba*, *Abies cephalonica* and *Abies nebrodensis* (Pinaceae) (García Morales *et al.*, 2016). It was first recorded in Greece in August 1996 on *A. cephalonica* in the area Thracomacedones (Attica) (Stathas, 1997).

Its phenology and natural enemies were studied on Parnis Mountain (Attica) during 1998-1999, where the scale was found to be univoltine, oviparous and overwintered as second instar nymph. The average fecundity of the scale on Parnis Mountain was counted to 188.4 eggs per female adult (Stathas, 2001). In Messenia *N. graniformis*, it was found on *A. cephalonica* on Taygetus Mountain where it was studied from December 2005 to November 2007 (Fig. 10) (Stathas *et al.*, 2011). It is oviparous and biparental species and it completed one generation per year and overwintered as second instar nymphs. Regarding its natural enemies in Messenia, female adults of the scale were found parasitized by unidentified endoparasitic larvae on Taygetus Mountain, in June and November 2006. Moreover, the predators *E. quadripustulatus* and *C. bipustulatus* were found in the colonies of the scale on the infested fir trees (Stathas *et al.*, 2011). In other studies in Greece, the aphelinid parasitoids *Coccophagus lycimnia* (Walker), *Coccophagus* sp. Westwood and the encyrtid *Aphycoides* sp. Mercet, as well as the predators *Exochomus quadripustulatus* L. and *C. bipustulatus* were recorded on Parnis Mountain, County of Attica (Stathas, 2001).

Figure 10. *Nemolecanium graniformis* on *Abies cephalonica*: Second instar nymph (a) and female adults (b) on Taygetus mountain, Messenia, Greece (Photo by George Stathas).

Parthenolecanium corni (Bouché)

Parthenolecanium corni is distributed in 73 countries, infesting 109 plant species belonging to 48 families (García Morales *et al.*, 2016).

In Greece it is referred by Argyriou (1983) on *Prunus persica* L. (Rosaceae) and by Santas (1985) on *P. persica*, *Prunus armeniaca*, *Crataegus* spp. (Rosaceae) and *Corylus avellana* L. (Betulaceae) close to Grevena (Northwestern Greece). Its distribution in Greece is not well known, but it has been found in Central and Northern Greece. The adults excrete honeydew which is exploited by honeybees. *Parthenolecanium corni* had one generation per year on *C. avellana*. It overwintered as second instar nymph, the adults appeared early in April and the crawlers in the middle of June. The second instar nymphs appeared in mid-July and by the end of October all the scale population was in this stage. The fecundity of the scale fluctuated between 700 and 1100 eggs per female (Santas, 1985).

Natural enemies of *P. corni* in Greece included the parasitoids *Coccophagus lycimnia* (Wlk.) (Hymenoptera: Aphelinidae), *Metaphycus insidiosus* (Merc.) (Hymenoptera: Encyrtidae) and the predators *Scutellista cyanea* Motsch. (Hymenoptera: Pteromalidae), *Leucopis alticeps* Czerny (Diptera: Chamaemyiidae), *Eubletnma scitula* (Ramb.) (Lepidoptera: Noctuidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and

Chilocorus hipustulatus (L.) (Coleoptera: Coccinellidae) (Santas, 1985). The hyperparasites *Pachyneuron concolor* (Forst) (Hymenoptera: Pteromalidae) and *Marietta picta* (André) (Hymenoptera: Aphelinidae) were also recorded (Santas, 1985).

In Messenia, *P. corni* was found in the area Asprochoma close to Kalamata on *Morus alba* in July 2005. The population of the scale consisted of ovipositing females. Female adults of the scale were deposited to the collection of the Hungarian Academy of Sciences. Superparasitism of female adults *P. corni* by an unidentified parasitoid species was also observed in the above infestation (Fig. 11) (Stathas and Kartsonas, unpublished data).

Parthenolecanium persicae (Fabricius)

Parthenolecanium persicae is distributed in 59 countries on several host plants of 54 genera, belonging to 33 families. Most of its hosts belong to the families Fabaceae and Rosaceae (García Morales *et al.,* 2016). In Greece, it was recorded by Kozár (1985) on *Morus* sp. in northern Greece and on *Viburnum tinus* (L.) (Adoxaceae) in Athens (Stathas, 2004b).

In Messenia, *P. persicae* was found on *Vitis vinifera* L. (Vitaceae) in the province Arfara in October 2000 (Stathas *et al.,* 2003; Stathas, 2004b). In studies of *P. persicae* in vineyards *cv.* Rodites during the years 2001-2002 it was found to be parthenogenic, ovipososting, univoltine species. Although *P. persicae* appears to be largely parthenogenic, two male adults of *P. persicae* were collected from vineyards in the Hunter Valley (Australia) during early September (Rakimov *et al*., 2013). The scale overwintered as a second instar nymph. Female adults appeared in April and oviposition took place from early May to late June. Crawlers hatched in May and during the rest of the summer period the population consisted of first and second instar nymphs. From late September until the following spring the population consisted of second instar nymphs. Regarding natural enemies of *P. persicae,* two parasitoids were recorded, *Metaphycus* sp. (Hymenoptera: Encyrtidae), which parasitized the scale up to 34.5%, and the predator *Chilocorus bipustulatus* (Stathas *et al.,* 2003).

Physokermes hemicryphus (Dalman)

Physokermes hemicryphus is distributed in 28 countries on conifers of the families Pinaceae (on species of genera *Abies, Picea*

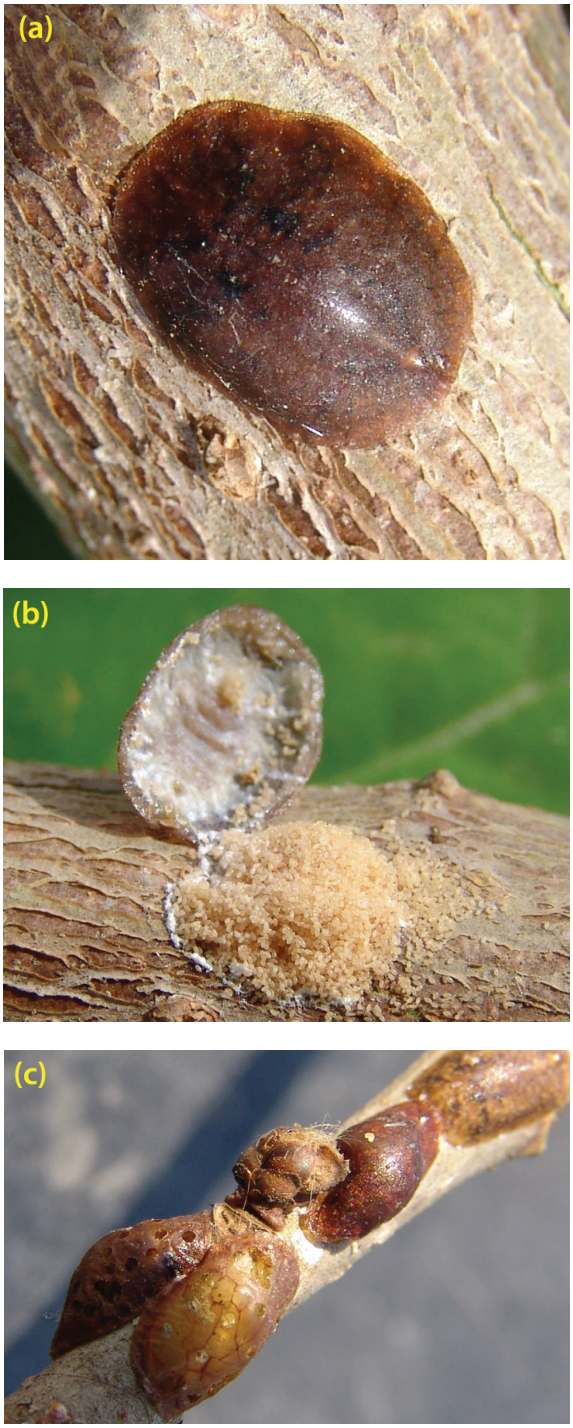

Figure 11. *Parthenolecanium corni* on *Morus alba*. Dorsal (a) and ventral (b) view of ovipositing female adults, parasitized females (c) in Kalamata, Greece (Photo by George Stathas).

and Tsuga) and Cupressaceae (on *Juniperus* sp.) (García Morales *et al.,* 2016).

In central Greece it has been recorded on *Abies cephalonica* and *A. borisii regis,* where the honeydew excretions of the scale is used in beekeeping as a main source for honey production in the country (Argyriou, 1983; Santas, 1988; Gounari *et al.,* 2004). In Messenia, *P. hemicryphus* was recorded on Taygetus Mountain infesting *A. cephalonica* (Fig. 12). The biology and ecology of the scale was studied in this area during the years 2004 - 2006 (Stathas *et al.,* 2011). It is an oviparous biparental and univoltine species, infesting the nodes of the annual growth of the trees. It overwintered as second instar nymph. Young female adults secrete honeydew from the middle of May until the beginning of July (Fig. 12). On Taygetus Mountain, ovipositing females laid 70-280 eggs in July 2006 and the mean fecundity was 193.9±44.6 eggs per female. The crawlers appeared from the end of August until the middle of September.

Concerning the natural enemies on Taygetus Mountain, the parasitoid *Pseudorhopus testaceus* (Ratzeburg) (Hymenoptera: Encyrtidae) was found to parasitize female adults of *P. hemicryphus*. Percentage parasitism was 24% in July 2005 and 47.8% in July 2006 with 1–4 or more parasitoid adults emerging per parasitized female adults. The predators *Chilocorus bipustulatus* (L.), *Exochomus quadripustulatus* (L.) and *Scymnus* sp. (Coleoptera: Coccinallidae). *Exochomus quadripustulatus* and *Scymnus* sp. are also reported as predators of the scale in western and central Greece (Santas, 1988). The role of *E. quadripustulatus* against other coccids in Greece such as *Saissetia oleae* Olivier has been studied by Katsoyannos (1976).

Physokermes inopinatus Danzig and Kozár

Physokermes inopinatus is recorded in Austria, Hungary, Greece, Romania, Sweden and Ukraine. Its hosts are species of genus *Abies* and *Picea* (Pinaceae). It causes damage to the host trees by its feeding on the sap from the needles as well by its honeydew secretions, on which sooty mold is created (García Morales *et al.,* 2016).

The first record of *P. inopinatus* in Greece was on *Abies cephalonica* on Taygetus Mountain, in Messenia (Stathas and Kozár, 2010). The phenology of the scale was studied on infested fir trees in this area from July 2006 to June 2008. The scale settles on the base of young shoots and needles. It is oviparous and biparental. It completed one generation per year and overwintered as second instar nymphs. Pre-ovipositing females appeared during May and June. Eggs were laid from mid-June to early August. Hatching of crawlers occurred during July and August. Larvae and adults of *C. bipustulatus* were observed on colonies (Stathas and Kozár, 2010). The coccinellid predator C. *bipustulatus* is included among the natural enemies of *P. inopinatus* by Kosztarab and Kozár (1988).

Protopulvinaria pyriformis (Cockerell)

Protopulvinaria pyriformis is a serious pest of fruit trees and ornamentals in several tropical and subtropical countries, totally recorded in 42 countries, infesting plants of 60 genera, belonging to 36 families (García

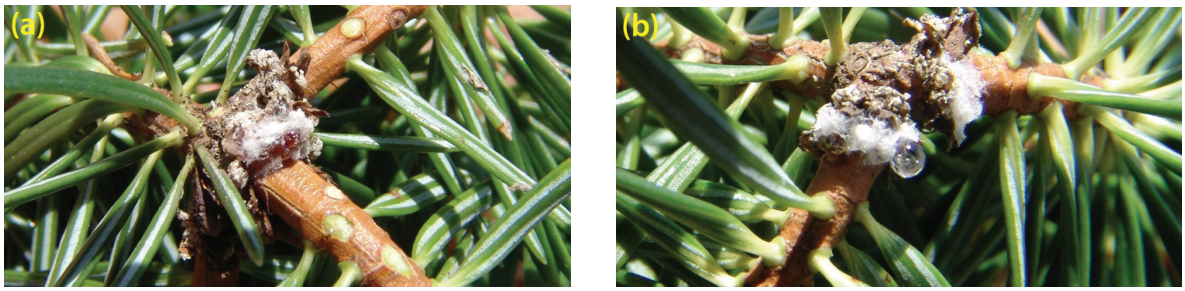

Figure 12. Young female adults of *Physokermes hemicryphus* on nodes of *Abies cephalonica* partially covered by white waxy filaments (a) and totally covered by filaments and honeydew excretion (b) on Taygetus mountain, Messenia, Greece (Photo by George Stathas).

Morales *et al.,* 2016).

In Greece, it was recorded for the first time on *Laurus nobilis* L. (Lauraceae) in Kalamata (Fig. 13), Messenia, in 2003 (Ben-Dov, *et al.,* 2003). Later, *P. pyriformis* was found on more host plants in Messenia. In June 2007, it was found on *Hedera helix* L. (Araliaceae) at the area of Almyros, Messenia and in May 2008 on *Citrus aurantium* L. (Rutaceae) in the city of Kalamata (Fig. 13). On both of the above host plants, *P. pyriformis* was found to settle mainly on the lower leaf surface (Stathas *et al.,* 2008).

The phenology, biology and natural enemies of the scale were studied in Messenia during the years 2003 – 2005 (Stathas *et al.,* 2009). It settles mainly on the lower leaf surface producing increased amounts of honeydew throughout the year. It is parthenogenetic and oviparous developing several overlapping generations per year. It overwintered under all developmental stages (egg, first and second instar nymph, adult).

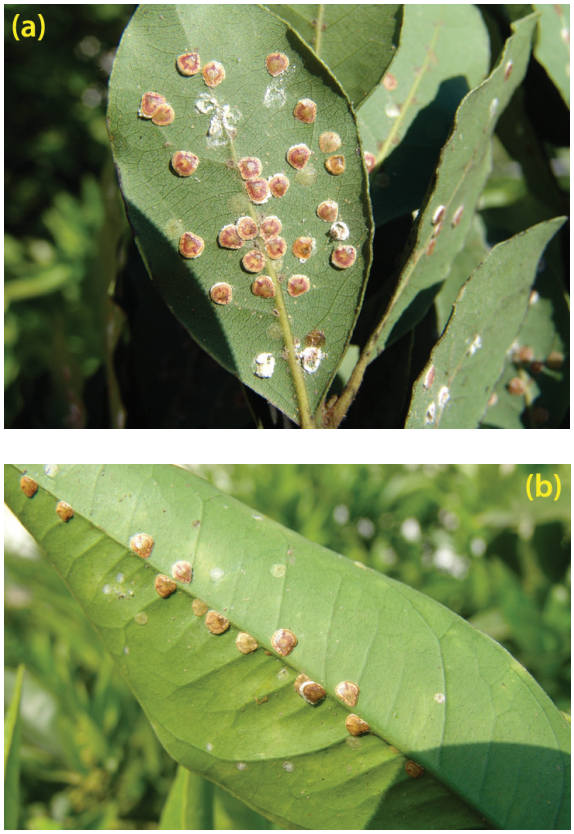

Figure 13. *Protopulvinaria pyriformis* on *Laurus nobilis* (a) and on *Citrus aurantium* (b) in Kalamata, Greece (Photo by George Stathas).

The life cycle was estimated to last in nature about 52 days during winter and 29-33 days during summer. The main natural enemy of the scale was the parasitoid *Metaphycus helvolus* (Compere) Hymenoptera: Encyrtidae). The parasitism rate reached 31.2% while encapsulation of the parasitoid eggs occurred in up to 23% of the adult scales. The number of the encapsulated eggs ranged from 1 to 5 eggs per scale individual. The coccinellid predator *C. bipustulatus* was also recorded as a natural enemy of the scale in Kalamata.

PSEUDOCOCCIDAE

Phenacoccus madeirensis Green

Phenacoccus madeirensis is widely spread in 83 countries, recorded on host plants belonging to 150 genera of 54 plant families (García Morales *et al.,* 2016).

In Greece it was recorded by Papadopoulou and Chryssohoides (2012) in June 2010 in the regions of Thessaloniki, Xanthi and Kavala (Northern Greece) on *Onicum basilicum* L. (Lamiaceae) and by Szita (*et al.,* 2017) in 2014 in the island Kefalonia (Western Greece) on *Campanula* sp.

In Messenia, *P. madeirensis* was found in Kalamata in May 2014 on *Aloysia citriodora* Palau (Verbenaceae) and on July of the same year on *Osteospermum jucundum* (Phillips) (Asteraceae) (Fig. 14). *Osteospermum jucundum* recorded for the first time as host plant

Figure 14. Infestation of *Phenacoccus madeirensis* on *Osteospermum jucundum* in Kalamata, Greece (Photo by George Stathas).

of *P. madeirensis* (Stathas *et al.,* 2015b).

As far as the phenology is concerned, *P. madeirensis* completed 5-6 generations per year in Sicily; it overwintered mainly as first and second instar nymphs however, female adults were also found (Sinacori, 1995).

Planococcus vovae (Nasonov)

Planococcus vovae is distributed to 38 countries, infesting plants of the families Araceae (genus: *Anthurium),* Cupressaceae (genus: *Calocedrus, Chamaecyparis, Cupressus, Juniperus and Thuja),* Lauraceae (genus: *Laurus*) and Taxaceae (genus: *Taxus*) (García Morale *et al.,* 2016).

The presence of *P. vovae* in Greece is referred by Cox (1989) and Cox and Ben-Dov, (1986). Milonas and Kozár (2008) recorded the scale on *Cupressus leylandii* (Jacks. and Dallim.) in Kifissia (Attica) in 2004. In Messenia, female adults of *P. vovae* were found on *Juniperus oxycedrus* L. in June 2006 on Taygetus Mountain (Stathas *et al.,* 2011). The low population of the infestation of the scale found on Taygetus, was not adequate to study the biology of the scale in this area. In Italy *P. vovae* develops two annual generation (Francardi and Covassi, 1992).

KERMESIDAE

Kermes echinatus Balachowsky

Kermes echinatus has been recorded only in Israel and in Greece. Its host plants are the species *Quercus calliprinos* Webb., *Quercus coccifera* L. and *Quercus ilex* L. (Fagaceae) (Spodek *et al.,* 2014; García Morales *et al.,* 2016).

In Greece, *K. echinatus* was found on *Q. coccifera* in Crete in April 2010 and again in June 2011 (Porcelli and Pellizzari, 2014). In Messenia, it was recorded on *Q. ilex* in Kalamata, in November 2012 (Fig. 15). In December of the same year, the scale was recorded in Athens on *Quercus ilex* which was reported as host of this scale for the first time (Stathas *et al.,*2013; Stathas *et al.,* 2018).

The phenology and the natural enemies of *K. echinatus* on *Q. ilex* were studied in Kalamata during the years 2015 – 2017 (Stathas *et al.,* 2018). *Kermes echinatus* is a univoltine, oviparous and biparental species. It overwintered as first instar nymph on the branches of the infested trees and it was developed to second instar by the middle of April and to third instar until the end of May. The immature males (larvae and pupae) were observed from the end of April until the end of

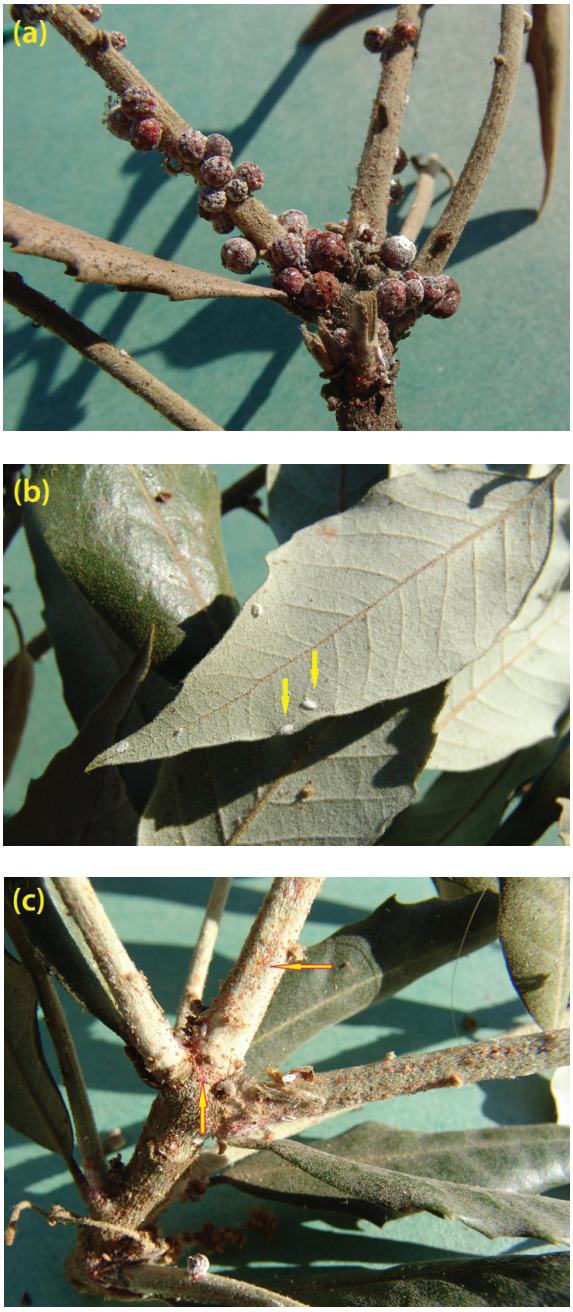

Figure 15. *Kermes echinatus* on *Quercus ilex.* (a): oviparous female adults, (b): scale covers of male nymphs, (c): crawlers dispersed on branches in Kalamata, Greece (Photo by George Stathas).

May, while the pre-ovipositing and ovipositing adults were recorded during May and June. The hatching of crawlers occurred by the end of June, which remained under this instar until the April of the next year. Natural enemies included the predator *Chilocorus bipustulatus* and the parasitoids *Metaphycus gennaroi* Guerrieri and Noyes, The hyperparasite *Cheiloneurus claviger* Thomson (Hymenoptera: Encyrtidae) was also found parasitizing the scale. The total parasitization rate of both parasitoids reached to 21% (Stathas *et al.,* 2018).

Concluding remarks

Twenty species of scale insects were recorded on agricultural, ornamental and forest plant species in the wider area of Messenian Province between the years 2000 and 2020. Nine of them belong to the family Diaspididae, eight to Coccidae, two to Pseudococcidae and one to Kermesidae. Some of these scale species or their parasitoids are recorded for the first time in Greece. For some other, this is the first report of several host plant species.

First records of these scale species in Greece include: *Physokermes inopinatus* on Taygetus mountain; *Protopulvinaria pyriformis* in Kalamata (Ben-Dov *et al.,* 2003); *Kermes echinatus* in Messenia (the scale has not been previously recorded in continental Greece, but only in Crete) (Stathas *et al.,* 2013; Porcelli and Pellizzari, 2014).

First records of host plant species include: *Physokermes inopinatus* was recorded on *A. cephalonica* (Stathas and Kozár, 2010); *Kermes echinatus* was recorded on *Quercus ilex*; the scale was previously recorded only on *Q. calliprinos* and *Q. coccifera* (Stathas *et al.,* 2013). *Chrysomphalus aonidum* was recorded for the first-time infesting species of the families Cucurbitaceae and Solanaceae (mass reared on *C. maxima* and *S. tuberosum*) and the plant *F. bensamina* (Stathas *et al.,* 2002; Stathas and Kozár, 2008). Infestation by *P. madeirensis* was recorded on *Osteospermum jucundum* (Stathas *et al.,* 2015b).

First reports of parasitoids of the scale species include: *Aphytis debachi* found on *D. echinocacti* is the first record of this ectoparasistoid in Europe; *Plagiomerus diaspidilis* in Kalamata is the first report of this ectoparasitoid in Greece (Japoshvili *et al.*, 2010).

The data on biology, phenology and ecology of the scale insects provided in this article, could contribute to their effective control. The knowledge of the time of appearance of the most susceptible stages of the scale to chemicals, the number of generations developed per year, their fecundity and the evaluation of the action of their natural enemies, is necessary and essential when planning an effective program of their Integrated Pest Management.

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Literature cited

Argyriou, L.C., Sravraki, H.G. and Mourikis, P.A. 1976. *A list of Recorded Entomophagous Insects of Greece*. Benaki Phytopathological Institute, Athens, Greece, 73pp.

Argyriou, L.C. 1983. Faunal analysis of some scale insects in Greece. *Proceedings of the 10th International Symposium of Central European Entomofaunistics,* Budapest, 15-20 August 1983 364-367.

Argyriou, L.C., and Santorini, A.P. 1980. On the phenology of *Ceroplastes rusci* L. (Hom. Coccidae) on fig-trees in Greece. *Mededlingen van de Rijksfaculteit Landbouwwetenschappen te Gent*, 45: 593-601.

Argyriou, L.C. and Mourikis, P.A., 1981. Current status of citrus pests in Greece. *Proceedings of the International Society of Citriculture*, Tokyo 9-12 November, International Society of Citriculture, Okitsu, Japan, Vol. 2: 623-627.

Balachowsky, A.S. 1954. Les cochenilles Paléarctiques de la tribu des Diaspidini. *Memmoires Scientifiques de l'Institut Pasteur.* Paris; *Institut Pasteur Publ.*

Belguendouz, R., Biche, M., and Bendifallah, L. 2017 Study of Trophic Relationships Between Diaspididae Family Species - Host Plants - Predators and Parasitoids in Algeria. *Journal of Fundamental and Applied Sciences*, 9(1): 119-135.

Ben-Dov, Y., Stathas, G.J. and Malliarou, J.S. 2003. The pyriform scale, *Protopulvinaria pyriformis* (Cockerell) (Hemiptera: Coccidae) in Greece. *Agrotiki Erevna*, 26(2): 89-91.

Cox, J.M. 1989. The mealybug genus *Planococcus* (Homoptera: Pseudococcidae). *Bulletin British Museum (Natural History). Entomology*, 58(1): 1-78.

Cox, J.M., and Ben-Dov, Y. 1986. Planococcine mealybugs of economic importance from the Mediterranean Basin and their distinction from a new African genus (Hemiptera: Pseudococcidae). *Bulletin of Entomological Research,* 76: 481-489.

DeBach, P. 1964. Some species of *Aphytis* Howard (Hymenoptera: Aphelinidae) in Greece. *Annales de l'Institut Phytopathologique Benaki*, 7(1): 5-18.

Francardi, V. and Covassi, M.V. 1992. Note bio-ecologiche sul *Planococcus vovae* (Nasonov) dannoso a *Juniperus* spp. in Toscana (Homoptera Pseudococcidae). [Biological and ecological notes on *Planococcus vovae* (Nasonov) (Homoptera Pseudococcidae living on *Juniperus* spp. in Tuscany. *Redia*, 75: 1-20.

Garcia Mari, F., Soto, A., Hernandez Penadès, P., Rodrigo, E. and Rodriguez Reina, J.M. 2000. Una nueva cochinilla aperece en los cítricos valencianos, *Chrysomphalus aonidum*. *Phytoma Espaňa*, 117: 35-40.

García Morales, M., Denno, B.D., Miller, D.R., Miller, G.L., Ben-Dov, Y. and Hardy, N.B. 2016. ScaleNet: A literature-based model of scale insect biology and systematics. Database. doi: 10.1093/database/bav118. http://scalenet.info. (Accessed: 4/1/2021).

Germain, J.F. and Matile Ferrero, D. 2005. Les co-chenilles sous serres en France : inventaire illu-stré. III – Les Diaspididae. *Phytoma*, 583: 32-35.

Google map 1: https://www.google.gr/maps/place/Messenia/@37.0837231,21.9086108,10z/data=!4m5!3m4!1s0x136102cd91712287:0x110575be25d07f19!8m2!3d37.1424653!4d21.9522491 (accessed on 18/05/2021).

Google map 2: https://www.google.gr/ maps/@38.2349212,22.0384587,7z (accessed on 18/05/ 2021).

Gordh, G. and Lacey, L. 1976. Biological studies of *Plagiomerus diaspis* Crawford, a primary internal parasite of diaspidid scale insects (Hymenoptera: Encyrtidae; Homoptera: Diaspididae). *Proceedings of the Entomological Society of Washington*, 78: 132–144.

Gounari, S., Mathiopoulos, M., Stathas, G.J. and Emmanouel, N. 2004. Aspects on bioecology and phenology of *Physokermes hemicryphus* (Dalman) in the county of Euritania – Greece. In *Proccedings of the X- International Symposium on Scale Insect Studies (ISSIS-X)*. 19-23 April, Adana, Turkey, 339p.

Hadzibejli, Z.K. 1967. Ecological characteristics of the genus *Eulecanium* Ckll. in the fauna of Gruzia. *Trudy Instituta Zashchita Rastenii Gruzinskoy SSR* 19: 59-63.

Hall, W.J. 1922. Observations on the Coccidae of Egypt. *Bulletin, Ministry of Agriculture, Egypt, Technical and Scientific Service*, 22: 1-54.

Herting, B. and Simmonds, F.J. 1972. A catalogue of parasites and predators of terrestrial arthropods. Farnham Roy., U.K. 210 pp.

Jansen. M.G.M. 2004. An updated list of scale insects (Hemiptera: Coccoidea) from importinterceptions and greenhouses in The Netherlands. *Proc. X International Symposium on Scale insects studies*, Adana, Turkey, 147-165 pp.

Japoshvili, G.O., Stathas, G.J. and Kampouris, S.G. 2010. Natural enemies of *Diaspis echinocacti* in Greece and first records of *Aphytis debachi* and *Plagiomerus diaspidis*. *Phytoparasitica*, 38: 121-123.

Katsoyannos, P., 1976. Etude d' un predatéur: *Exochomus quadripustulatus* l. (Col.: Coccinellidae) en vue d' un éventuelle utilization contre *Saissetia oleae* Olivier (Ho.: Coccoidea-Coccidae) dans les oliveraies de la Grèce. Doct. Ing. Thesis Acad. Montpellier, Univ. Sci. et Techn. Du Langueloc, France, 144 pp.

Katsoyannos, P. 1993. Phenology and natural enemies of *Lineaspis striata* Newstead (Homoptera: Diaspididae) in Attica. *Proceedings of the 5th Pan-Hellenic Congress of Entomology.* 8-10 November, Athens, Greece, 201–205.

Katsoyannos, P. 1996. *Integrated Insect Pest Management for Citrus in Northern Mediterranean countries*. Benaki Phytopathologica lInstitute, Athens, Greece, 110 pp.

Koroneos, J. 1934. *Les Coccidae de la Grèce sur tout du Pélion (Thessalie). I. Diaspinae.* Athens. 95p.

Kosztarab, M.P. and Kozár, F., 1988. *Scale Insects of Central Europe*. Akademiai Kiado Budapest, Hungary 456 pp.

Kozár, F. 1985. New data to the knowledge of scale-insects of Bulgaria, Greece, and Rumania (Homoptera: Coccoidea). *Acta Phytopathologica Academiae Scientiarum Hungaricae,* 20: 201-205.

Kozár, F., Paloukis, S. and Papadopoulos, N. 1991. New Scale Insects (Homoptera: Coccoidea) in the Greek Entomofauna. *Entomologia Hellenica*, 9: 63-68.

Kozstarab, M. and F. Kozár. 1988. *Scale insects of central Europe.* Akademiai Kiadó, Budapest, 456pp.

Kyparissoudas, D.S. 1992. Flight of White Peach Scale, *Pseudaulacaspis pentagona*, males and time of crawler appearance in Northern Greece. *Entomologia Hellenica*, 10: 21—24.

Milonas, P.G. and Kozár, F. 2008. Check list of mealybugs (Homoptera: Pseudococcidae) in Greece:

three new records. *Hellenic Plant Protection Journal*, 1(1): 35-38.

Paloukis, S.S. 1967. *Prospaltella berlesei* How. (Hymenoptera-Aphelinidae), an entomoparasite specifique of *Pseudaulacaspis pentagona* Targ. (Coccidae), pest of peaches trees in Macedonia. *Geoponica,* 159-161.

Paloukis S.S. and Mentzelos I. 1971. Contribution a l'étude de la bio-écologie et de la lutte de *Pseudaulacaspis (Diaspis) pentagona* Targ. (Homoptera: Diaspididae) ennemi du pêcher en Macedoine Centrale (Grèece). Stationde Rècherche pour la Protection des Plantes, Thessalonique, Grèece, Bull. I.

Paloukis, S.S. 1979. *The main scale insects of fruit trees in Northern Greece*. [in Greek]. Thessaloniki, 148p.

Panis, A., 1981. Les cochenilles circum-Méditerranéennes des arbres d'alignement et brisevent (Homoptera, Coccoidea). *Sixièmes Journées Phytiatrie and Phytopharmacie Circum-Mediterranéennes*, 1-11.

Panis, A. and Pinet, C. 1999. A study of two *Plagiomerus* species (Hymenoptera: Encyrtidae) parasitising diaspidid scales (Coccoidea) in glasshouses in France. *Entomologica*, 33: 423–427.

Papadopoulou, S. and Chryssohoides, C. 2012. *Phenacoccus madeirensis* Green, 1923 (Homoptera: Pseudococcidae) on *Ocimum basilicum*: a new geographical record for Greece. *EPPO Bulletin*, 42(1): 146–147.

Pellizzari, G. and Vacante, V. 2007. Una nuova cocciniglia sulgi agrumi in Italia: il *Chrysomphalus aonidum* (Linnaeus) (Hemiptera: Diaspididae). *Informatore Phytopathologico*, 45-47.

Pellizzari, G., Rainato A. and Stathas, G.J. 2010. Description of the immature female instars of *Ceroplastes rusci* (Linnaeus) (Hemiptera: Coccidae). *Zootaxa,* 2556: 40-50.

Pellizzari, G., Porcelli, F., Seljak, G. and Kozár, F. 2011. Some additions to the Scale insect fauna (Hemiptera: Coccoidea) of Crete with a check list of the species known from the island. *Journal of Entomological and Acarological Research Ser. II*, 43(3): 291-300.

Porcelli, F. and Pellizzari, G. 2014. Description of female nymphal instars and adult female of *Kermes echinatus* Balachowsky (Hemiptera, Coccoidea, Kermesidae) based on specimens from Crete and mainland Greece, with a discussion on geographical variation. *Zootaxa*, 3878(1): 061–074.

Rakimov, A., Ben-Dov, Y., White, V., and Hoffmann, A.A. 2013. Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. *Australian Journal of Entomology* 52: 371–378.

Reiderne, S.K. and Kozár, F. 1994. Recently appeared scale insect species (Homoptera, Coccoidea, Diaspididae) on glasshouse ornamentals in Hungary. *Novenyvedelem*, 30: 423-427.

Rosen, D. and DeBach, P. 1979. Species of *Aphytis* of the world (Hymenoptera: Aphelinidae). The Hague, the Netherlands: Dr. W. Junk BV –Publishers.

Santas, L.A. 1983. Insects producing honeydew exploited by bees in Greece. *Apidologie* 14: 93-103.

Santas, L.A. 1985. *Parthenolecanium corni* (Bouché) an orchard scale pest producing honeydew foraged by bees in Greece. *Entomologia Hellenica*, 3: 53-58.

Santas, L.A. 1988. *Physokermes hemicryphus* (Dalman) a fir scale insect useful to apiculture in Greece. *Entomologia Hellenica,* 6: 11-22.

Sinacori, A. 1995. Bio-ethological observations on *Phenacoccus madeirensis* Green (Coccoidea: Pseudococcidae) in Sicily. *Israel Journal of Entomology*, 29: 179-182.

Spodek, M., Ben-Dov, Y. and Mendel, Z. 2014. The scale insects (Hemiptera: Coccoidea) of oak trees (Fagaceae: *Quercus* spp.) in Israel. *Israel Journal of Entomology*, 43: 95-124

Stathas, G.J. 1997. First record of *Nemolecanium graniformis* Wünn) (Homoptera: Coccidae) in Greece. *Annales de l'Institut Phytopathologique Benaki*, 18: 57-59.

Stathas, G.J. 2001. The scale *Nemolecanium graniformis* (Wünn) (Homoptera: Coccidae) in Greece. *Anzeiger für Schädlingskunde (Journal of Pest Science)*, 74: 57–59.

Stathas, G.J. and Kontodimas, D.C. 2001. Ecological data of the scale *Targionia vitis* on grapes in Southern Greece. *Annalsdel' Institut Phytopathologique Benaki*,19: 134-139.

Stathas, G.J., Eliopoulos, P.A., Kontodimas, D.C. and Siamos D.Th. 2002. Adult morphology and life cycle under constant temperatures of the predator *Rhyzobius lophanthae* Blaisdell (Col., Coccinellidae). *Anzeiger für Schädlingskunde (Journal of Pest Science),* 75: 105-109.

Stathas, G.J., Eliopoulos, P.A., Bouras, S.L., Economou, L.P. and Kontodimas, D.C. 2003. The scale *Parthenolecanium persicae* (Fabricius) on grapes in Greece. IOBC/WPRS Bulletin, 26(8): 253-257.

Stathas, G.J. 2004a. Ecological data of *Lepidosaphes gloverii* (Hemiptera: Diaspididae) in Greece. *Entomologia Hellenica*, 15: 19-26.

Stathas, G.J. 2004b. First record of the scale *Parthenolecanium persicae* on *Viburnum tinus* and *Vitis vinifera* in Greece. *Annals of the Benaki Phytopathological Institute,* 20(1): 57-59.

Stathas, G.J., Eliopoulos, P.A., Porichi, E.E., Katsanis, A.G. and Beratis, V.N. 2005. The scale insect *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) on orange trees in Messenia. Abstracts of 11th Panhellenic Entomological Congress, Karditsa, October 11-14, p 9.

Stathas, G.J. 2008. The scale insect *Dynaspidiotus abietis* (Schrank) on *Abies cephalonica* (Pinace-

ae). *Entomologia Hellenica,* 17: 28-33.

Stathas, G.J. and Kozár, F. 2008. *Chrysomphalus aonidum* as a pest of citrus in Greece. *Entomologia Hellenica,* 16 (2005-2006): 16-21.

Stathas, G.J., Kartsonas, E.D. and Kontodimas D.C., 2008. New hosts for the pyriform scale *Protopulvinaria pyriformis* (Cockerell) (Hemiptera: Coccidae) in Greece. *Entomologia Hellenica,* 17 (2007-2008): 56-59.

Stathas, G.J., Eliopoulos, P.A., Japoshvili, G.O. and Kontodimas, D.C. 2009. Phenological and ecological aspects of *Protopulvinaria pyriformis* (Cockerell) (Hemiptera: Coccidae) in Greece. *Journal of Pest Science*, 82: 33-39.

Stathas, G.J. and Kozár, F. 2010. First record of *Physokermes inopinatus* Danzig & Kozár 1973 (Hemiptera: Coccidae) in Greece. *Hellenic Plant Protection Journal*, 3: 7-8

Stathas, G.J., Eliopoulos, P.A., Salmas, I.C. and Kozár, F. 2011. Data on ecology of some Hemiptera species recorded in the forest of Taygetus Mountain, Peloponnesus, Greece. *Phytoparasitica*, 39: 377–383.

Stathas, G.J., Kartsonas, E.D. and Skouras, P.J. 2013. First record of *Kermes echinatus* Balachowsky (Hemiptera, Coccoidea, Kermesidae) on *Quercus ilex*. *Entomologia Hellenica*, 22(1): 19-22.

Stathas, G.J. 2015. The scale insect *Dynaspidiotus abieticola* (Koroneos) (Hemiptera: Diaspididae) on the Taygetus mountain in Greece. *Hellenic Plant Protection Journal,* 8: 63-65.

Stathas, G.J., Skouras, P.J. and Kontodimas, D.C. 2015a. Data on ecology of the purple scale *Lepidosaphes beckii* (Newman) on citrus in Greece. *Bulletin OEPP/EPPO,* 45(1): 128-132.

Stathas, G.J., Kartsonas, E.D. and Darras, A.I. 2015b. Record of *Phenacoccus peruvianus* Granara de Willing and *Phenacoccus madeirensis* (Hemiptera: Pseudococcidae) on new host ornamental plants in Greece. *Hellenic Plant Protection Journal*, 8: 12-14.

Stathas, G.J., Kartsonas, E.D., Skouras, P.J. and Darras, A.I. 2018. Data on the ecology of *Kermes echinatus* Balachowsky (Hemiptera, Coccomorpha, Kermesidae) on *Quercus ilex* (Fagaceae) in Greece. *Phytoparasitica*, (46): 491-498.

Stathas, G.J., Kostriva, A., Skouras, P.J., Kontodimas, D.C. and Karipidis, C.F. 2020. Data on biology and ecology of *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Hemiptera: Diaspididae) on fruit trees in the Peloponnese. *Journal of Agronomy and Animal Industries,* 15 (2): 142–149.

Szita, É., Fetykó, K., Konczné Benedicty, Z., Kozár, F., Partsinevelos, G.K., Milonas, P.G. and Kaydan, M.B. 2017. Data on the scale insect (Hemiptera: Coccomorpha) fauna of Greece, with description of two new species. *Zootaxa* 4329(5): 463–476.

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ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

Επισκόπηση των κοκκοειδών εντόμων (Hemiptera: Coccomorpha) και των φυσικών εχθρών τους σε καλλιεργούμενα, καλλωπιστικά και δασικά φυτικά είδη στην ευρύτερη περιοχή της Μεσσηνίας, 2000 – 2020

Γ.Ι. Σταθάς, Ε.Δ. Κάρτσωνας, Α.Ι Δάρρας και Π.Ι. Σκούρας

Περίληψη Αναφέρονται τα κοκκοειδή έντομα (Hemiptera: Coccomorpha) τα οποία κατεγράφησαν στην ευρύτερη περιοχή της Μεσσηνίας (Πελοπόννησος) κατά την εικοσαετία 2000 - 2020. Τα είδη αυτά βρέθηκαν σε καλλιεργούμενα, καλλωπιστικά και δασικά φυτικά είδη. Στα δεκαπέντε από τα είκοσι αναφερθέντα είδη, μελετήθηκε η βιολογία, η φαινολογία και οι φυσικοί εχθροί τους στη Μεσσηνία. Τα είδη αυτά ανήκουν σε τέσσερις οικογένειες Diaspididae: *Aonidiella aurantii* (Maskell), *Chrysomphalus aonidum* (L.), *Diaspis echinocacti* (Bouché), *Dynaspidiotus abieticola* (Koroneos), *D. abietis* (Schrank), *Lepidosaphes beckii* (Newman), *L. gloverii* (Packard), *Lineaspis striata* (Newstead), *Targionia vitis*

(Signoret), Coccidae: *Ceroplastes rusci* (L.), *Eulecanium sericeum* (Lindinger), *Nemolecanium graniformis* (Wünn), *Parthenolecanium corni* (Bouché), *P. persicae* (Fabricius), *Physokermes hemicryphus* (Dalman), *P. inopinatus* Danzig and Kozár, *Protopulvinaria pyriformis* (Cockerell), Pseudococcidae: *Phenacoccus madeirensis* Green, *Planococcus vovae* (Nasonov) και Kermesidae: *Kermes echinatus* Balachowsky.

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Investigation of the effects of household processing on the reduction rate of chlorpyrifos, metalaxyl and diazinon residues in orange fruit

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Summary The effect of the household processing on the reduction rate of chlorpyrifos, metalaxyl and diazinon residues in contaminated oranges has been investigated and the processing factors were determined. The evaluation included validation parameters, matrix effect (ME %), reduction behavior and processing factors (PFs). Validation parameters were successfully applied; the three pesticides showed satisfactory recovery (70–120%) and precision (relative standard deviation - RSD<20%); they also exhibited no matrix effect. The most effective process in the pesticide residues reduction was juicing, followed by pulping while the washing process was less efficient in removing all pesticide residues; sonication showed a high reduction rate with both chlorpyrifos and diazinon. The processing factors (PFs) were generally less than one which indicates that all processes can reduce pesticide residues in oranges. The results could guide the safe and reasonable use of chlorpyrifos, diazinon, and metalaxyl. These processes contribute substantially to reduce consumer exposure to pesticide residues in oranges.

Additional keywords: GC/MS, QuEChERS, matrix effect, oranges, pesticides, Processing Factor

Introduction

Oranges are among the fruits most widely accepted as they are an excellent source of vitamin C, fiber, and antioxidants (Barrose *et al.,* 2012). Analysis of 141 pesticide residues in 31 orange samples from Egypt by Malhat *et al.* (2017) showed 66.7% of them being contaminated with 8 different pesticide residues, including fenpropathrin and chlorpyrifos, which were also detected in this work.

Chlorpyrifos is an organophosphorus insecticide and acaricide that is widely used to combat pests infesting citrus fruits (European Food Safety Authority, 2011; Jardim and Caldas, 2012). Two organophosphorus pesticides, chlorpyrifos (5.3%) and diazinon (10.5%) were found by Iñigo-Nuñez *et al.* (2010) in 19 orange juice samples from markets in Madrid (Spain). Knezevic, *et al.* (2012) studied 103 pesticides (including isomers) in 105 commercial orange samples in Croatia from 2007 to 2009, and diazinon residues were found up to levels of 0.28 mg.kg^{-1}.

Effects of food processing on pesticide residues have been reviewed comprehensively over the last decade (González-Rodríguez *et al.*, 2011) and the literature review shows that most household processing treatments lead to considerable reductions in residue levels in the prepared food, particularly through washing and peeling, fermentation, refrigeration singly or in combination. The behavior of residues in storage and processing can be rationalized in terms of the physico-chemical properties of the pesticide and the nature of the process (Vinita *et al.*, 2013).

Bonnechere *et al.* (2012) studied the processing factors of several pesticides and degradation products in carrots by household and industrial processing, such as washing, peeling, blanching, microwave cooking, pasteurization and sterilization. The levels of six pesticide residues and eight associated degradation products were quantified. The washing step allowed decreasing

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the concentration of residues for all pesticides up to ~ 90%. It was the most effective step to remove pesticide residues from carrots. The second process, peeling, resulted in a reduction comparable to washing. The blanching step, combining heat with a large quantity of water, enhanced the elimination of residues (maximum 50%). After cutting and washing, the residual concentrations were below 5 ppb.

Processing factors (PFs) are the ratio of residue concentrations after processing to those in the raw commodity. PF values greater than 1 indicate an increase in pesticide residue concentrations during processing; PF values less than 1 indicate decreases (Ramezani and Shahriari, 2014). PFs depend on both the crop and the physicochemical properties of pesticides (González-Rodríguez *et al*., 2011), especially water solubility and the water–octanol partition coefficient (Kow). Pesticides with high water solubility and with low Kow and Koc are mostly transferred to the juice, but those with low solubility and high Kow and Koc are retained on some fruit skins. Peeling and storage were found to be two important processing procedures that may remarkably reduce non-systemic pesticide residues in some fruits and vegetables (Ramezani and Shahriari, 2014; Jiang *et al*., 2013).

Validation parameters such as sensitivity, linearity, specificity, trueness, precision, limit of detection (LOD), limit of quantitation (LOQ), and matrix effect are commonly evaluated in pesticide residues determination. Chromatographic techniques established that matrix effect is significant when higher than ± 20% (Sherif *et al.,* 2016; SANTE/11813/2017). In recent years, it has been essential to use matrix-matched calibration in routine procedure for the analysis of pesticides in food by chromatographic methods to avoid an error caused by the presence of matrix effect (Kwon *et al.* 2012). In previous study, in which the validation of the GC-MS method was the main focus, QuEChERS was employed as a sample preparation technique for the determination of chlorpyrifos and metalaxyl residues in tomatoes matrices (Hassan *et al.,* 2019).

Lozowicka *et al.* (2016) determined the processing factor (PF) for 16 pesticides processing techniques in strawberries. Washing with ozonized water was demonstrated to be a more effective reduction strategy, displaying percentage ranges from 36.1 to 75.1% compared to washing with tap water (respective ranges fluctuated from 19.8 to 68.1%). Boiling decreased the residues of the most compounds with reductions ranging from 42.8 to 92.9%. Ultrasonic cleaning lowered residues for all analyzed pesticides with removal above 90%. According to Bajwa and Sandhu (2014), ultrasonic cleaning and boiling were the most effective treatments for the reduction of 16 pesticide residues in raw strawberries, resulting in a lower health risk exposure.

The aim of the present study was to evaluate validation parameters, matrix effect and the impact of several processing techniques on the reduction rate of chlorpyrifos, metalaxyl and diazinon pesticide residues on orange fruits.

Materials and Methods

Organic Oranges were bought from BIO COMPANY®, GmbH, Rheinstrasse, Berlin, Germany. Commercial plant protection products were used in artificial contamination of oranges: Ridomil 72 (8% metalaxyl), Helban 48 (48% chlorpyrifos), Basudin 60 EC (diazinon) were purchased from Al-Mukhtar for pesticides and chemicals, Monufia Governorate, Egypt. Metalaxyl (1000 µg/ml) dissolved in acetone was supplied from SPEX-CertiPrep, New Jersey, USA. Chlorpyrifos 1000 µg/mL in methanol, Diazinon 1000 µg/mL in methanol were supplied from Restek, Pennsylvania, USA. Acetonitrile (HPLC grade, assay 99.9%) was obtained from Merck KGaA, Darmstadt, Germany. Hydrogen peroxide solution 31% (Ultrapure), anhydrous Sodium bicarbonate and acetic acid were purchased from Sigma-Aldrich, St. Louis, USA. The QuEChERS kit consisted of extraction packets and dispersive SPE kit suited to

the orange matrix, were purchased from Agilent Technologies, California, USA.

Instruments

The equipment used in processing and sample preparations were: a laboratory Blender (Waring, Stamford, USA), an analytical balance, model ME 104 (Mettler Toledo, Greifensee, Switzerland), a Vortex-mixer, (VELP scientific, Usmate Velate, Italy), centrifuge (BOECO, Hamburg, Germany), and Ultrasonic Tabletop Cleaner, model P 230 (CREST Ultrasonics, Penang, Malaysia).

Extraction and clean-up

The samples were treated according to the QuEChERS methodology [EN 15662: (2008)].

GC-MS analysis

Qualitative and quantitative determination of pesticide residues were performed on a 7890B gas chromatograph coupled to an Agilent 5977A mass detector (Agilent Technologies, Wilmington, USA). The chromatographic analytical conditions are shown in Table 1.

Method validation and matrix effect

The proposed method was validated following the European Commission guideline (SANTE/11813/2017). The parameters assessed were selectivity, specificity, linearity, LOD, LOQ, precision, recovery (trueness) and matrix effect.

The linearity was studied during the construction of the analytical curves obtained using analytical solutions of the mixture of the pesticides prepared in pure solvent and the extract of the orange matrix in the concentration range from 0.001 to 2 mg /L. The studies to evaluate the recovery of the pesticides were made in orange samples devoid of pesticides residues, which were fortified five times with an analytical solution containing the pesticides under study, at two different concentration levels. The study of repeatability of the instrument was evaluated with five injections in the chromatographic system for each level of concentration of the analytical solutions in pure solvent and in the extract of the matrix.

LODs and LOQs have been calculated based on two analytical parameters: the residual standard deviation of the matrix cal-

Table 1. GC/MS instrumental and analytical condition for analysis of metalaxyl, chlorpyrifos and diazinon residues.

Column	Zebron ZB-5MS Crossbond (30 m, 0.25 mm internal diameter, 0.25 mm film thickness) from Phenomenex®, Torrance, CA, USA.
Inlet	Multimode inlet (MMI) operated in splitless mode
Inlet liner	An Agilent ultra-inert splitlesss single taper liner with glass wool (p/n 5190-2293)*
Carrier gas	Helium with purity grade 6 for 9 s,
Flow rate	1 mL/min (constant flow mode)
Inlet temperature	250°C
Injection volume	2 µL
Purge flow to split vent	50 mL/min at 0.75 min
Oven temperature program	60°C (1 min), 20°C/min to 170°C (0 min), 5°C/min to 285°C
Mass detector	operated in electron impact (EI) ionization mode at 70 eV.
Scan mode	mass spectra for stock solutions of metalaxyl, chlorpyrifos and diazinon was collected at the rate of 1.5 scans/s over the mass range (m/z) of 40–550
Quantitative measurement	was carried out at Selected ion monitoring SIM mode **
The temperatures of the transfer line, ion source, quadrupole	285°C, 250°C, 150°C

*Zhao and Mao (2011); Zhao (2013). **Refer to Table 3 for settings in detail

ibration curve (σ) and the slope of the calibration curve as in Eq (1) &Eq (2) (Ellison *et al.,* 2000).

LOD= 3.3σ/slope Eq (1)
LOQ= 10σ/slope Eq (2)

The precision of the method was expressed as the relative standard deviation (RSD) for repeatability of 5 replicates of spiked blank orange samples (raw, juice, and pulp) at two concentration levels of 0.03 and 0.5 mg/ kg for metalaxyl, 0.01 and 0.5 mg/ kg for chlorpyrifos and diazinon. The percentage recoveries (R %) for chlorpyrifos, diazinon and metalaxyl were calculated according to the European Commission guideline (SANTE/11813/2017). Acceptable mean recoveries are those within the range of 70–120%.

Matrix effect (ME %) was determined by comparing the slopes obtained from solvent calibration and matrix-matched calibration curves. ME % was calculated according to Guedes *et al.* (2015):

$$ME = \left[1 - \left(\frac{stope\ of\ solvent}{stope\ of\ matrix\ calibration}\right)\right] {}^{*}100 \quad \text{Eq (3)}$$

Preparation of contaminated samples

Orange fruits were contaminated by immersion into the pesticide's solution. Organic oranges were manually washed in distilled water and dried with filter paper, then dipped into a dipping solution containing 0.5 g Ridomil WP 72 WP, 2 ml of Helban 48 EC and 2 ml Basudin 60 EC per liter of water to obtain a sufficient quantity of suspension. The samples were immersed for one hour until the pesticide residue levels did not increase. The contaminated oranges were air dried for 24 h at room temperature before processing (Hassan *et al.,* 2019).

Processing treatments

Processing treatments for removal of pesticide residues from contaminated oranges samples are illustrated in Table 2. After each process, three representative replicates were taken from each treatment for residue analysis. After each experiment, the samples were extracted immediately and analyzed, the percentages of reduction were calculated and compared with control.

Processing factors

Processing factors (PFs) were calculated for all transformation steps by a ratio between the pesticide residue concentration (mg kg^{-1}) in the processed commodity and the pesticide residue concentration (mg kg^{-1}) in the raw, non-processed commodity. If a PF is lower than one, it indicates the reduction of a pesticide, while if higher than 1, it indicates a concentration in regulatory practice, regardless of changes in volume or weight for the processed food. (Aurore *et al.,* 2012; Bonnechère *et al.*, 2012). PFs were cal-

Table 2. Processing treatments for removal of pesticide residues from contaminated oranges samples.

Treatment	Description
Peeling & Juicing	Group 1: Pulp Group 2: Juice
Washing	Group 1: washing contaminated oranges with tap water for 10 min Group 2: washing with 10% sodium bicarbonate solution (w/v) for 10 min Group 3: washing with 4% acetic acid solution(v/v) for 10 min Group 4: washing with 1% H_2O_2 solution (v/v)
Sonication in ultrasonic bath (45 kHz) filled with distilled water	Group 1(UB1): sonication for 15 min Group 2(UB2): sonication for 30 min Group 3(UB3): sonication for 60 min

culated with the following equation (Timme and Walz-Tylla, 2004):

$$PFs = \frac{\text{Residues in processed product (mg/kg)}}{Residues\ in\ raw\ agricultural\ commodity\ (mg/kg)} \quad \text{Eq (4)}$$

Results and Discussion

Optimization of separation conditions using GC/MS

The presence of matrix interferences from the orange fruit samples was evaluated by monitoring the specific ions for each pesticide at the retention time interval expected for their elution (Table 3). For qualitative analysis a full scan mass spectrum range form 40–550(m/z) was applied (Pano-Farias *et al.,* 2017).

For quantitative pesticide analysis, selected ion monitoring (SIM) mode was used and analyte peaks from both product ions in the extracted ion chromatograms fully overlapped. The monitored ions for each compound were the m/z 137, 152, 153, 304 for diazinon, m/z 132, 105, 142, 192, 297 for metalaxyl and m/z 197, 199, 314 for chlorpyrifos. Pesticides were also identified on the basis of their retention time consistency with the ones acquired by standard and spiked extract solutions injections (chlorpyrifos 14.63 min, diazinon 11.72 min and metalaxyl 13.59 min).

Analytical method validation parameters

Linearity in solvent and orange matrix extract for metalaxyl, diazinon and chlorpyrifos (Table 4) was acceptable, as indicated by the values of regression coefficient (r^2) that were > 0.99 for all compounds in solvent, and in the matrix (De Sousa *et al.,* 2012; Domínguez *et al.,* 2014). According to the European Commission guideline SANTE/11813/2017, the acceptable RSD should be ≤20%, where the fit of calibration inspected by calculation of the residuals avoids over-reliance on the correlation coefficient. The ME % (suppression or enhancement) is the signal increase or loss of an analyte in matrix standard solution compared to a matrix-free one. For metalaxyl, chlorpyrifos and diazinon ME % were -14.16, -12.1, -5.48, respectively, categorized by Ferrer *et al.* (2011) as no matrix effect (Table 4). These results were in agreement with the findings reported by Ferrer *et al.* 2011 and Chawla *et al.* (2017).

The analytical method was used to evaluate recovery, precision, LODs and LOQs for determining metalaxyl, chlorpyrifos and di-

Table 3. Optimized MS Parameters of pesticides metalaxyl, chlorpyrifos, diazinon.

Compound	Diagnostic ions (m/z)	Quantification ions (m/z)	Retention time (min)
Metalaxyl	132, 105, 192,279,206.1	206.1	13.59 ± 0.2
Chloropyrifos	197, 199, 314	314	14.63 ± 0.2
Diazinon	137, 152, 153, 304	304.1	11.7 1± 0.2

Table 4. Matrix effect (ME %) and linearity parameters in solvent and orange matrix extract for metalaxyl, diazinon and chlorpyrifos.

Pesticide	Linear range (mg/kg)	Solvent		Matrix match orange		
		Slope	r^2	Slope	r^2	ME%
Metalaxyl	0.03: 2	342610	0. 9981	300110	0.9979	-14.16
Chlorpyrifos	0.01: 2	432881	0.9994	386196	0.9990	-12.1
Diazinon	0.01: 2	425793	0.9992	403666	0.9991	-5.48

Regression coefficient (r^2)

azinon residues in orange (Table 5). The obtained LODs and LOQs were based on matrix matched calibration data at low levels of concentrations as we spike blank orange matrix with pesticides at conc. levels 5, 10, 25, 50, 100 mg/kg each in 5 replicates. Subjected to all the method preparation and injection steps, we found the detector response correlating to its concentration (peak area), calculating the average using data analysis function to calculate LOQ and LOD. The LOD and LOQ for metalaxyl were 0.01 mg/ kg, and 0.03 mg/ kg respectively, while LOD and LOQ for both chlorpyrifos and diazinon were 0.003 mg/ kg, and 0.009 mg/ kg, respectively.

Before pesticide residue analysis, recovery experiments were carried out on orange fruit, orange juice, and orange pulp. Recovery and precision data are shown in Tables 5 and 6. Residue analysis of the recovery experiments showed that mean recovery percentages were highest in orange fruit than in orange juice and in orange pulp for all pesticides. Also, chlorpyrifos had the highest recovery rates and the lowest RSD % than all pesticides. The recovery percentage (70–120%) and precision of all pesticide residue analyses (RSD ≤20%) were within the satisfactory limits recommended by the EC guideline SANTE/11813/2017.

Quantification using calibration was performed by means of calibration curves using the peak area of the most intense transition of metalaxyl (*m/z* 220). Calibration plots were linear, with regression coefficients greater than 0.99. The LOQ of the method were 17 µg kg^{-1} while the LOD were 8.5 µg kg–1.

Table 5. Validation parameters for metalaxyl, diazinon and chlorpyrifos in orange fruit.

	Pesticide	LOD[a]	LOQ[b]	Recovery and precision(*n=5)						
				FLc	Whole fruit*		Juice*		Pulp*	
					R%[d]	RSD%[e]	R%	RSD%	R%	RSD%
Orange	Metalaxyl	0.01	0.03	0.03	108.5	10.8	89.6	11.1	99.5	10.5
				0.5	97.7	8.6	88.3	8.3	94.6	9.8
	Chlorpyrifos	0.003	0.009	0.01	114.3	2.7	112.0	4.1	106.8	3.6
				0.5	100.2	3.5	107.0	2.2	103.0	2.8
	Diazinon	0.003	0.009	0.01	99.5	8.3	111.4	5.8	106.3	7.9
				0.5	97.0	6.4	100.5	2.9	102.2	4.8

[a]Limit of detection (mg/kg), [b]Limit of quantification (mg/kg), [c]Fortification Level,
R[d] %: Percentage recovery = CE/CM× 100;
CE: the experimental concentration, CM: the spiked concentration
[e]Relative Standard Deviation, *n: no of replicates

Table 6. Repeatability and Reproducibility (precision) expressed as %RSD of peak areas for spiked samples.

	Repeatability		Reproducibility	
	Level 1	Level 2	Level 1	Level 2
Diazinon	1.51	1.36	8.00	9.07
Chlorpyrifos	1.78	1.82	3.66	5.12
Metalaxyl	3.02	2.71	10.00	11.0

Level 1= 0.01 mg/kg for diazinon and chlorpyrifos, 0.03 mg/ kg for metalaxyl;
Level 2= 0.1 mg/kg for all pesticides under study

Physicochemical properties of metalaxyl, diazinon and chlorpyrifos

The behavior of pesticide residues in processing can be rationalized in terms of the physico-chemical properties of the pesticide and the nature of the process (Vidisha and Jaswinder, 2013). Table 7 shows the physicochemical properties of metalaxyl, diazinon and chlorpyrifos. Both diazinon and chlorpyrifos are non-systemic, while metalaxyl is systemic, thus it is absorbed by the plant surface and enters the plant transport system.

Regarding water solubility, it has been shown (Table 7) that pesticides with high water solubility and with low Kow and Koc are mostly transferred to the juice. Those with low solubility and high Kow and Koc are retained on the fruit skin. Peeling and storage were found to be two essential processing procedures that may remarkably reduce non-systemic pesticide residues in some fruits and vegetables (Ling *et al*., 2011).

Effect of various household processes

The study of the reduction of pesticides during household processes of orange fruit allows calculating the PFs for the tested pesticides, which are necessary to refine the risk assessment of these frequently detected pesticides.

Table 8 shows the reduction of the first treatment by household processes (raw orange, orange juice, and orange pulp) on metalaxyl, chlorpyrifos and diazinon residues. The juicing process was the most effective process in removing all pesticides; metalaxyl residues were reduced by 95% in orange juice and 93% in orange pulp, while chlorpyrifos and diazinon residues were reduced by 98%, 97% in orange juice and 90%, 86% in orange pulp, respectively.

Washing showed the less efficient removal of all pesticide residues. The residues of metalaxyl after washing with running water were reduced by 26%. Washing with 1% H_2O_2 solution reduced metalaxyl residues by 5%, and the reduction after washing with both 2% baking soda and acetic acid were 2%. The reduction levels for chlorpyrifos and diazinon were 33,29% with 1% H_2O_2, 3,5% with baking soda, and 12,12% with 4% acetic acid. The less efficient removal of metalaxyl residues from orange by washing may be due to the distinct nature of orange peels, the high log Kow values of metalaxyl and its behavior as a systemic pesticide penetrating through the peel into the flesh. On the other hand, both chlorpyrifos and diazinon residues were eliminated from the orange more successfully as non-systemic pesticides, both physically (minor part) and chemically (major part).

Similar to our study, other investigations have shown that there were differences in efficacy of removing individual pesticides from fruits and vegetables by washing. Rani *et al.* (2013) reported that by washing of tomatoes with water, chlorpyrifos residues were reduced by 41 to 44% (Duirk and Collette, 2006). Wanwimolruk *et al.* (2017) reported on the effect of washing with running water on

Table 7. Physicochemical characteristics of pesticides metalaxyl, chlorpyrifos and diazinon.

Pesticide	Category	Mode of action	Molecular Formula	Mwt (g/mol)	SW (mg/l)	log Kow
Metalaxyl	Fungicide	Systematic	$C_{15}H_{21}NO_4$	279.33	8.4 (at 22°C)	1.75
Chlorpyrifos	Insecticide, Acaricide	Non Systematic (Cholinsterase inhibition)	$C_9H_{11}Cl_3NO_3PS$	350.6	1.4 (at 25°C)	4.70
Diazinon	Insecticide, Acaricide, Nematicide	Non-Systematic	$C_{12}H_{21}N_2O_3PS$	304.345	40 (at 25°C)	3.81

Sw: water solubility; Kow: Octanol-Water Partition Coefficient; Mwt: Molecular Weight

Table 8. Residue levels (mg/ kg), reduction percentage and experimental processing factors of diazinon, metalaxyl and chlorpyrifos in oranges.

Processing factors	Diazinon			Metalaxyl			Chlorpyrifos		
	C ± SD*	Re% ± SD	PF ± SD	C ± SD*	Re% ± SD	PF ± SD	C ± SD*	Re% ± SD	PF ± SD
Con	4.09 ± 0.08	-	-	1.98 ± 0.02			3.57 ± 0.073		
Pu	0.57 ± 0.03	86 ± 0.60	0.14 ± 0.01	0.14 ± 0.01	93 ± 0.51	0.07 ± 0.01	0.35 ± 0.015	90 ± 0.43	0.1 ± 0.004
J	0.12 ± 0.01	97 ± 0.13	0.03 ± 0.001	0.09 ± 0.007	95 ± 0.36	0.05 ± 0.004	0.088 ± 0.007	98 ± 0.19	0.02 ± 0.002
W	2.98 ± 0.08	27 ± 1.90	0.73 ± 0.02	1.46 ± 0.03	26 ± 1.27	0.74 ± 0.013	2.53 ± 0.026	29 ± 0.74	0.71 ± 0.007
H_2O_2 1%	2.90 ± 0.10	29 ± 2.44	0.71 ± 0.02	1.88 ± 0.021	5 ± 1.05	0.95 ± 0.01	2.38 ± 0.025	33 ± 0.71	0.67 ± 0.007
$NaHCO_3$ 10%	3.88 ± 0.11	5 ± 2.64	0.95 ± 0.03	1.94 ± 0.015	2 ± 0.77	0.98 ± 0.008	3.46 ± 0.018	3 ± 0.28	0.97 ± 0.003
acetic acid 4%	3.60 ± 0.10	12 ± 2.44	0.88 ± 0.02	1.94 ± 0.01	2 ± 0.51	0.98 ± 0.005	3.14 ± 0.03	12 ± 0.86	0.88 ± 0.009
UB1	2.45 ± 0.12	40 ± 2.90	0.60 ± 0.03	1.68 ± 0.012	15 ± 0.58	0.85 ± 0.006	1.37 ± 0.01	61 ± 0.28	0.39 ± 0.003
UB2	1.02 ± 0.01	75 ± 0.14	0.25 ± 0.001	1.47 ± 0.023	26 ± 1.17	0.74 ± 0.012	0.64 ± 0.01	82 ± 0.29	0.18 ± 0.003
UB2	0.28 ± 0.03	94 ± 0.75	0.06 ± 0.01	1.35 ± 0.02	32 ± 1.00	0.68 ± 0.01	0.16 ± 0.01	96 ± 0.30	0.04 ± 0.003

Con: Control, Pu: pulp, J: juice, W: washing with tap water, H_2O_2 1%: washing with H_2O_2 1%(v/v), $NaHCO_3$ 10%: washing with $NaHCO_3$ 10% (w/v), acetic acid 4%: washing with acetic acid 4%(v/v), UB1: sonication 15min, UB2: sonication 30 min, UB3: sonication 60 min
C: mean concentration of 3 replicates (mg/kg); *number of replicates = 3; SD: Standard division; Re%: Reduction %; PF: Processing Factor

pesticide residue removal from tomatoes, in which both carbofuran and fenobucarb residues were reduced by 58% and 40%, respectively, although without statistical significance ($p > 0.2$). In the same study, cypermethrin and λ-cyhalothrin residues were removed by 27% by washing with running water although there was no significant difference ($p > 0.5$) between the mean concentrations of pesticides after washing *vs.* unwashed samples. In the study by Andrade *et al.* (2015) in cucumber, washing with water reduced the residues of procymidone by 24%, while 85% was eliminated with the removal of the peel, even though this pesticide is systemic. Chlorpyrifos residues translocated into the internal tissue may not be removed physically and chemically (Pugliese *et al.,* 2004). These disparities in efficacy of washing with water may be due to water solubility of pesticides.

Sonication in the ultrasonic bath was effective in eliminating pesticide residues in orange samples and sonication time (for 15, 30, and 60 min) significantly influenced the effect. Sonication removed 98% and 94% of chlorpyrifos and diazinon residues, respectively, after 60 minutes, while the percentage of reduction reached 82% and 75% after 30min. Metalaxyl residues were eliminated by 32% after 60 minutes of sonication. Zhang *et al.* (2012) reported that sonication could effectively remove phorate residues in apple juice. Helmy *et al.* (2019) reported that sonication treatment effectively removed chlorpyrifos residues in tomato matrices, but not metalaxyl residues.

Processing factors (PFs)

The processing factors (PFs) for the household processes of oranges, which are necessary to refine the risk assessment of frequently detected pesticides, were lower than one. The lowest PFs were found in juicing (0.05, 0.02, 0.03 in metalaxyl, chlorpyrifos and diazinon, respectively) followed by sonication (0.04 and 0.06, respectively in chlorpyrifos and diazinon). On the other hand, the highest PFs were found in the washing treatments for metalaxyl, ranging from 0.74 to 0.98. The PFs for both chlorpyrifos and diazinon were lower than for metalaxyl in all treatments. The results are in agreement with those previously reported by Ramezani and Shahriari (2015) and Bajwa and Sandhu (2014). We conclude that the status of pesticide residues, mainly related to the physicochemical properties of the pesticides, affects their removal from oranges.

Conclusion

The tested QuEChERS and GC-MS method for the separation of metalaxyl, chlorpyrifos and diazinon in orange fruit was properly validated using orange samples and the results indicate that this method is specific, accurate and reproducible. The expanded uncertainty of the method is acceptable according to SANCO/12495/2011 guideline. The proposed method was also found to be suitable for different kind of orange fruits. The effects of different household processes indicated that the levels of metalaxyl, chlorpyrifos and diazinon residues can be reduced significantly by juicing, followed by peeling and sonication. The percentage reduction was lower in metalaxyl than in chlorpyrifos. The less efficient removal of metalaxyl residues from orange by washing may be due to the distinct nature of orange. All pesticides showed no matrix effect. The PF values for all treatments were lower than one, with the lowest for juicing, followed by sonication in all of pesticides. The household processes can substantially contribute to reduce consumer exposure to pesticides.

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Literature Cited

Abdel Ghani S.B., and Abdallah, O.I. 2016. Method validation and dissipation dynamics of chlorfenapyr in squash and okra. *Food Chemistry*, 194: 516-521.

Andrade, G.C., Monteiro, S.H., Francisco, J.G., Figueiredo, L.A., Rocha, A.A. and Tornisielo, V.L. 2015. Effects of types of washing and peeling in relation to pesticide residues in tomatoes. *Journal of the Brazilian Chemical Society,* 26(10): 1994–2002.

Bonnechère, A., Hanot, V., Jolie, R., Hendrickx, M., Bragard, C., Bedoret, T. and Van Loco, J. 2012. Processing Factors of Several Pesticides and Degradation Products in Carrots by Household and Industrial Processing. *Journal of Food Research*, 1(3): 68-83.

Bajwa, U. and Sandhu, K.S. 2014. Effect of Handling and Processing on Pesticide Residues in Food-A Review. *Journal of Food Science and Technology,* 52(2): 201-220.

Banerjee, K., Utture, S., Dasgupta, S., Kandaswamy, C., Pradhan, S., Kulkarni, S. and Adsule, P. 2012. Multiresidue determination of organic contaminants including pesticides, polychlorinated biphenyls, and polyaromatic hydrocarbons in fruits and vegetables by gas chromatography-etriple quadrupole mass spectrometry with introduction of semi quantification. *Journal of Chromatography A*, 270: 283.

Barros, H.R.M., Ferreira, T.A.P.C. and Genovese, M.I. 2012. Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food Chemistry,* 134(4): 1892-1898.

Chawla, S., Patel, H.K., Gor, H.N., Vaghela, K.M., Solanki, P.P. and Shah, P.G. 2017. Evaluation of matrix effects in multiresidue analysis of pesticide residues in vegetables and spices by LC-MS/MS. *Journal of AOAC International*, 100(3): 616–623.

De Sousa, F.A., Guido Costa, A.I., De Queiroz, M.E.L.R., Teófilo, R.F., Neves, A.A. and De Pinho, G.P. 2012. Evaluation of matrix effect on the GC response of eleven pesticides by PCA. *Food Chemistry*, 135(1): 179–185.

Domínguez, A.M., Placencia, F., Cereceda, F., Fadic, X. and Quiroz, W. 2014. Analysis of tomato matrix effect in pesticide residue quantification through QuEChERS and single quadrupole GC/MS. *Chilean Journal of Agricultural Research*, 74(2): 148–156.

Duirk, S.E. and Collette T.W. 2006. Degradation of chlorpyrifos in aqueous chlorine solutions: Pathways, kinetics, and modeling. *Environmental Science and Technology,* 40: 546–51.

Ellison, S.R.L, Rosslein, M. and Williams, A. 2000. Editors. Quantifying uncertainty in analytical measurements. 3rd ed. EURACHEM/CITAC.

EN 15662: 2008. Foods of plant origin: Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile. Extraction/partitioning and clean-up by dispersive SPE.

EU Commission. 2017. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANTE/11813/2017. European Commission health and Consumer Protection Directorate-General, Brussels, Belgium.

Ferrer, C., Lozano, A., Agüera, A., Girón, A.J. and Fernández-Alba, A.R. 2011. Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables. *Journal of Chromatography A,* 1218(42): 7634–7639.

Guedes J.A., Silva R.deO., Lima, C.G., Milhome, M.A. and do Nascimento R.F. 2016. Matrix effect in guava multiresidue analysis by QuEChERS method and gas chromatography coupled to quadrupole mass spectrometry. *Food Chemistry*, 199: 380–386.

González-Rodríguez, R.M., Rial-Otero, R, Cancho-Grande B., Gonzalez-Barreiro, C. and Simal-Gándara, J. 2011. Review on the fate of pesticides during the processes within the food-production chain. *Critical Reviews in Food Science and Nutrition*, 51: 99–114.

Hassan, H., Elsayed, E., El-Raouf, A.E.R.A. and Salman, S.N. 2019. Method validation and evaluation of household processing on reduction of pesticide residues in tomato. *Journal fur Verbraucherschutz und Lebensmittelsicherheit.* 14(1): 31–39.

Iñigo-Nuñez, S., Herreros, M.A., Encinas, T., and Gonzalez-Bulnes, A. 2010. Estimated daily intake of pesticides and xenoestrogenic exposure by fruit consumption in the female population from a Mediterranean country (Spain). *Food Control*, 21(4): 471-477.

Jardim, A.N.O. and Caldas, E.D. 2012. Brazilian monitoring programs for pesticide residues in food Results from 2001 to 2010. *Food Control,* 25(2): 607–616.

Jiang, Y., Shibamoto, T., Li, Y. and Pan, C. 2013. Effect of household and commercial processing on acetamiprid, azoxystrobin and methidathion residues during crude rapeseed oil production. *Food Additives and Contaminants: Part A*, 30(7): 1279e1286.

Knezevic, Z., Serdar, M. and Ahel, M. 2012. Risk assessment of the intake of pesticides in Croatian diet. *Food Control*, 23(1): 59-65.

Kwon, H., Lehotay S.J. and Geis-Asteggiante L. 2012. Variability of matrix effects in liquid and gas chromatography-mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops. *Journal of Chromatography* A, 1270: 235–45.

Ling, Y., Wang, H., Yong, W., Zhang, F., Sun, L., Yang, M. and Chu, X. 2011. The effects of washing and cooking on chlorpyrifos and its toxic metabo-

lites in vegetables. *Food Control*, 22(1), 54-58.

Lozowicka, B., Jankowska, M., Hrynko, I. and Kaczynski, P. 2016. Removal of 16 pesticide residues from strawberries by washing with tap and ozone water, ultrasonic cleaning and boiling. *Environmental Monitoring and Assessment*, 188: 1–19.

Malhat, F., Boulangé, J., Abdelraheem, E., Abd Allah, O., Abd El-Hamid, R. and Abd El-Salam, S. 2017. Validation of QuEChERS based method for determination of fenitrothion residues in tomatoes by gas chromatography–flame photometric detector: Decline pattern and risk assessment. *Food Chemistry*. 229: 814–819.

Pano-Farias, N.S., Ceballos-Magaña, S.G., Muñiz-Valencia, R. and Gonzalez, J. 2017. Validation and assessment of matrix effect and uncertainty of a gas chromatography coupled to mass spectrometry method for pesticides in papaya and avocado samples. *Journal of Food and Drug Analysis*, 25(3): 501–509.

Polat, B. and Tiryaki, O. 2019. Assessing washing methods for reduction of pesticide residues in Capia pepper with LC-MS / MS. *Journal of Environmental Science and Health, Part B*, 55(1): 1–10.

Pugliese, P., Moltó, J.C., Damiani, P., Marín, R., Cossignani, L. and Mañes, J. 2004. Gas chromatographic evaluation of pesticide residue contents in nectarines after non-toxic washing treatments. *Journal of Chromatography* A. 1050(2): 185–191.

Ramezani, M.K. and Shahriari, D. 2014. Dissipation behavior, processing factors and risk assessment for metalaxyl in greenhouse-grown cucumber. *Pest Management Science*, 71(4): 579–583.

Rani, M., Saini, S. and Kumari, B. 2013. Persistence and effect of processing on chlorpyriphos residues in tomato (*Lycopersicon esculantum* Mill.). *Ecotoxicology and Environmental Safety*, 95: 247–252.

Reiler, E., Jørs, E., Bælum, J., Huici, O., Alvarez Caero, M.M. and Cedergreen, N. 2015. The influence of tomato processing on residues of organochlorine and organophosphate insecticides and their associated dietary risk. *Science of the Total Environment*, 6(2): 262–269.

SANCO Document /12571/2013. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. <http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf>

Timme, G. and Walz-Tylla, B. 2004. Effects of food preparation and processing on pesticide residues in commodities of plant origin. In Pesticides residues in food and drinking water: human exposure and risks. 121–148.

Tomer, V. and Sangha, J.K. 2013. Vegetable processing at household level: Effective tool against pesticide residue exposure. *Toxicology and Food Technology*, 6(2): 43–53.

Wanwimolruk, S., Duangsuwan, W., Phopin, K., Boonpangrak, S. 2017. Food safety in Thailand 5: the effect of washing pesticide residues found in cabbages and tomatoes. *Journal of Consumer Protection and Food Safety*, 12:209–221

Xu, W., Li, H., Guan, Q., Shen, Y., and Cheng, L. 2017. A rapid and simple liquid chromatography-tandem mass spectrometry method for the measurement of testosterone, androstenedione, and dehydroepiandrosterone in human serum. *Journal of Clinical Laboratory Analysis*, 31(5), e22102.

Zhang, Y., Zhang, Z., Chen, F., Zhang, H., Hu, X. 2012. Effect of sonication on eliminating of phorate in apple juice. *Ultrasonics Sonochemistry,* 19: 43–48. doi:10.1016/j.ultsonch.2011.05.014

Zhao, L. and Mao, D. 2011. "Analysis of Pesticides in Food by GC/MS/MS using the Ultra Inert Liner with Wool" Application note, Agilent Technologies, Inc., Publication number 5990-7706 EN.

Zhao, L. 2013. Evaluating Inert Flow Path Components and Entire Flow Path for GC/MS/MS Pesticide Analysis. Application note, Agilent Technologies, Inc.

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Διερεύνηση της επίδρασης διαφόρων μεθόδων οικιακής επεξεργασίας στη μείωση των υπολειμμάτων chlorpyrifos, metalaxyl και diazinon σε καρπούς πορτοκαλιάς

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Περίληψη Εξετάστηκε η επίδραση διαφόρων μεθόδων οικιακής επεξεργασίας στη μείωση των υπολειμμάτων chlorpyrifos, metalaxyl και diazinon σε καρπούς πορτοκαλιάς και προσδιορίστηκαν οι συ-

ντελεστές επεξεργασίας. Η αξιολόγηση περιελάμβανε παραμέτρους επικύρωσης της αναλυτικής μεθόδου συμπεριλαμβανομένης της επίδρασης μήτρας, τη συμπεριφορά μείωσης των υπολειμμάτων, και τους συντελεστές επεξεργασίας. Σε ότι αφορά τις παραμέτρους επικύρωσης της αναλυτικής μεθόδου, η ανάκτηση των τριών δραστικών ήταν ικανοποιητική (70–120%), όπως και η ακρίβεια (σχετική τυπική απόκλιση - RSD <20%), ενώ δεν παρουσιάστηκε επίδραση μήτρας. Η πιο αποτελεσματική επεξεργασία στη μείωση των υπολειμμάτων ήταν η χυμοποίηση, ακολουθούμενη από την πολτοποίηση ενώ η διαδικασία του πλυσίματος των καρπών ήταν λιγότερο αποτελεσματική στην απομάκρυνση των υπολειμμάτων όλων των δραστικών. Η κατεργασία με υπερήχους έδειξε υψηλό ποσοστό μείωσης τόσο στο chlorpyrifos όσο και στο diazinon. Οι συντελεστές επεξεργασίας ήταν γενικά μικρότεροι της μονάδας, γεγονός που υποδηλώνει ότι όλες οι διεργασίες μπορούν να μειώσουν τα υπολείμματα των εν λόγω δραστικών στα πορτοκάλια. Τα αποτελέσματα είναι χρήσιμα για την ασφαλή και ορθολογική χρήση των chlorpyrifos, diazinon και metalaxyl. Οι πρακτικές που μελετήθηκαν μπορούν να συμβάλλουν ουσιαστικά στη μείωση της έκθεσης των καταναλωτών σε υπολείμματα φυτοπροστατευτικών προϊόντων στα πορτοκάλια.

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Volatile organic compound profiling of *Capsicum annuum* var. *longum* grown under different concentrations of nitrogen

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Summary Emission of volatile organic compounds (VOCs) in plants is triggered by several biotic and abiotic factors, such as nutrient deficiency, environmental stress, and pathogenic attacks. For instance, plants suffering from limited or excessive nitrogen (N) supply may experience internal stress which can ultimately lower their stability and immunity making them susceptible to infection and infestation. In this study, VOCs from *Capsicum annuum* var. *longum* (Solanaceae) exposed to nitrogen (1.8 g/L, 4.5 g/L, and 9 g/L urea) were extracted using a 100 µm Solid Phase Microextraction (SPME) fiber coated with polydimethylsiloxane (PDMS). Using Gas Chromatography-Mass Spectrometry (GC-MS), extracted VOCs from N-treated plants were identified as Butanoic acid, 3-hexenyl ester, (E)-; Butanoic acid, hexyl ester; Hexanoic acid, 3-hexenyl ester, (Z)-; Hexanoic acid, 4-hexen-1-yl ester; cis-3-Hexenyl cis-3-hexenoate and 4-Pentenoic acid 2-methyl-, hexyl ester. Among these volatiles, butanoic acid, 3-hexenyl ester showed the most distinctive peak from the N-treated plants in comparison with the untreated. In addition, the Green Leaf Volatiles (GLV) 3-Hexenal; 2-Hexenal; 3-Hexen-1-ol, (Z)-; 2-Hexen-1-ol, (E) and 1-Hexanol were also detected from the N-treated plants. The identification of plant volatiles provides useful information that can be used in agricultural practices and plant phenotyping.

Additional keywords: Butanoic acid, GC-MS, Green Leaf Volatiles, Polydimethylsiloxane, Solid Phase Microextraction Fiber

Introduction

The *Capsicum* genus (Solanaceae) represents a diverse plant group where varieties have been produced from different climatic conditions and cultivation periods (Gaytan *et al.,* 2017). Among the 20-27 identified species (Walsh and Hoot, 2001), *Capsicum chinense, Capsicum frutescens, Capsicum annuum, Capsicum baccatum*, and *Capsicum pubescens* are globally cultivated (Heiser and Pickersgill, 1969). Among these five reared capsicums, *C. annuum* is the most extensively cultivated across the globe. Varieties of *C. annuum* are further categorized based on its fruit shape, namely, *C. annuum* var *glabriusculum, C. annuum* var *abbreviatum, C. annuum* var *grossum, C. annuum* var *anuum and C. annuum* var *accuminatum* (Zhigila *et al*., 2014). *C. annuum* generally grows in areas with subtropical to tropical climate where there is a temperature and an annual rainfall range of 20-25°C and 120-850 mm, respectively. *C. annuum* var. *longum*, commonly known as Green long chili or "siling haba", originated from tropical America and has been widely cultivated in the Philippines for food purposes, predominantly used as a spice and for seasoning, and medicinal uses (McBride, 2016). *Capsicum* usually requires four to five months before it reaches complete growth (Bargavi and Elumalai, 2010) while yield is also significantly affected by nutrient availability (Anitha and Geethakumari, 2006). Some of the key factors affecting the growth and productivity of capsicums are pests, diseases, temperature, soil characteristics, and water availability (Bhutia *et al*., 2018). The tillage, irriga-

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tion, spacing, and sowing strategies can also have an impact on the development of capsicums (Sharma and Kumar, 2017; Islam *et al.,* 2011).

Nitrogen, one of the essential nutrients in plant productivity, is usually supplied as fertilizer given directly to the soil or blended with the irrigation water. Upon contact with soil, N-fertilizers, such as urea, begin to breakdown and hydrolyze into ammonium and carbon dioxide. Although the addition of urea can increase dry weight and enhance drought tolerance of plants (Gou *et al.,* 2017), their response to the availability of nitrogen varies in plant species, cultivars, plant organs, and tissues (Effan *et al*., 2019; Moreau *et al*., 2015). This variation can be attributed to the plants' efficiency to absorb nitrogen and carbon, which can be measured as radiation use efficiency, root weight ratio, nitrogen uptake efficiency, and morphogenic efficiency (Moreau *et al*., 2015).

In *C. annuum*, the introduction of nitrogen is reported to have led to an increased pod number (83.47%), seed number (19.27%), plant height (42.7%), branch number (37.7%) and leaf number (63.5%) (Molla *et al*., 2019). High accumulation of nitrogen can trigger the release of growth hormone factors such as cytokines and gibberellins resulting in an increased plant height. The phenological properties, such as flowering, maturity, and pod settings of hot pepper plants are also affected by nitrogen (Molla *et al*., 2019). The addition of appropriate N concentration could improve production yield in *C. annuum*, however, excessive nitrogen may damage its reproductive structures (Aliyah, 2000). Besides, differences in available nitrogen levels significantly affect plant VOC production (Holopainen and Gershenzon, 2010) while deficiency alters VOC emission (Lou and Baldwin, 2004). For instance, tomato plants exposed at high nitrogen concentration were able to produce various terpenes, such as β-caryophyllene, α-terpinene, β-pinene, (+)-4-carene, α-copaene, p-cymene, β-phellandrene, and α-humulene (Islam *et al.,* 2017).

Approximately 1,700 of the 100,000 phytochemicals are classified as volatile organic compounds (VOCs) (Holopainen and Gershenzon, 2010; Spinelli *et al*., 2011). As plants remain the top VOC emitter, its annual emission reaches around 760 Tg (°C) across the globe (Samburova *et al.,* 2019). Its crucial role in plants can be attributed to the plant-plant and plant-biota interaction to act as a defense mechanism against invasive insects, alleviation of environmental stress, and signal between and within plants (Spinelli *et al*., 2011). These volatile emissions could be traced from the bursting of plant organs with stored VOCs due to herbivory or de novo synthesis as a response to damaged organs (Holopainen and Gershenzon, 2010). In some cases, VOCs are released by carnivorous plants to attract their prey (Kreuzwieser *et al*., 2014). Some of the most common VOCs identified from plants include isoprene, monoterpenes, and sesquiterpenes (Samburova *et al*., 2019). Its emission can greatly vary depending on the plant species, type of organ, stage of development, and ecological conditions (Jassbi *et al*., 2010).

The main classes of VOCs previously identified from different species of capsicums belong to aliphatic branched-chain hydrocarbons, terpenes, aldehydes, and alcohols (Ziino *et al*., 2009). Previous studies also identified the different VOCs that are naturally produced in chilli pepper plants. For instance, a total of 64 plant volatiles identified as terpenes and its derivatives, pyrazines, alcohols, aldehydes, ketones, esters, hydrocarbons, and carotenoid derivatives were detected from powdered chilli pepper fruits using HS-SPME/GC-MS method (Cirlini *et al*., 2019). These VOCs, in combination with non-VOCs are often associated with the fruit's flavor and aroma giving it its unique taste and smell (Eggink *et al*., 2012a, 2012b; Cirlini *et al*., 2019). Similarly, a variety of VOCs were also detected from chili pepper seeds (Silva *et al*., 2013). From these studies, only few reported the influence of elevated nitrogen concentrations on the profile of VOCs in capsicums. Therefore, this study aims to determine the effects of nitrogen nutrition on the VOCs profile of *C. annuum* var. *longum*.

Materials and Methods

Plant cultivation, nitrogen treatment, and sample collection

Forty-five-day old seedlings of *C. annuum* var. *longum* were acquired from the Bureau of Plants Industry, Manila, Philippines. Plants were allowed to grow for 4 weeks in a nursery at De La Salle University, Manila for the experimental process. Plants received similar treatments such as watering and occasional rotation to attain uniform treatments. For nitrogen (N) treatments, urea was used as N source. Three concentrations were prepared by dissolving 1.8 g (1x), 4.8 g (2.5x) and 9 g (5x) of urea in 1L distilled water. The N-treatment started when 500 mL of N solutions were introduced to the soil. Untreated plants were given the same amount of water (0x). Control and treated *C. annuum* var. *longum* leaves were collected after 4 weeks. All treatments were conducted in triplicates.

Volatile organic compound analysis

From each plant, a total of three leaves were collected from the top, middle, and bottom parts of the plant. Then, samples were acclimatized for 1 hour before the analysis. Collected leaf samples were placed in a 500 mL Pyrex® Erlenmeyer flask sealed with parafilm and covered with aluminum foil. The flask served as a headspace chamber for VOC analysis (Tavera *et al*., 2018). Volatile organic compounds were extracted using a Supelco® 100 µm Polydimethysiloxane Solid Phase Microextraction fiber (SPME). PDMS fiber was selected in this study to include both low and high molecular mass volatile compounds in addition to the short desorption time and thermal stability of the fiber as reported by the group of Perera *et al*. (2020). Initially, the fibers were conditioned at 250°C for 30 min to remove chemical residues. The flasks containing the samples and the fiber were heated at 30°C-40°C for 15 min to initiate the release of VOCs (Silva *et al*. 2017). While the samples were heating, the fiber was exposed inside the flask to collect VOCs from the headspace. For the control, the SPME fibers were exposed to flask without the leaf samples (Tavera *et al*., 2018).

Gas Chromatography-Mass Spectrometry

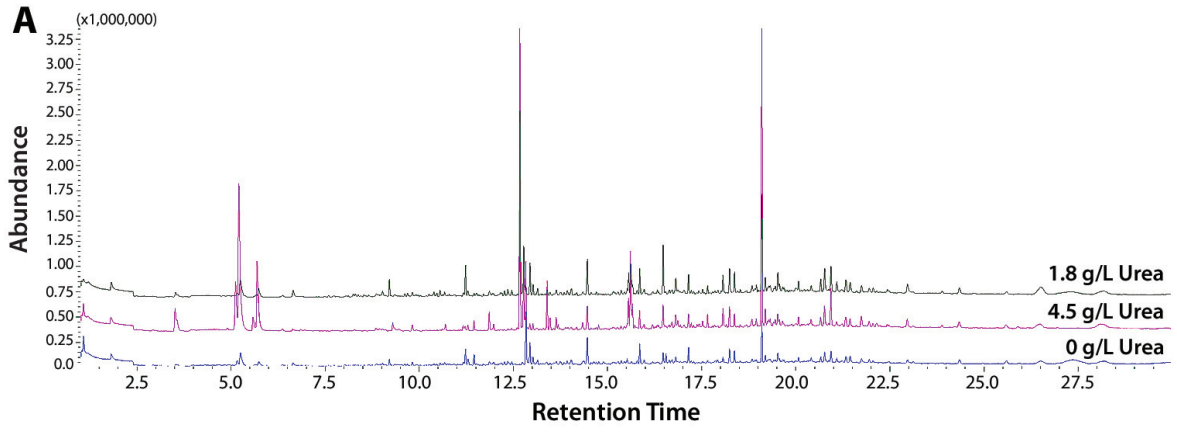
The analysis of VOCs was performed using Gas Chromatography-Mass Spectrometry (Shimadzu GC-MS QP2020) equipped with SH-Rxi-5Sil MS capillary column (30m x 0.25 mm x 0.25 µm). The samples were passed through a pre-installed SPME Inlet liner and Merlin Microseal Septa General Purpose Kit. The injection temperature was set to 250°C with an initial temperature of 50°C for 5 minutes and a ramping temperature of 1°C per minute until it reached 200°C with a constant helium gas flow rate of 1 mL/min. The mass spectra of the N treated samples were compared with the control and the distinct VOC mass spectra found were identified using the NIST 2017 Mass Spectral Library and Wiley Registry 11th edition (Tavera *et al.,* 2018).

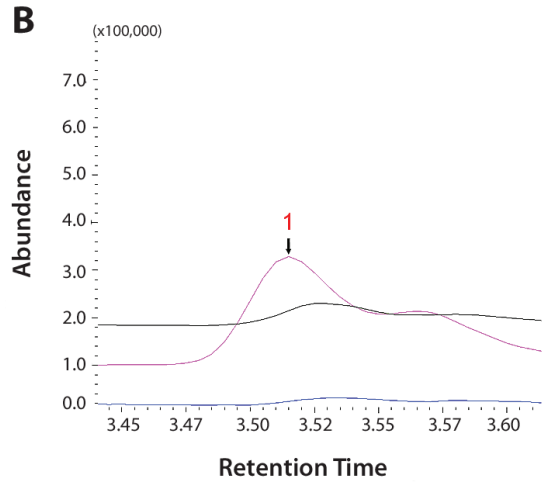
Results

The effect of high N-supply on the VOC profile of *C. annuum* var. *longum* is presented in Figure 1. After the four-day treatment, the plants which received urea higher than 1.8 g/L concentration started to exhibit curling and wrinkling of leaves. The plants treated with 5x N dried up within 48 h and were not included for further analysis. This type of response is typical in plants when urea is suddenly introduced. N-treated plants also developed black spots on the leaves which may be caused by a weakened innate defense system or N toxicity. Furthermore, aphids and whiteflies appeared on the N-treated plants four days after treatment. On the other hand, the untreated control plants showed normal growth and absence of insects, suggesting that the N supply may have caused the observed responses and the attraction of insects to N-treated plants. The sensitivity of *C. annuum* var. *longum* to N-treatment further suggests that this species is a very good candidate for N-stress studies.

Figure 1. Representatives of *C. annuum* var. *longum* treated with 0 g/L, 1.8g/L, 4.5 g/L and 9 g/L Urea after 48 h supply.

The VOCs collected from the leaves of N-treated setups (1x and 2.5x) and compared to those of the control (0x) are shown in Figure 2A-F. In general, the VOCs common in N-treated and untreated *C. annuum* var. *longum* belonged to the group of alkanes, sesquiterpenes, carboxylic acids, aldehydes, ketones, alcohols, and alkenes. Among these plant volatiles, Butanoic acid, 3-hexenyl ester [peak #6], (E)-, Butanoic acid, hexyl ester [peak #7], Hexanoic acid, 3-hexenyl ester, (Z)- [peak #8], Hexanoic acid, 4-hexen-1-yl ester [peak #9], cis-3-Hexenyl cis-3-hexenoate [peak #10], and 4-Pentenoic acid





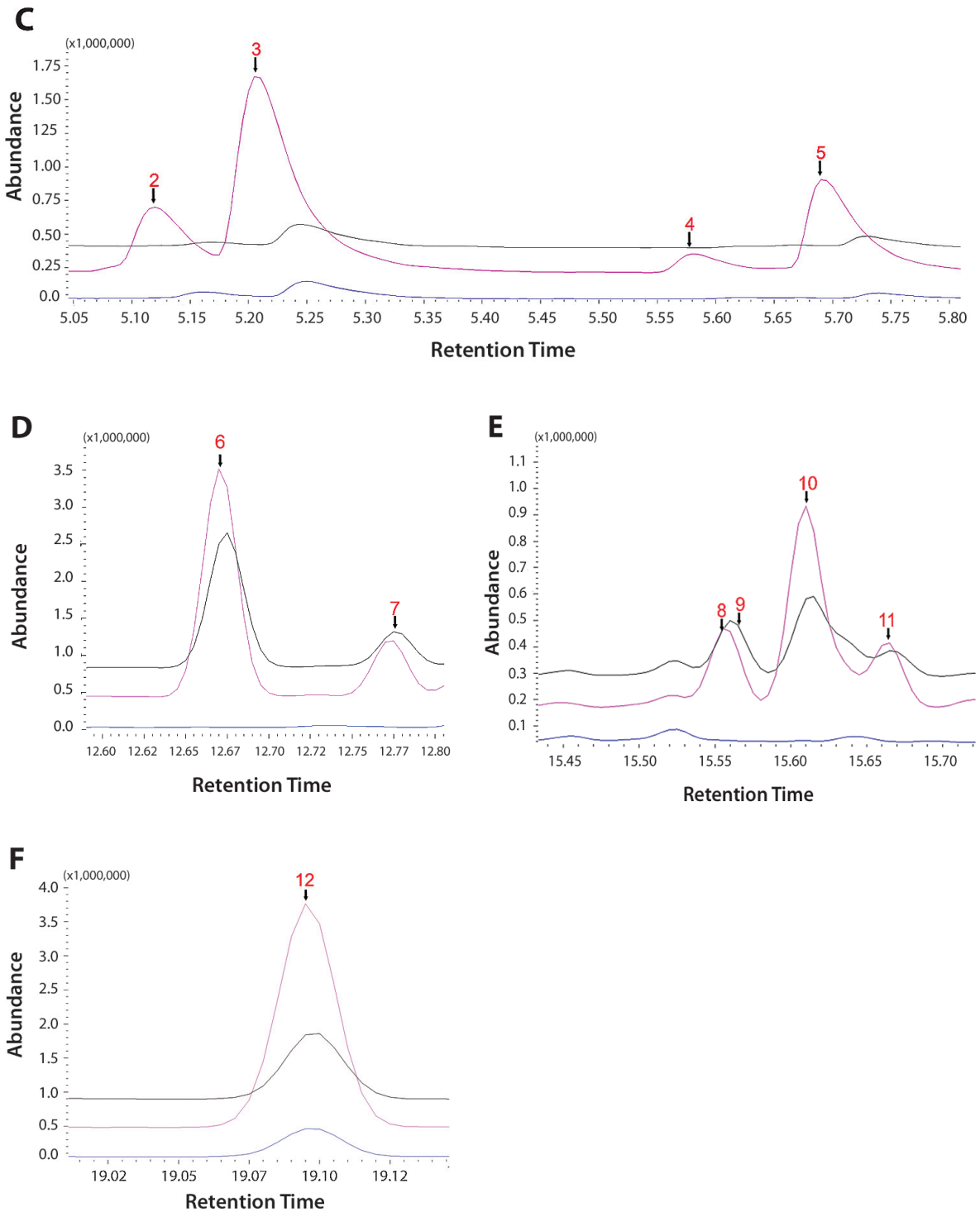


Figure 2. (A) GC-MS analysis of the volatile organic compounds identified from *C. annuum* var. *longum*. Magnified version of the chromatograms with retention times are shown in (B) 3.40 – 3.60 (C) 5.05 – 5.80 (D) 12.60 – 12.80 (E) 15.45 – 15.70 (F) 19.02 – 19.12. The detected compounds were 3-Hexenal [1, 3.527], 2-Hexenal [2, 5.120], 3-Hexen-1-ol, (Z)- [3, 5.207] and 2-Hexen-1-ol, (E)- [4, 5.581], 1-Hexanol [5, 5.692], Butanoic acid, 3-hexenyl ester [6, 12.776], (E)-, Butanoic acid, hexyl ester [7, 12.674], Hexanoic acid, 3-hexenyl ester, (Z)- [8, 15.557], Hexanoic acid, 4-hexen-1-yl ester [9, 15.561], cis-3-Hexenyl cis-3-hexenoate [10, 15.61315], and 4-Pentenoic acid 2-methyl-, hexyl ester [11, 15.666] and Octane, 1,1'-oxybis- [12, 19.096]. Samples for 1x, 2.5x and control are shown as black, purple and blue lines, respectively.

2-methyl-, hexyl ester [peak #11] were the ones detected from N-treated plants.

Other plant volatiles, such as 3-Hexenal [peak #1], 2-Hexenal [peak #2], 3-Hexen-1-ol, (Z)- [peak #3], 2-Hexen-1-ol, (E)- [peak #4] and 1-Hexanol [peak #5], which are part of the green leaf volatile synthesis were detected as shown in peak areas on the GC-MS analysis (Fig. 2A-F). The matching factor and structure of the plant volatiles are shown in Table 1. Among these volatiles, only 2-hexenal, 3-Hexen-1-ol, (Z)- and 1-Hexanol were identified from the control samples. The 3-Hexen-1-ol, (Z)- and 1-Hexanol identified from the treated samples have increased peak areas, whereas 2-hexenal only increased in plants treated with 2.5x urea. On the other hand, 3-hexenal was identified only from plants treated with 1x and 2.5x urea, whereas 2-Hexen-1-ol, (E)- was only identified in plants treated with 2.5x urea. Also, the peak area of Octane, 1,1'-oxybis- [peak #12] increased as N concentration increased. These results suggest that the VOCs detected were in response to the increased concentration of N. The characteristics of each VOCs, including the retention time, matching factor, and structure can be found in Table 1.

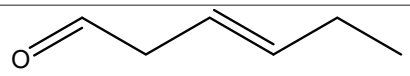
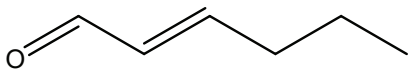
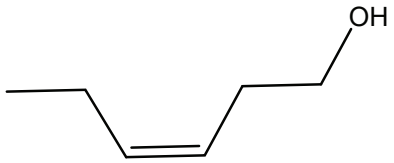
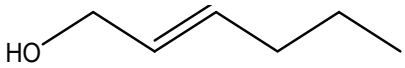
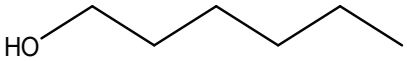
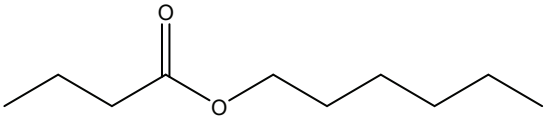
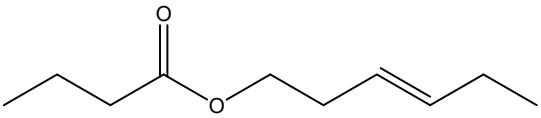
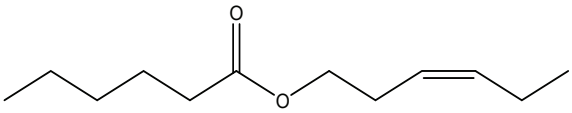
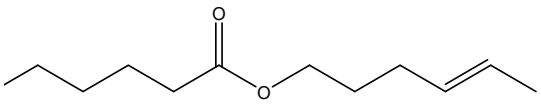
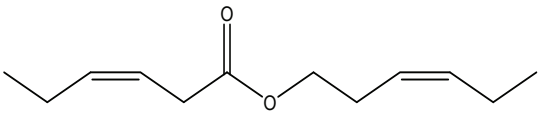
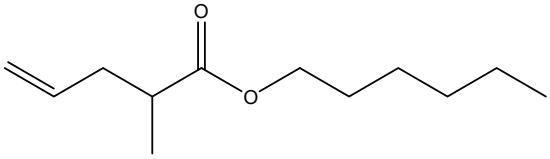
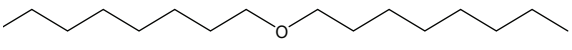
Discussion

In general, plants respond to changes in the concentration of exogenous nitrogen (N) present in soil from molecular up to the physiological level. These changes elicit an appropriate response that may benefit or harm the plants (Redillas *et al*., 2011; Wahocho *et al*., 2016; Jansson *et al.,* 1986; Jauset *et al.,* 1998; Lu *et al.,* 2007; Veromann *et al*., 2013; Islam *et al.,* 2017). The application of nitrogen in chili plants in an appropriate amount can affect the production of carbohydrates, protein, ash contents, fibers, fats, and nutrients. An increased nitrogen level (up to 250 Kg/ha Urea) in the cultivation of capsicum can improve the plant's height, number of branches, number of days before flowering, and length of fruits (Wahocho *et al.,* 2016). However, improper application of nitrogen may trigger negative effects on the plants. For instance, increased N in plants could increase the population of insects which often leads to insect infestation and herbivory (Jansson *et al.,* 1986; Jauset *et al.,* 1998; Lu *et al.,* 2007; Veromann *et al*., 2013; Islam *et al.,* 2017). In our study, we observed that whiteflies and aphids became attracted to chili plants when treated with N. The silverleaf whitefly (*Bemisia tabaci*) is one of the most common insect pests in chili plants. It serves as a vector to transmit *Begomovirus* which can hamper the growth and development of the plant (Firdaus *et al.,* 2011; Salas *et al.,* 2015; Saad *et al.,* 2015). Similarly, the green peach aphid (*Myzus persicae)* and the cotton aphid (*Aphis gossypii*) have been reported as pests and viral vectors in Chili plants (Talaga-Taquinas *et al.,* 2020). This attraction of insects to plants with higher nitrogen content has also been documented in other plants, such as potato and tomato plants (Jansson *et al.,* 1986; Jauset *et al.,* 1998; Islam *et al.,* 2017; Veromann *et al*., 2013). In tomato plants, the greenhouse whitefly (*Trialeurodes vaporarium*) has shown a high preference for plants with higher N content for egg-laying and oviposition compared to plants with lower N content (Jauset *et al.,* 1998). Green peach aphids infestation has also been detected in potato plants with high N content (Jansson *et al.,* 1986). Similarly, pollen beetles and cabbage seed weevils became more attracted to oilseed rape plants when high N concentration was introduced to the plant (Veromann *et al*., 2013).

The emergence of black spots on chili plants treated with 2.5x N has also been reported in case of oilseed rape plants exposed to high nitrogen concentration (Veromann *et al.,* 2013). The appearance of black spots may have been due to either a weakened innate defense system or N toxicity. However, further studies are required to confirm the cause of the emergence of these black spots on N-treated chili plants.

It is widely known that plants release VOCs as a response to abiotic stresses, to reduce their negative impact, and trigger

Table 1. Plant volatile organic compounds identified from *C. annum* var. *longum* treated with 1x and 2.5x urea. The retention time, matching factor and structure of the VOCs are shown in the figure.

Peak Number	Retention time (min)	Match Factor	Compound	Structure
1	3.527	95	3-Hexenal	
2	5.120	97	2-Hexenal	
3	5.207	97	3-Hexen-1-ol, (Z)-	
4	5.581	98	2-Hexen-1-ol, (E)-	
5	5.692	97	1-Hexanol	
6	12.674	97	Butanoic acid, hexyl ester	
7	12.776	96	Butanoic acid, 3-hexenyl ester, (E)-	
8	15.557	93	Hexanoic acid, 3-hexenyl ester, (Z)-	
9	15.561	93	Hexanoic acid, 4-hexen-1-yl ester	
10	15.613	97	cis-3-Hexenyl cis-3-hexenoate	
11	15.666	83	4-Pentenoic acid 2-methyl-, hexyl ester	
12	19.096	95	Octane, 1,1'-oxybis-	

plant-to-plant interactions i.e. water stress (Salerno *et al.,* 2017), drought stress (Hansen and Seufert, 1999), salt stress (Teuber *et al*., 2008), oxidative stress (Vuorinen *et al*., 2004), increased temperature (Maleknia *et al.,* 2009), light intensity (Hansen and Seufert, 2003), mechanical damage (Kirstine and Galbally, 1998; 2004), and nutrient deficiency (Gouinguene and Turlings, 2002). A range of plant volatiles has been identified in this study that may function in defense against a wide array of stresses i.e. Butanoic acid, 3-hexenyl ester, (E)-; Butanoic acid, hexyl ester; Hexanoic acid, 3-hexenyl ester, (Z)-; Hexanoic acid, 4-hexen-1-yl ester; cis-3-Hexenyl cis-3-hexenoate; and 4-Pentenoic acid 2-methyl-, hexyl ester. The different compounds detected in this study that have hexanoic acid-containing moiety may play an important role in the plant's defense system. Studies report that hexanoic acid is a priming inducer that triggers plants defense against fungi (Aranega-Bou *et al*., 2014; Finiti *et al*., 2014). For instance, treatment with hexanoic acid helps the plant to regulate oxidative stress caused by plant-pathogen interaction. This oxidative stress protection was previously reported in tomato plants infected with *Botrytis cinerea* (Finiti *et al*., 2014). The protective property of hexanoic acid was also reported to be effective against *Pseudomonas syringae* (Vicedo *et al*., 2009). Similarly, a total of 16 VOCs were detected from tomato plants treated with different concentration of N. These include an alkane (Farnesan), Sesquiterpene (α-Humulene, β-Caryophyllene, α-Cedrene, Longifolene, α-Copaene, d-Elemene), Alkyl aldehyde (Nonanal), Monoterpene (β-Phellandrene, p-Cymene, α-terpiene, (+)-4-carene, Myrcene, β-pinene, α-pinene), and Alkyl aldehyde (Heptanal) (Islam *et al.,* 2017). Among these VOCs, the nonanal and p-cymene were also detected in chili plants, however, both were detected on treated and untreated plants.

The green leaf volatiles (GLV) with an increased peak in the N- treated capsicum plants were 2-Hexenal, 1-Hexanol, and 3-Hexen-1-ol, (Z)-. The detection of the 3-Hexenal, 2-Hexenal, 3-Hexen-1-ol, (Z)- and 2-Hexen-1-ol, (E)- suggest that the GLV synthesis is active in the N-treated plant. The synthesis of GLV begins when galactolipids and phospholipids in the thylakoid membranes are digested with lipase enzymes releasing linoleic acid; the oxidation of a diene catalyzed by the 13-Lipoxygenase produces 13-hydroperoxide, which will then undergo further oxidation to produce (Z)-3-Hexenal or n-Hexenal through isomerization; when treated with aldehyde reductase, aldo/keto reductase or alcohol dehydrogenase 3-Hexen-1-ol, (Z)- and 2-Hexen-1-ol, (E)- can be produced, respectively (Kunishima *et al*,. 2016). The emission of (Z)-3-Hexen-1-ol in plants can have different functions such as the enhancement of defense against insect pests e.g. the (Z)-3-Hexen-1-ol function to elicit a defense system in tea plants, *Camellia sinensis,* against the attack by the tea geometrid *Ectropis oblique* by increasing VOC emission and the activity of polyphenol oxidase. In return, the release of VOCs attracted a parasitoid of the tea geometrid (Xin *et al.,* 2016). The (Z)-3-Hexen-1-ol is also known to attract other beneficial insects, such as *Stethorus p. picipes, Orius tristicolor, Anagrus daanei,* Syrphidae, Braconidae, and Micro-Hymenoptera (James, 2005). Similarly, the detected 4-Pentenoic acid, 2-methyl-, hexylester, in plant treated with nitrogen, initiates plants' mechanism for insect control (Janzantti *et al*., 2012; Tewari *et al*., 2014). On the other hand, the detected hexan-1-ol was previously observed to have a repellent effect on *Ceutorhynchus assimilis* (Smart and Blight, 1997). Nevertheless, the GLVs detected in this study suggest the presence of compounds that establish an insect-plant relationship (Smart and Blight, 1997; James, 2005; Xin *et al.,* 2016; Kunishima *et al*,. 2016).

In conclusion, excessive concentration of nitrogen weakens the overall plant health making it more susceptible to herbivory and infestation (Islam *et al.,* 2017; Veromann *et al.,* 2013). In this study, we have identified a range of volatiles that may function in defense against a wide array of stresses. The majority of the detected volatiles were pro-

duced by plants in response to nitrogen-induced insect infestation and the presence of black spots. Although these volatiles have been reported in previous studies, this is the first study that reports their involvement in *C. annuum* when exposed to increased nitrogen conditions. We believe that the results obtained from this study will contribute in understanding the mechanisms behind volatile emission in *C. annuum* var. *longum* in response to N-nutrition. Also, the profile of plant volatiles is useful especially in the field of agriculture to avoid infestation and disease outbreak.

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Literature Cited

Aliyu, L. 2000. Effect of organic and mineral fertilizers on growth, yield, and composition of pepper (*Capsicum annuum* L.). *Biological Agriculture and Horticulture*, 18(1): 29-36.

Anitha, S. and Geethakumari, V.L. 2006. Nutrient management in chilli (*Capsicum annuum* L.) based cropping system. *Indian Journal of Crop Science*, 1(1 and 2): 209-210.

Aranega-Bou, P., de la O Leyva, M., Finiti, I., García-Agustín, P. and González-Bosch, C. 2014. Priming of plant resistance by natural compounds. Hexanoic acid as a model. *Frontiers in Plant Science*, 5: 488.

Bargavi, R. and Elumalai, S. 2010. *In vitro* clonal propogation of *Capsicum annum* L. var.(Arka lohit and Arka suphal) for fruit borer disease resistance. *Biosciences, Biotechnology Research Asia,* 7(1): 281-8.

Cirlini, M., Luzzini, G., Morini, E., Folloni, S., Ranieri, R., Dall'Asta, C. and Galaverna, G., 2019. Evaluation of the volatile fraction, pungency and extractable color of different Italian *Capsicum annuum* cultivars designed for food industry. *European Food Research and Technology*, 245(12): 2669-2678.

Effah, E., Holopainen, J.K. and McCormick, A.C. 2019. Potential roles of volatile organic compounds in plant competition. *Perspectives in Plant Ecology, Evolution and Systematics*, 38: 58-63.

Eggink, P.M., Maliepaard, C., Tikunov, Y., Haanstra, J.P.W., Bovy, A.G. and Visser, R.G.F., 2012b. A taste of sweet pepper: Volatile and non-volatile chemical composition of fresh sweet pepper (*Capsicum annuum*) in relation to sensory evaluation of taste. *Food Chemistry*, 132(1): 301-310.

Eggink, P.M., Maliepaard, C., Tikunov, Y., Haanstra, J.P.W., Pohu-Flament, L.M.M., de Wit-Maljaars, S.C., Willeboordse-Vos, F., Bos, S., Benning-de Waard, C., De Grauw-van Leeuwen, P.J. and Freymark, G., 2012a. Prediction of sweet pepper (*Capsicum annuum*) flavor over different harvests. *Euphytica*, 187(1): 117-131.

Finiti, I., de la O Leyva, M., Vicedo, B., Gómez-Pastor, R., López-Cruz, J., García-Agustín, P., Real, M.D. and González-Bosch, C. 2014. Hexanoic acid protects tomato plants against *Botrytis cinerea* by priming defense responses and reducing oxidative stress. *Molecular Plant Pathology*, *15*(6): 550-562.

Firdaus, S., Van Heusden, A., Harpenas, A., Supena, E. D., Visser, R.G., and Vosman, B. 2011. Identification of silverleaf whitefly resistance in pepper. *Plant Breeding*, 130(6): 708-714.

Gaytán, V.G., Merino, F.C., Téllez, L.I., Castillo, G. and Morales, S.G. 2017. The Chilhuacle Chili (*Capsicum annuum* L.) in Mexico: Description of the Variety, Its Cultivation, and Uses*, International Journal of Agronomy*, v. 2017, Article ID 5641680

Gou, W., Zheng, P., Tian, L., Gao, M., Zhang, L., Akram, N.A. and Ashraf, M. 2017. Exogenous application of urea and a urease inhibitor improves drought stress tolerance in maize (*Zea mays* L.). *Journal of Plant Research,* 130(3): 599-609.

Gouinguené, S.P. and Turlings, T.C. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, 129(3): 1296-1307.

Hansen, U. and Seufert, G. 1999. Terpenoid emission from *Citrus sinensis* (L.) OSBECK under drought stress. *Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere*, 24(6): 681-687.

Hansen, U. and Seufert, G. 2003. Temperature and light dependence of β-caryophyllene emission rates. *Journal of Geophysical Research: Atmospheres*, 108(D24).

Heiser Jr, C.B., and Pickersgill, B. 1969. Names for the Cultivated Capsicum Species (Solanaceae). *Taxon*, 18 (3): 277–283.

Hiltpold, I., Toepfer, S., Kuhlmann, U. and Turlings, T.C. 2010. How maize root volatiles affect the efficacy of entomopathogenic nematodes in controlling the western corn rootworm? *Chemoecology,* 20(2): 155-162.

Holopainen, J.K., and Gershenzon, J. 2010. Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, 15(3): 176-184.

Islam, M., Saha, S., Akand, H. and Rahim, A. 2011. Effect of spacing on the growth and yield of sweet pepper (*Capsicum annuum* L.). *Journal of Central European Agriculture,* 12(2): 328-335.

Islam, M.N., Hasanuzzaman, A.T.M., Zhang, Z.F., Zhang, Y. and Liu, T.X. 2017. High Level of Nitrogen Makes Tomato Plants Releasing Less Volatiles and Attracting More *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Frontiers in Plant Science*, 8: 466.

James, D. G. 2005. Further field evaluation of synthetic herbivore-induced plan volatiles as attractants for beneficial insects. *Journal of Chemical Ecology*, 31(3): 481-495.

Jansson, R. K., and Smilowitz, Z. 1986. Influence of nitrogen on population parameters of potato insects: abundance, population growth, and within-plant distribution of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae). *Environmental Entomology*, *15*(1): 49-55.

Janzantti, N., Macoris, M., Garruti, D. and Monteiro, M. 2012. Influence of the cultivation system in the aroma of the volatile compounds and total antioxidant activity of passion fruit, *LWT - Food Science and Technology*, 46: 511-518

Jassbi, A.R., Zamanizadehnajari, S. and Baldwin, I.T. 2010 Phytotoxic volatiles in the roots and shoots of *Artemisia tridentata* as detected by headspace solid-phase microextraction and gas chromatographic-mass spectrometry analysis. *Journal of Chemical Ecology*, 36(12): 1398-1407.

Jauset, A.M., Sarasua, M.J., Avilla, J., and Albajes, R. 1998. The impact of nitrogen fertilization of tomato on feeding site selection and oviposition by Trialeurodes vaporariorum. *Entomologia Experimentalis et Applicata*, 86(2): 175-182.

Kirstine, W.V. and Galbally, I.E. 2004. A Simple Model for Estimating Emissions of Volatile Organic Compounds from Grass and Cut Grass in Urban Airsheds and Its Application to Two Australian Cities, *Journal of the Air and Waste Management Association,* 54(10): 1299-1311.

Kirstine, W.V., Galbally, I.E., Ye, Y. and Hooper, M. 1998. Emissions of volatile organic compounds (primarily oxygenated species) from pasture, *Journal of Geophysical Research*, 103: 10605-10619.

Kreuzwieser, J., Scheerer, U., Kruse, J., Burzlaff, T., Honsel, A., Alfarraj, S., Georgiev, P., Schnitzler, J.P., Ghirardo, A., Kreuzer, I. and Hedrich, R. 2014. The Venus flytrap attracts insects by the release of volatile organic compounds. *Journal of Experimental Botany*, 65(2): 755-766.

Kunishima, M., Yamauchi, Y., Mizutani, M., Kuse, M., Takikawa, H. and Sugimoto, Y. 2016. Identification of (Z)-3:(E)-2-hexenal isomerases essential to the production of the leaf aldehyde in plants. *Journal of Biological Chemistry*, 291(27): 14023-14033.

Lou, Y. and Baldwin, I.T. 2004. Nitrogen supply influences herbivore-induced direct and indirect defenses and transcriptional responses in *Nicotiana attenuata*. *Plant Physiology*, 135(1): 496-506.

Lu, Z.X., Yu, X.P., Heong, K.L., and Cui, H.U. 2007. Effect of nitrogen fertilizer on herbivores and its stimulation to major insect pests in rice. *Rice Science*, 14(1): 56-66.

Maleknia, S.D., Vail, T.M., Cody, R.B., Sparkman, D.O., Bell, T.L. and Adams, M.A., 2009. Temperature-dependent release of volatile organic compounds of eucalypts by direct analysis in real time (DART) mass spectrometry. *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, 23(15): 2241-2246.

McBride, M.F. 2016. Extraction of resins from *Capsicum annuum* var. *longum* (Siling haba) for the study of their potential anti-microbial activities. *Journal of Chemical and Pharmaceutical Research,* 8(3): 117-127.

Molla, T., Abera, G. and Beyene, S. 2019. Effects of Nitrogen Fertilizer and Mulch Application on Growth Performance and Pod Yields of Hot Pepper (*Capsicum annuum* L.) under Irrigated Condition. *International Journal of Plant and Soil Science*, 27(5): 1-15.

Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejicek, A., Strbik, F., Philippot, L. and Mougel, C. 2015. Plant traits related to nitrogen uptake influence plant-microbe competition. *Ecology*, 96(8): 2300-2310.

Perera, R.M., Marriott, P.J., Galbally, I.E. 2002. Headspace solid-phase microextraction—Comprehensive two-dimensional gas chromatography of wound induced plant volatile organic compound emissions. *Analyst*, 127(12): 1601-1607.

Redillas, M.C.F.R., Jeong, J.S., Strasser, R.J., Kim, Y.S., and Kim, J.K. 2011. JIP analysis on rice (*Oryza sativa* cv Nipponbare) grown under limited nitrogen conditions. *Journal of the Korean Society for Applied Biological Chemistry*, 54(5): 827-832.

Saad, K.A., Roff, M.M., Hallett, R.H., and Idris, A.B. 2015. Aphid-induced defences in chilli affect preferences of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Scientific Reports*, *5*(1): 1-9.

Salas, R.A., Gonzaga, Z.C., Wu, D.L., Luther, G., Gniffke, P.A., and Palada, M.C. 2015. Effects of physical barrier and insect growth regulator on whitefly control and yield of chili pepper (*Capsicum annuum* L.). *Journal of Food and Nutrition Sciences*, *3*(1-2): 13-19.

Salerno, G., Frati, F., Marino, G., Ederli, L., Pasqualini, S., Loreto, F., Colazza, S. and Centritto, M. 2017. Effects of water stress on emission of volatile organic compounds by *Vicia faba*, and consequences for attraction of the egg parasitoid

Trissolcus basalis. *Journal of Pest Science*, 90(2): 635-647.

Samburova, V., McDaniel, M., Campbell, D., Wolf, M., Stockwell, W.R. and Khlystov, A. 2019. Dominant volatile organic compounds (VOCs) measured at four Cannabis growing facilities: pilot study results. *Journal of the Air and Waste Management Association*, 69(11): 1267-1276.

Sharma, R. and Kumar, R. 2017. Growth, flowering and yield of chilli,(*Capsicum annuum* L.) as influenced by spacing and growing conditions. *Indian Journal of Pure and Applied Biosciences*, 5(5): 524-527.

Silva, É.A.S., Saboia, G., Jorge, N.C., Hoffmann, C., dos Santos Isaias, R.M., Soares, G.L. and Zini, C.A. 2017. Development of a HS-SPME-GC/MS protocol assisted by chemometric tools to study herbivore-induced volatiles in *Myrcia splendens*. *Talanta*, 175: 9-20.

Silva, L.R., Azevedo, J., Pereira, M.J., Valentão, P. and Andrade, P.B. 2013. Chemical assessment and antioxidant capacity of pepper (*Capsicum annuum* L.) seeds. *Food and Chemical Toxicology*, 53: 240-248.

Smart, L.E., and Blight, M.M. 1997. Field discrimination of oilseed rape, *Brassica napus* volatiles by cabbage seed weevil, Ceutorhynchus assimilis. *Journal of Chemical Ecology*, 23(11): 2555-2567.

Spinelli, F., Cellini, A., Marchetti, L., Nagesh, K.M. and Piovence, C. 2011. Emission and Function of Volatile Organic Compounds in Response to Abiotic Stress. *Department of Fruit Tree and Woody Plant Sciences*, 16: 367-393.

Tálaga-Taquinas, W., Melo-Cerón, C. I., Lagos-Álvarez, Y. B., Duque-Gamboa, D. N., Toro-Perea, N. and Manzano, M. R. 2020. Identification and life history of aphids associated with chili pepper crops in southwestern Colombia. *Universitas Scientiarum*, 25(2): 175-200.

Tavera, M.A.A., Lago, J.C.A., Magalong, V.K.D., Vidamo, G.A.V., Carandang, J.S.R., Amalin, D.M., Janairo, J.I.B. 2018. Effect of *Aspidiotus rigidus* infestation on the volatile chemical profile of the host plant *Garcinia mangostana*. *Hellenic Plant Protection Journal*, 11(1): 1-8.

Teuber, M., Zimmer, I., Kreuzwieser, J., Ache, P., Polle, A., Rennenberg, H. and Schnitzler, J.P. 2008. VOC emissions of Grey poplar leaves as affected by salt stress and different N sources. *Plant Biology*, 10(1): 86-96.

Tewari, S., Leskey, T.C., Nielsen, A.L., Piñero, J.C., and Rodriguez-Saona, C.R. 2014. Use of pheromones in insect pest management, with special attention to weevil pheromones. In *Integrated Pest Management* (pp. 141-168). Academic Press.

Veromann, E., Toome, M., Kännaste, A., Kaasik, R., Copolovici, L., Flink, J., Kovács, G., Narits, L., Luik, A. and Niinemets, Ü. 2013. Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. *Crop Protection*. 43: 79-88.

Vicedo, B., Flors, V., de la O Leyva, M., Finiti, I., Kravchuk, Z., Real, M.D., García-Agustín, P. and González-Bosch, C. 2009. Hexanoic acid-induced resistance against Botrytis cinerea in tomato plants. *Molecular Plant-Microbe Interactions*, 22(11):1455-1465.

Vuorinen, T., Nerg, A.M., Holopainen, J.K. 2004. Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signalling. *Environmental Pollution,* 131(2): 305-311.

Wahocho, N.A., Zeshan Ahmed, S., Jogi, Q., Talpur, K.H. and Leghari, S.J. 2016. Growth and productivity of chilli (*Capsicum annuum* l.) under various nitrogen levels. *Science International*, 28(2): 1321-1326.

Walsh, B.M. and Hoot, S.B. 2001. Phylogenetic Relationships of *Capsicum* (Solanaceae) Using DNA Sequences from Two Noncoding Regions: The Chloroplast atpB-rbcL Spacer Region and Nuclear waxy Introns. *International Journal of Plant Sciences*, 162(6): 1409–1418.

Xin, Z., Li, X., Li, J., Chen, Z. and Sun, X. 2016 Application of chemical elicitor (Z)-3-hexenol enhances direct and indirect plant defenses against tea geometrid *Ectropis obliqua*. *BioControl*, 61(1): 1-12.

Zhigila, D.A., Abdul Rahaman, A.A., Kolawole, O.S. and Oladele, F. 2014. Fruit Morphology as Taxonomic Features in Five Varieties of *Capsicum annuum* L. Solanaceae, *Journal of Botany*, v. 2014, Article ID 540868.

Ziino, M., Condurso, C., Romeo, V., Tripodi, G. and Verzera, A. 2009. Volatile compounds and capsaicinoid content of fresh hot peppers (*Capsicum annuum* L.) of different Calabrian varieties. *Journal of the Science of Food and Agriculture*, 89(5): 774-780.

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Προσδιορισμός πτητικών οργανικών ενώσεων σε φυτά *Capsicum annuum* var. *longum* που αναπτύσσονται κάτω από διαφορετικές συγκεντρώσεις αζώτου

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Περίληψη Η έκλυση πτητικών οργανικών ενώσεων (VOC) στα φυτά ενεργοποιείται από διάφορους βιοτικούς και αβιοτικούς παράγοντες, όπως η ανεπάρκεια σε θρεπτικά στοιχεία, η περιβαλλοντική καταπόνηση και οι προσβολές από παθογόνα και εχθρούς. Για παράδειγμα, η ελλειπής ή υπερβολική παροχή αζώτου (N) στα φυτά είναι δυνατό να προκαλέσει εσωτερική καταπόνηση που θα μειώσει τη σταθερότητα και την ανοσία τους καθιστώντας τα ευπαθή σε προσβολές από παθογόνα και εχθρούς. Στην παρούσα εργασία πραγματοποιήθηκε εκχύλιση των πτητικών οργανικών ενώσεων από φυτά Capsicum annuum var. longum (Solanaceae) μετά από εφαρμογή αζώτου (1,8 g/L, 4,5 g/L, και 9 g/L ουρία) με τη χρήση 100 μm ίνας Στερεάς Φάσης Μικροεκχύλισης (Solid Phase Microextraction, SPME) επικαλυμμένης με πολυδιμεθυλοσιλοξάνιο (PDMS). Με τη χρήση αέριας χρωματογραφίας-φασματομετρίας μάζας (GC-MS) οι πτητικές ενώσεις, που εκλύθηκαν από τα φυτά στα οποία είχε γίνει εφαρμογή αζώτου, προσδιορίστηκαν ως 3-εξενυλεστέρας του βουτανοϊκού οξέος, (E)-εξυλεστέρας του βουτανοϊκού οξέος, (Z)-3-εξενυλ εστέρας του εξανοϊκού οξέος, 4-εξεν-1-υλεστέρας του εξανοϊκού οξέος, cis-3-εξενυλ-cis-3-εξενοϊκός εστέρας, και εξυλεστέρας του 2-μεθυλ-4-πεντενοϊκού οξέος. Μεταξύ αυτών των πτητικών ενώσεων, ο 3-εξενυλεστέρας του βουτανοϊκού οξέος έδειξε την πιο χαρακτηριστική κορυφή (peak) στα φυτά που είχαν δεχθεί επέμβαση με άζωτο σε σύγκριση με το μάρτυρα. Επιπλέον, στα φυτά αυτά ανιχνεύθηκαν τα πτητικά πράσινων φύλλων (Green Leaf Volatiles, GLV) 3-εξενάλη, 2-εξανάλη, (Z)-3-εξεν-1-όλη, (E)-2-εξεν-1-όλη, και 1-εξανόλη. Ο προσδιορισμός των πτητικών ουσιών στα φυτά παρέχει χρήσιμες πληροφορίες που μπορούν να αξιοποιηθούν σε γεωργικές πρακτικές και στη φαινοτύπιση των φυτών.

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Lethal and sublethal effects of several plant compounds compared to spiromesifen against *Tetranychus turkestani*

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Summary *Tetranychus turkestani* Ugarov and Nikolski is one of the main agricultural pests of southwestern Iran and some other tropical regions. In the present study, fumigant activity of three essential oils extracted from *Rosmarinus officinalis* L., *Mentha longifolia* L. and *Eucalyptus globulus* Labill., and contact toxicity of two biopesticides (Tondexir and Palizin) on *T. turkestani* mite females were investigated and compared with that of spiromesifen, a chemical acaricide. Also, sublethal effects of the tested compounds at 50% lethal concentration (LC_{50}) were estimated on the biological parameters of *T. turkestani*. The LC_{50} values for *E. globulus*, *R. officinalis* and *M. longifolia* essential oils were 12.50, 11.52 and 4.00 µl/l air and for spiromesifen, Tondexir and Palizin were 10.98, 327.34 and 858.13 ppm, respectively. All tested compounds significantly reduced adult female longevity, equally to the chemical acaricide spiromesifen. Fecundity also decreased in all treatments and this reduction was even higher for plant essential oils than the other compounds. Palizin, *E. globulus* and *M. longifolia* significantly reduced the hatchability of *T. turkestani* eggs similarly to spiromesifen. According to the results, the tested plant compounds are effective against *T. turkestani* and may be applied as suitable alternatives to synthetic pesticides against this crop pest.

Additional keywords: biological parameters, biopesticide, Palizin, spider mite, Tondexir

Introduction

Tetranychus turkestani Ugarov and Nikolski, strawberry spider mite, (Acari: Tetranychidae) is a severe pest in some parts of tropical regions and southwestern Iran (Zhang, 2003; Karami-Jamour and Shishehbor, 2012). It is a polyphagous pest and has been reported from 270 host plants worldwide (Jeppson *et al.,* 1975; Migeon and Dorkeld, 2006-2013). The mite feeds in a piercing-sucking manner on the underside of leaves, covers leaves by web, thus destroying plant cells and tissue. The feeding causes yellow chlorotic spots on leaves and decreases photosynthetic rate of plants (Martinez-Ferrer *et al.,* 2006; Mohammadi *et al.,* 2015).

In Iran, synthetic acaricides are mainly applied against spider mites in various crops (Nikpay *et al.,* 2016; Morteza *et al.,* 2017; Ziaee *et al.,* 2017). However, high propagation potential, short generation time, and arrhenotokous parthenogenesis, combined with repeated acaricide use, led to the *build-up of* resistance among mite populations. Finding alternative control methods that are sustainable and environmentally friendly is necessary (Lee *et al.,* 2003; Van Leeuwen *et al.,* 2005; Pree *et al.,* 2005; Osakabe *et al.,* 2009).

Plant essential oils can be considered as an alternative for controlling spider mites, due to their compatibility with biological control agents and shorter residual effect, while they are safer to humans (Chiasson *et al.,* 2004; Hincapié *et al.,* 2008; Hussein *et al,* 2013). As a result, the application of botanical pesticides and their natural compounds has been considered for pest control in recent years (Isman, 2000). *Rosmarinus officinalis* L., *Mentha longifolia* L. (both Lamiaceae) and *Eucalyptus globulus* Labill. (Myrtaceae) are considered as three of the most valuable aromatic and medicinal plants. The lethal and sublethal impact of essential oils extracted

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from these plants have been demonstrated on tetranychid mites in the past. Miresmailli *et al*. (2006) and Choi *et al*. (2004) pointed out that *R. officinalis* essential oils was effective against the phytophagous mite *Tetranychus urticae* Koch. Motazedian *et al*. (2012) stated that the essential oil of *M. longifolia* possesses a repellent effect against *T. urticae*. The results of repellent, antifeedent and toxic effects of *E. globulus* essential oil on *T. urticae* showed that *E. globulus* essential oil can be considered as a natural agent against *T. urticae* (Hussein *et al.,* 2013).

Botanical pesticides have also been reported as a source of bioacaricides for the control of tetranychid mites (Azaizeh *et al.,* 2007; Nikpay *et al.,* 2016). Tondexir contains pepper extract (TON) and Palizin is coconut extract (PAL). They are two botanical pesticides recently used in Iran against various pests, including spider mites (Kabiri *et al.,* 2013; Honarmand *et al.,* 2016; Mirfakhraie and Mohamadian, 2017; Sohrabi *et al.,* 2019).

Evaluation of lethal and sublethal effects of any pesticide on spider mites is essential for assessing the toxicity of the pesticide and probability of resistance development in the mite population (Stark and Banks, 2003; Mohammadi *et al.,* 2016). Therefore, in this study, the lethal and sublethal effects of several plant compounds including *R. officinalis*, *M. longifolia*, and *E. globulus* essential oils and two biopesticides (Tondexir and Palizin) were evaluated against *T. turkestani* females and compared with that of Spiromesifen, a commercial chemical acaricide.

Materials and Methods

Mite culture

Tetranychus turkestani population used for the bioassays was reared in the laboratory on cowpea, *Vigna unguiculata* (L.) Walp. at 27±1°C, 65±5% R.H. and a 16:8 L:D photoperiod.

Test compounds

Rosmarinus officinalis, *M. longifolia*, and *E. globulus* essential oils of 100% purity were tested which were provided by the Essential Herbal Pharmaceutical Company (Golestan, Iran). The bioacaricides used were Palizin SL 65% (PAL) and Tondexir EC 85% (TON), provided by Kimia Sabzavar Co., Iran. Spiromesifen, as commercial formulation Oberon® (SC 24%; Bayer CropScience), was purchased from Giah Bazr Alvand Company, Iran.

Bioassay tests

Fumigant toxicity of R. officinalis, M. longifolia, and E. globulus essential oils on adult females of T. turkestani.

The fumigant toxicity of the essential oils was assessed in plastic Petri dishes (6 cm diameter, which offers 75 ml air space). Leaf discs of bean plants (3 cm in diameter) were placed with their dorsal side inside the Petri dishes on wet cotton. Ten adult females of mite were separately placed on the leaf plant discs using a soft paintbrush. Filter paper disks (Whatman No. 1,2 cm in diameter) were treated with concentrations of the essential oils and placed in the Petri dishes. Five concentrations containing 1.33, 6.67, 13.33, 33.33, 66.67 µl/l air of *R. officinalis* and *E. globulus*, and 0.13, 1.33, 6.67, 13.33, 26.67 µl/l air of *M. longifolia* were used for the experiments with three replications, based on preliminary experiments and acetone was applied as a solvent. The caps of the Petri dishes were covered with parafilm to hinder the release of essential oils. In the control, filter papers were treated with acetone alone. The mortality was estimated using a stereomicroscope after 24 h of exposure. Mites were considered as dead, when no reaction was observed after fine paintbrush stimulation. The bioassays were conducted at 27±1°C, 65±5% R.H. and a 16:8 L:D photoperiod.

Contact toxicity of Tondexir, Palizin, and spiromesifen acaridides against adult females of T. turkestani

Based on preliminary experiments, five concentrations containing 200, 320, 500, 900, 1500 ppm for TON and 100, 250, 500, 1000, 2500 ppm for PAL, and 10, 100, 250,

500, 1000 ppm in case of spiromesifen were used against the adult females of mites. Leaf discs of bean (3 cm diameter) were immersed in acaricide concentrations for 10 seconds and distilled water served as the control treatment. They were subsequently dried at room temperature for 30 min, then transferred in Petri dishes (6 cm in diameter) on wet cotton. Three replications were used for each concentration. Then, ten adult females were introduced into each Petri dish, ventilated through a hole of 1-cm diameter that was covered with organza net. Experiments were conducted at 27±1°C, 65±5% R.H. and a photoperiod of 16:8 L:D in a growth chamber. Mortality was determined under a stereomicroscope after 24 h of exposure.

Sublethal effects

Sublethal effects of the six tested compounds at 50% lethal concentration (LC_{50}) on biological parameters of *T. turkestani,* including adult longevity, fecundity and hatchability were studied. About 100 adult females (<24 h old) were exposed to LC_{50} of each essential oil (12.50, 11.52 and 4.00 µl/l for *E. globulus*, *R. officinalis,* and *M. longifolia*, respectively) and acaricide (10.98, 327.34 and 858.13 ppm for spiromesifen, TON and PAL, respectively) using the bioassays described above. Untreated mites were used as control. After 24 h, 20 alive *T. turkestani* females were randomly selected for each treatment and transferred individually to new untreated leaves inside Petri dishes on water-soaked cotton. Leaf discs were provided daily for each female mite (Li *et al.,* 2017). Then the adult longevity, oviposition and egg hatch were estimated. To study each compound's sublethal effects on the hatching rate, 30 eggs in six replicates were randomly selected and incubated under the conditions described above for hatching. Experiments were conducted at 27±1°C, 65±5% R.H. and a photoperiod of 16:8 L:D in a growth chamber.

Data analysis

Lethal concentration value (LC_{50}) and its corresponding 95% fiducial limits (FL) for each essential oil and pesticides were estimated using Polo-PC software. Lethal concentration ratios were performed to estimate the compounds' relative potency as described by Robertson *et al*. (2007). The log- probity curves were created using Microsoft Excel 2007. Data from the sublethal bioassays were analyzed using analysis of variance (ANOVA). The Duncan's Multiple Range Test separated means at 0.05 probability (SAS Institute, 2003). The percentage data were transformed to the square root of arcsine, and longevity data were transformed to square-root, before analysis.

Results

Fumigant toxicity of essential oils

The toxicity of essential oils on *T. turkestani* adults 24 h after exposure significantly increased with increasing concentrations (for *E. globulus*: $F = 14.69$; d.f.= 4, 10; $p = 0.0003$; *R. officinalis*: $F = 22.71$; d.f. = 4, 10; $p < .0001$; and for *M. longifolia*: $F = 5.68$; d.f. = 4, 10; $p = 0.0119$) (Figure 1). LC_{50} values of the tested essential oils are presented in Table 1. According to the LC_{50} values, *M. longifolia* was more effective than *E. globulus* and *R. officinalis* against *T. turkestani* adults (Table 1).

Contact toxicity of bioacaricides

The toxicity of acaricides significantly increased when *T. turkestani* adults were exposed to increasing concentrations of the tested compounds for 24 h (for Tondexir: $F = 12.98$; d.f.= 4, 10; $p = 0.0006$; Palizin: $F = 5.58$; d.f. = 4, 10; $p = 0.0126$; and for Spiromesifen: $F = 35.50$; d.f. = 4, 10; $p < .0001$) (Figure 2). The LC_{50} values of the three acaricides tested are presented in Table 2. These values and the confidence limits of toxicity ratios revealed that these acaricides' toxicity was significantly different for *T. turkestani* adults. The chemical acaricide Spiromesifen was significantly more effective than the two bioacaricides Tondexir and Palizin against *T. turkestani* adults (Table 2).

Sublethal effects

The sublethal effects of different com-

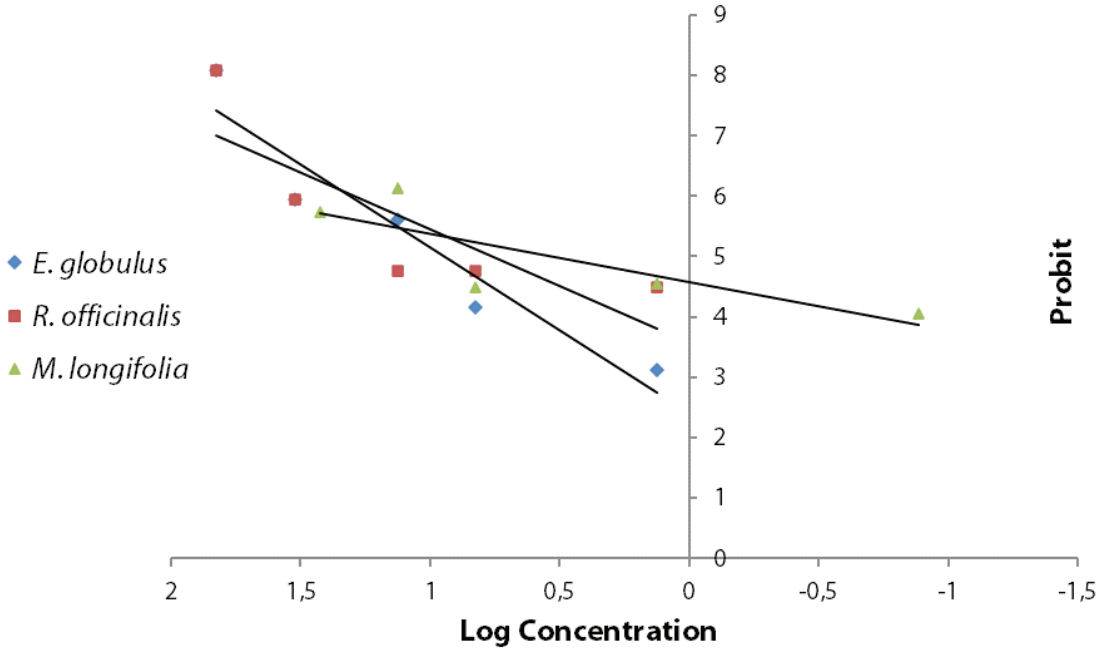

Figure 1. Log- Probity curve of plant essential oils of *Eucalyptus globulus, Rosmarinus officinalis* and *Mentha longifolia* on the strawberry spider mite *Tetranychus turkestani*.

Table 1. Probit analysis and lethal concentration ratios (% 95 fiducial limits) of *Eucalyptus globulus*, *Rosmarinus officinalis* and *Mentha longifolia* essential oils against the adult females of *Tetranychus turkestani*.

Parameters	Essential oil		
	E. globulus	*M. longifolia*	*R. officinalis*
Slope ± SE	3.01 ± 0.71	0.79 ± 0.27	1.82 ± 0.60
LC_{50} (95%FL) (µl/l air)	12.50 (1.40-30.12)	4.00 (0.54-29.64)	11.52 (3.87-16.73)
χ^2 (df=3)	8.04	15.36	13.45
RP[a]	-	0.572 (0.313-1.044)	-
RP[b]	-	-	1.005 (0.606-1.665)
RP[c]	-	-	1.757 (0.871-3.541)

[a] Relative potency for LC_{50} of *M. longifolia*/ *E. globulus*
[b] Relative potency for LC_{50} of *R. officinalis*/ *E. globulus*
[c] Relative potency for LC_{50} of *R. officinalis*/ *M. longifolia*

pounds on biological parameters of *T. turkestani* adults are shown in Table 3. All tested compounds significantly reduced the fecundity ($F = 58.76$; $df = 6, 133$; $P < 0.0001$) and longevity ($F = 7.37$; $df = 6, 133$; $P < 0.0001$) of the treated females compared with the control. The reduction in the number of eggs was significantly greater in the female mites treated with the essential oils *E. globulus*, *M. longifolia* and *R. officinalis* than

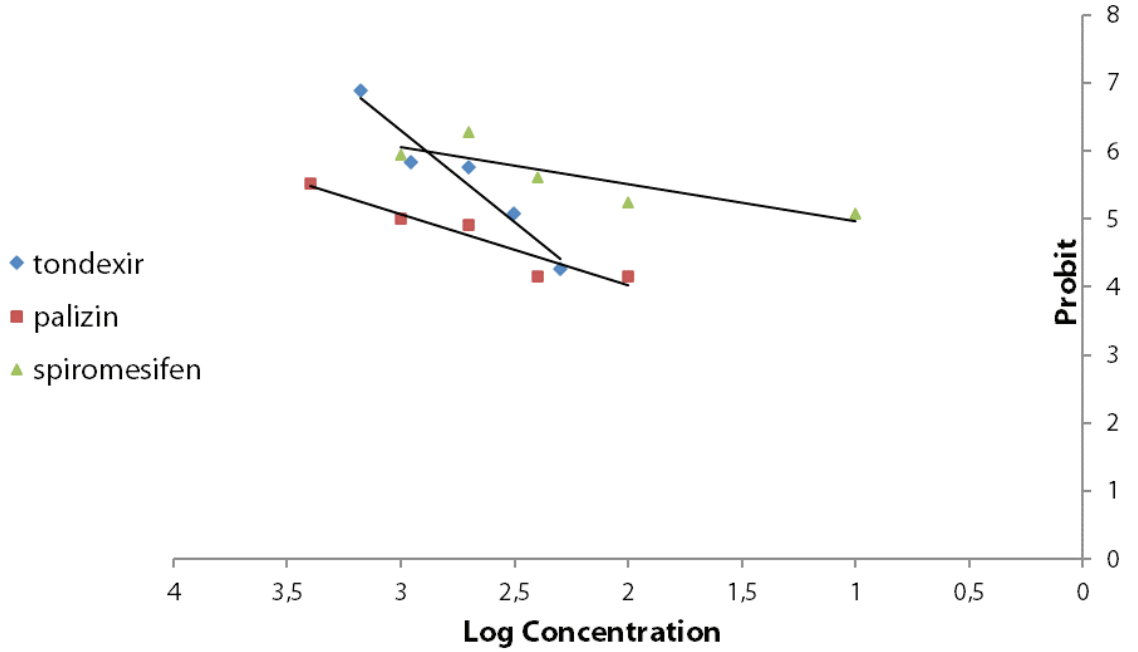

Figure 2. Log- Probity curve of three acaricides (Tondexir, Palizin, spiramesifen) on the strawberry spider mite T*etranychus turkestani.*

Table 2. Probit analysis and lethal concentration ratios (% 95 fiducial limits) of Tondexir, Palizin and spiromesifen against the adult females of *Tetranychus turkestani.*

Parameters	Compound		
	Tondexir	Palizin	spiromesifen
Slope ± SE	2.60 ± 0.43	1.04 ± 0.23	0.52 ± 0.16
LC_{50} (95%FL) (ppm)	327.34 (248.02-402.91)	858.13 (538.85-1689)	10.98 (0.23-37.20)
χ^2 (df=3)	3.14	2.02	3.24
RP[a]	-	3.143 (1.798-5.494)	-
RP[b]	-	-	0.030 (0.005-0.177)
RP[c]	-	-	0.010 (0.002-0.060)

[a] Relative potency for LC_{50} of Palizin/Tondexir
[b] Relative potency for LC_{50} of spiromesifen/Tondexir
[c] Relative potency for LC_{50} of spiromesifen/Palizin

in females treated with the chemical acaricide spiromesifen. No significant difference was observed in longevity between the females treated with spiromesifen and the females treated with tested plant compounds. The percentage of egg hatchability was also significantly affected by the treatments (F = 3.34; df = 6, 35; P = 0.0104). Compared with the control, the females treated with spiromesifen and three plant compounds, Palizin, *E. globulus* and *M. longifolia* had significantly lowered hatchability (Table 3).

Table 3. Sublethal effects of different compounds on *Tetranychus turkestani* adults (Mean ± SE)[a].

Treatment	Number of eggs per female	% Egg hatch	Female longevity (days)
Control	20.15 ± 1.27 a	100.00 ± 0.00 a	15.95 ± 0.68 a
Tondexir	16.10 ± 0.64 bc	82.33 ± 11.20 abc	10.45 ± 0.84 bc
Palizin	17.2 ± 0.70 b	78.66 ± 5.89 bc	12.05 ± 0.95 b
Spiromesifen	13.90 ± 0.46 c	72.16 ± 6.60 c	10.30 ± 1.05 bc
Eucalyptus globulus	10.3 ± 1.13 d	77.83 ± 5.64 bc	11.35 ± 1.00 b
Mentha longifolia	1.2 ± 0.09 e	79.28 ± 0.32 bc	7.90 ± 0.69 c
Rosmarinus officinalis	8.80 ± 0.88 d	88.83 ± 11.16 ab	9.70 ± 1.02 bc

[a] Means in each column followed by the same letter were not significantly different at the 0.05 level when tested by Duncan's Multiple Range Test.

Discussion

Essential oils are phytochemicals with repellent and toxic properties against mites (Cavalcanti *et al.,* 2010; Sertkaya *et al.,* 2010; Attia *et al.,* 2012; Camilo *et al.,* 2017). Our results clearly showed that essential oils from the aromatic plants *R. officinalis*, *M. longifolia*, and *E. globulus* possess acaricidal activity against *T. turkestani* adults. The fumigant toxicity of *M. longifolia* was higher than that of *E. globulus* and *R. officinalis*. Furthermore, mite mortality increased with essential oil concentration. As shown in a previous study, the essential oil of *M. longifolia* is active against *T. urticae* with an LC_{50} value of 20.08 µl/l air (Motazedian *et al.,* 2012). Aissaoui *et al*. (2019) reported substantial contact toxicity of *E. globulus* essential oil against different stages of *T. urticae* and a continuous increase in mortality rates of spider mite larvae and adults was observed with increasing concentration. In another study, Hussein *et al*. (2013) examined the contact activity of three plant essential oils, including that of *E. globulus* against females of *T. urticae*. They found that *E. globulus* essential oil is effective with an LC_{50} value of 2.202%. Efficacy of rosemary oil when applied as an acaricide to control two-spotted spider mite has been reported in previous studies (Miresmailli & Isman, 2006; Miresmailli *et al*. 2006). In the study conducted by Choi *et al*. (2004), rosemary oil was not acutely toxic (mortality <60%) against *T. urticae* compared with oil of eucalyptus.

Spiromesifen is one of the most promising chemical acaricides for managing spider mites. It is an acetyl-CoA-carboxylase inhibitor, a lipid metabolism enzyme (Kontsedalov *et al.,* 2009; Sato *et al.,* 2011; Reddy & Latha, 2013). Our results suggested that spiromesifen was the most toxic acaricide to adult mites with an LC_{50} value of 10.98 ppm, which was lower than the LC_{50} (12.53 ppm) reported by Kumari *et al*. (2017) against *T. urticae*. Based on our results, the bioacaricides TON and PAL were toxic to adult mites. Antonious *et al*. (2007) indicated that the pesticide TON has lethal effects on *T. urticae*, which could be attributed to pepper extract as an active agent in this formulation. Our results also indicate that PAL was the least toxic (LC_{50} = 858.13 ppm) against *T. turkestani,* i.e. 78 times less effective than spiromesifen. In another study, Mirfakhraie and Mohamadian (2017) reported the repellency effect of PAL and contact toxicity of TON against females of *T. urticae* with an LC_{50} value of 2130.91 ppm.

Our findings revealed that all the compounds at LC_{50} significantly affected *T. turkestani* females' biological parameters compared to control. The chemical acaricide spiromesifen and all three plant compounds caused adverse effects on *T. turkestani* fe-

males' longevity. Reduced female longevity led to a reduction in reproductive capacity. The highest significant decrease in fecundity rate by 94% was observed after exposing newly emerged adults to *M. longifolia* essential oil. Similarly to our results, Momen *et al*. (2018) reported the great potential of *M. longifolia* to deterrent the oviposition of *T. urticae* by 99.4% decrease in egg production. In the study conducted by Mohamed *et al*. (2015), the extract of *M. longifolia* reduced *T. urticae* female longevity and ovipositional period and the total number of laid eggs. Furthermore, sublethal effects of plant essential oils on the fecundity and fertility of spider mites have been reported in the previous studies (Momen *et al.,* 2001; Lim *et al.,* 2011; Abd El-Moneim *et al.,* 2012; Gholamzadeh-Chitgar *et al.,* 2013; Esmaeily *et al.,* 2017).

Hatchability of *T. turkestani* eggs was also significantly reduced by spiromesifen and some plant compounds tested, including PAL, *E. globulus* and *M. longifolia*. However, TON and *R. officinalis* did not significantly affect the percentage of eggs hatched. Similarly, Ismail *et al*. (2011) reported that *R. officinalis* oil did not affect two-spotted spider female fertility. *There* are *no* other *studies* concerning the impact of TON and PAL on biological parameters of spider mites to the best of our knowledge. The chemical acaricide spiromesifen also reduced the fecundity and egg hatchability of *T. turkestani* to 31 and 28%, respectively. Similarly, Nauen *et al*. (2005) found that spiromesifen significantly reduced the fecundity of two-spotted spider mite females at the concentrations between 0.064 and 40 mg/l Marcic *et al*. (2009) also reported that the number of laid eggs and egg hatchability of *T. urticae* was significantly affected by 180 mg/l, 18 mg/l, and 1.8 mg/l concentrations of spiromesifen.

In summary, the present study showed that *M. longifolia, E. globulus* and *R. officinalis* essential oils and two bioacaricides (TON and PAL) had satisfactory effects against *T. turkestani* even at a low concentration level. Given the above results and the environmentally friendly properties of plant compounds, along with their low persistence in nature and low risk for humans and mammals, these compounds can be considered as reduced-risk pesticides and useful alternatives to synthetic chemicals for spider mite control. These laboratory findings need to be confirmed with further trials to investigate this bioinsecticide' sublethal effect on field populations of this pest. Furthermore, it is critical to test the safety of these compounds before practical use in *T. turkestani* control in commercial greenhouses.

Literature Cited

Abd El-Moneim, M.A., Fatma, S.A. and Turky, A.F. 2012. Control of *Tetranychus urticae* Koch by extracts of three essential oils of chamomile, marjoram and Eucalyptus. *Asian Pacific Journal of Tropical Biomedicine*, 2(1): 24-30.

Aissaoui, A.B., Zantar, S. and Elamrani, A. 2019. Chemical composition and potential acaricide of *Salvia officinalis* and *Eucalyptus globulus* on *Tetranychus urticae* Koch (Acarina: Tetranychidae). *Journal of Applied Chemistry and Environment Protection*, 4(1): 280-292.

Antonious, G.F., Meyer, J.E., Rogers, J.A. and Hu, Y.H. 2007. Growing hot pepper for cabbage looper, *Trichopulsia ni* (Hubner) and spider mite, *Tetranychus urticae* (Koch) control. *Journal of Environmental Science and Health*, 42(5): 559-567.

Attia, S., Grissa, K.L., Mailleux, A.C., Heuskin, S., Lognay, G. and Hance, T. 2012. Acaricidal activities of *Santolina africana* and *Hertia cheirifolia* essential oils against the two-spotted spider mite (*Tetranychus urticae*). *Pest Management Science*, 68(7): 1069-1076.

Azaizeh, H., Kobaisy, M., Dakwar, S., Saad, B., Shaqir, I. and Said, O. 2007. Botanical pesticides as a source of safe bioacaricides for the control of *Tetranychus cinnabarinus*. *Acta Phytopathologica et Entomologica Hungarica*, 42(1): 143-152.

Camilo, C.J., Alves Nonato, C.D.F., Galvão-Rodrigues, F.F., Costa, W.D., Clemente, G.G., Sobreira Macedo, M.A.C., Galvão Rodrigues, F.F. and Da Costa, J.G.M. 2017. Acaricidal activity of essential oils: a review. *Trends in Phytochemical Research*, 1(4): 183-198.

Cavalcanti, S.C.H., Niculau, E.S., Blank, A.F., Câmara, C.A.G., Araújo, I.N. and Alves, P.B. 2010. Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). *Bioresourse Technology,* 101(2): 829-832.

Choi, W.I., Lee, S.G., Park, H.M. and Ahn, Y.J. 2004. Toxicity of plant essential oils to *Tetranychus ur-*

ticae (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Journal of Economic Entomology*, 97: 553–558.

Chiasson, H., Bostanian, N.J. and Vincent, C. 2004. Acaricidal properties of a *Chenopodium*-based botanical. *Journal of Economic Entomology*, 97(4): 1373-1377.

Esmaeily, M., Bandani, A., Zibaee, I. and Sharifian, I. 2017. Sublethal effects of *Artemisia annua* L. and *Rosmarinus officinalis* L. essential oils on life table parameters of *Tetranychus urticae* (Acari: Tetranychidae). *Persian Journal of Acarology*, 6(1): 39-52.

Gholamzadeh-Chitgar, M., Khosravi, R., JalaliSendi, J. and Ghadamyari, M. 2013. Sublethal effects of *Thymus vulgaris* essential oil on life-table parameters of two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Archives of Phytopathology and Plant Protection*, 46: 781–788.

Hincapié, C.A., López, G.E. and Torres, R. 2008. Comparison and characterization of garlic (*Allium sativum* L.) bulbs extracts and their effect on mortality and repellency of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Chilean Journal of Agricultural Research*, 68(4): 317-327.

Honarmand, P., Nouri, G., Dastjerdi, H.R., Hassanpour, M. and Fathi, S.A.A. 2016. Lethal effect of three synthetic insecticides and two commercial botanical extracts on the Sunn pest, *Eurygaster integriceps* Puton under the field and laboratory conditions. *Plant Pest Research*, 6(3): 65-77.

Hussein, H., Reda, A. and Momen, F. 2013. Repellent, antifeedent and toxic effects of three essential oils on the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Acta Phytopathologica et Entomologica Hungarica,* 48(1): 177-186.

Isman, M.B. 2000. Plant essential oils for pest and disease management. *Crop Protection*, 19: 603–608.

Ismail, M.S., Ghallab, M., Soliman, M.F. and AboGhalia, A.H. 2011. Acaricidal activities of some essential and fixed oils on the two-spotted spider mite, *Tetranychus urticae*. *Egyptian Academic Journal of Biological Sciences, B. Zoology,* 3(1): 41-48.

Jeppson, L.R., Keifer, H.H. and Barker, E.W. 1975. Mites injurious to economic plants Berkeley: University of California Press. 614 pp

Kabiri, M., Raeis, A. and Amiri-Besheli, B. 2013. Comparison of the toxicity of three botanical insecticides and two chemical insecticides on *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) in laboratory and field conditions. *Journal of Agriculture and Crop Sciences*, 5(10): 1074-1079.

Karami-Jamour, T. and Shishehbor, P. 2012. Development and life table parameters of *Tetranychus turkestani* (Acarina, Tetranychidae) at different constant temperatures. *Acarologia*, 52(2): 113-122.

Kumari, S., Chauhan, U., Kumari, A. and Nadda, G. 2017. Comparative toxicities of novel and conventional acaricides against different stages of *Tetranychus urticae* Koch (Acarina, Tetranychidae). *Journal of the Saudi Society of Agricultural Sciences*: 16(2): 191-196.

Kontsedalov, S., Gottlieb, Y., Ishaaya, I., Nauen, R., Horowitz, R. and Ghanim, M. 2009. Toxicity of spiromesifen to the developmental stages of *Bemisia tabaci* biotype B. *Pest Management Science, Formerly Pesticide Science*, 65(1): 5-13.

LeOra Software. 1987. POLO-PC, user's guide to probit or logit analysis, LeOra Software Inc., Berkeley, CA.

Lee, Y., Song, M., Ahn, K., Lee, K., Kim, J. and Kim, G. 2003. Monitoring of acaricide resistance in two-spotted spider mite (*Tetranychus urticae*) populations from rose greenhouses in Korea. *Journal of Asia-Pacific Entomology*, 6: 91–96.

Li, Y.Y., Fan, X., Zhang, G.H., Liu, Y.Q., Chen, H.Q., Liu, H. and Wang, J.J. 2017. Sublethal effects of bifenazate on life history and population parameters of *Tetranychus urticae* (Acari: Tetranychidae). *Systematic and Applied Acarology*, 22(1): 148-159.

Lim, E.G., Roh, H.S., Coudron, T.A. and Park, C.G. 2011. Temperature-dependent fumigant activity of essential oils against two spotted spider mite (Acari, Tetranychidae). *Journal of economic entomology*, 104(2): 414-419.

Marčić, D., Ogurlić, I., Mutavdžić, S. and Perić P. 2009. The effect of spiromesifen on the reproductive potential of *Tetranychus urticae* Koch (Acari, Tetranychidae). *Pesticidi i Fitomedicina,* 2(3): 203-209.

Martínez-Ferrer, M.T., Jacas, J.A., Ripollés-Moles, J.L. and Aucejo-Romero, S. 2006. Approaches for sampling the two-spotted spider mite (Acari, Tetranychidae) on clementines in Spain. *Journal of Economic Entomology*, 99(4): 1490-1499.

Migeon, A. and Dorkeld, F. 2006-2013. Spider Mites Web, a comprehensive database for the Tetranychidae. http,//www.montpellier.inra.fr/CBGP/pmweb. Last accessed on May 2013.

Miresmailli, S., Bradbury, R. and Isman, M.B. 2006. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari, Tetranychidae) on two different host plants. *Pest Management Science, formerly Pesticide Science*, 62(4): 366-371.

Miresmailli, S. and Isman, M.B. 2006. Efficacy and persistence of rosemary oil as an acaricide against two-spotted spider mite (Acari, Tetranychidae) on greenhouse tomato. *Journal of Economic Entomology*, 99(6): 2015-2023.

Mirfakhraie, S. and Mohamadian, P. 2017. Effects of botanical insecticides sirinol, tondexir and repellency of palizin on two spotted spider mite (*Tetranychus urticae* Koch) in the laboratory conditions. *Plant Protection (Scientific Journal of Agriculture)*, 40(3): 1-12.

Motazedian, N., Ravan, S. and Bandani, A.R. 2012. Toxicity and repellency effects of three essential oils against *Tetranychus urticae* Koch (Acari, Tetranychidae). *Journal of Agricultural Science and Technology*, 14: 275-284.

Mohammadi, S., Seraj, A.A. and Rajabpour, A. 2015. Evaluation of six cucumber greenhouse cultivars for resistance to *Tetranychus turkestani* (Acari, Tetranychidae). *Journal of Crop Protection*, 4(4): 545-556.

Mohammadi, S., Ziaee, M. and Seraj, A.A. 2016. Sublethal effects of Biomite® on the population growth and life table parameters of *Tetranychus turkestani* Ugarov and Nikolskii on three cucumber cultivars. *Systematic and Applied Acarology*, 21(2): 218-226.

Momen, F.M., Amer, S.A.A. and Refaat, A.M. 2001. Repellent and oviposition-deterring activity of rosemary and sweet marjoram on the spider mites *Tetranychus urticae* and *Eutetranychus orientalis* (Acari, Tetranychidae). *Acta Phytopathologica et Entomologica Hungarica,* 36(1-2): 55-164.

Morteza, Z., Mousavi, S.B., Baghestani, M.A. and Aitio, A. 2017. An assessment of agricultural pesticide use in Iran, 2012-2014. *Journal of Environmental Health Science and Engineering*, 15: 1-10.

Mohamed, F.S., Hoda, E.H. Darwish, Z.E., Amer, S.A., Salama, A.B. and El-Desouky, M.E. 2015. Influence of some extracts from three Lamiaceae plants on toxicity, repellency and some biological aspects of *Tetranychus urticae* Koch (Acari, Tetranychidae). *Egyptian Journal of Biological Pest Control,* 25(1): p.255.

Momen, F.M., Abdelkader, M.M. and Fahim, S.F. 2018. Composition, repellent and fumigant toxicity of Mentha longifolia essential oil on *Tetranychus urticae* and three predatory mites of the family phytoseiidae (Acari, Tetranychidae, Phytoseiidae). *Acta Phytopathologica et Entomologica Hungarica,* 53(2): 221-232.

Nauen, R., Schnorbach, H.J. and Elbert, A. 2005. The biological profile of spiromesifen (Oberon)-a new tetronic acid insecticide/acaricide. *Pflanzenschuz-Nachrichten Bayer,* 58: 417–440.

Nikpay, A., Soleyman-Nejadian, E., Goldasteh, S. and Farazmand, H. 2016. Efficacy of Biomite® and GC-Mite® on *Oligonychus Sacchari* and its predator *Stethorus gilvifrons* on sugarcane, preliminary results. *International Sugar Journal*, 118: 454-458.

Osakabe, M.H., Usegi, R. and Goka, K. 2009. Evolutionary aspects of acaricide-resistance development in spider mites. *Psyche*: 1-11.

Pree, D.J., Whitty, K.J. and Van Driel, L. 2005. Baseline susceptibility and cross resistances of some new acaricides in the European red mite, *Panonychus ulmi. Experimental and Applied Acarology*, 37(3-4), p.165.

Reddy, D.S. and Latha, M.P. 2013. Efficacy of certain new acaricides against two spotted spider mite, *Tetranychus urticae* Koch on ridge gourd. *Pest Management in Horticultural Ecosystems,* 19(2): 199-202.

Robertson, J.L., Russell, R.M., Preisler, H.K. and Savin, N.E. 2007. Bioassay with Arthropods. Taylor and Francis Group, London.

SAS Institute. 2003. The SAS system for Windows, Release 9.0. SAS, Institute, Cary, NC.

Sato, M.E., Da Silva, M.Z., Raga, A., Cangani, K.G., Veronez, B., and Nicastro, R.L. (2011). Spiromesifen toxicity to the spider mite *Tetranychus urticae* and selectivity to the predator *Neoseiulus californicus*. *Phytoparasitica*, 39(5): p.437.

Sertkaya, E., Kaya, K. and Soylu, S. 2010. Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.)(Acarina, Tetranychidae). *Industrial Crops and Products,* 31(1): 107-112.

Stark, J.D. and Banks, J.E. 2003. Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology*, 48: 505–519.

Sohrabi, F., Jamali, F., Morammazi, S., Saber, M. and Kamita, S.G. 2019. Evaluation of the compatibility of entomopathogenic fungi and two botanical insecticides tondexir and palizin for controlling *Galleria mellonella* L. (Lepidoptera, Pyralidae). *Crop Protection*, 117: 20-25.

Van Leeuwen, T., Van Pottelberge, S. and Tirry, L. 2005. Comparative acaricide susceptibility and detoxifying enzyme activities in field-collected resistant and susceptible strains of *Tetranychus urticae*. *Pest Management Science, formerly Pesticide Science*, 61(5): 499-507.

Zhang, Z.Q. 2003. Mites of greenhouse, Identification, biology and control. *CABI International*, 256.

Ziaee, M., Nikpay, A., Koohzad-Mohammadi, P., and Behnam-Oskuyee, S. 2017. The toxicity of Biomite®, GC-mite®, Oberon® and Envidor® acaricides against sugarcane yellow mite, *Oligonychus sacchari* (Acari, Tetranychidae). *Persian Journal of Acarology*, 6: 137-141.

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Ακαρεοκτόνος και υποθανατηφόρος δράση διαφόρων ουσιών φυτικής προέλευσης έναντι του τετράνυχου *Tetranychus turkestani* σε σύγκριση με το συνθετικό ακαρεοκτόνο spiromesifen

F. Sohrabi και M. Ziaee

Περίληψη Ο τετράνυχος *Tetranychus turkestani* Ugarov and Nikolski είναι ένας από τους κύριους εχθρούς των καλλιεργειών στο νοτιοδυτικό Ιράν και άλλες τροπικές περιοχές. Στην παρούσα εργασία εξετάστηκαν η καπνιστική δράση τριών αιθέριων ελαίων που εξήχθησαν από φυτά δενδρολίβανου (*Rosmarinus officinalis* L.), άγριας μέντας (*Mentha longifolia* L.) και ευκάλυπτου (*Eucalyptus globulus* Labill.), και η δράση επαφής δύο ακαρεοκτόνων φυτικής προέλευσης (Tondexir και Palizin) σε ενήλικα θηλυκά άτομα του *T. turkestani*, σε σύγκριση με αυτή του συνθετικού ακαρεοκτόνου spiromesifen. Επίσης, εκτιμήθηκε η υποθανατηφόρος δράση των υπό δοκιμή αιθέριων ελαίων στη συγκέντρωση LC_{50}, σε διάφορες βιολογικές παραμέτρους του τετράνυχου. Οι τιμές LC_{50} για τα αιθέρια έλαια του ευκάλυπτου, του δενδρολίβανου και της άγριας μέντας ήταν 12,50, 11,52 και 4,00 μl/ l ατμού, ενώ για το spiromesifen, το Tondexir και το Palizin ήταν 10,98, 327,34 και 858,13 ppm, αντίστοιχα. Όλες οι δοκιμασθείσες ουσίες μείωσαν σημαντικά τη διάρκεια ζωής των ενήλικων θηλυκών ατόμων, σε ανάλογα επίπεδα με το spiromesifen. Επιπλέον, μειώθηκε η γονιμότητα σε όλες τις επεμβάσεις, με μεγαλύτερη μείωση αυτή στα αιθέρια έλαια. Το Palizin και τα αιθέρια έλαια του ευκάλυπτου και της άγριας μέντας μείωσαν σημαντικά την εκκόλαψη των ωών του τετράνυχου σε επίπεδα ανάλογα με το spiromesifen. Σύμφωνα με τα αποτελέσματα, οι δοκιμασθείσες φυτικές ουσίες είναι αποτελεσματικές έναντι του τετράνυχου *T. turkestani* και αποτελούν κατάλληλες εναλλακτικές ουσίες σε συνθετικά φυτοφάρμακα για την αντιμετώπισή του.

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Effects of gamma radiation and *Bacillus thuringiensis* on F_1 progeny of *Cydia pomonella*

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Summary The codling moth [*Cydia pomonella* L. (Lepidoptera: Tortricidae)] is the main pest in most apple orchards in Syria. It causes billions of dollars in loss of fruit crops every year. The present work examined the effects of gamma radiation and *Bacillus thuringiensis* (BT) on F_1 progeny of *C. pomonella*. The experimental design was based on two factors, namely F_1 offspring produced by males irradiated at a dose of 150 Gy, and artificial diet of BT-treated larvae. The first offspring of unirradiated and irradiated *C. pomonella* males, F1, were from parents treated with a commercial formulation of *Bacillus thuringiensis* BT (Dipel® 2X). F_1 progeny of unirradiated was significantly less susceptible to BT than that of irradiated parents. The results showed high mortality in F_1 progeny of *C. pomonella* when gamma radiation and BT were applied together. The LC_{50} in F_1 progeny was 2.5 ppm for irradiated parents, while it was 13 ppm for unirradiated ones. A significant reduction in the fecundity and egg hatchability of F_1 progeny of irradiated parents compared to unirradiated ones was observed. This study demonstrated that for F_1 progeny the combination between Inherited Sterility Technique and BT can be useful to achieve an integrated pest management program of codling moth.

Additional keywords: *Bacillus thuringiensis*, biocontrol, *Cydia pomonella*, radiation

Introduction

Apple is a very important fruit tree in Syria. The total area of apples is approximately 41,000 hectares. The codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) is one of the most important pests worldwide, including Syria, due to its distribution and economic impact on apple farms (Basheer *et al*., 2016; Idris *et al*., 2019a). The control of *C. pomonella* relies on the use of insecticides which are costly, non-selective, and environmentally unfriendly. In addition, *C. pomonella*, like other pests has already developed resistance to a wide spectrum of insecticides (Carpenter *et al*., 2010; Harba and Idris, 2018). Therefore, efforts for novel biocontrol methods are now the focus of intense studies.

The Sterile Insect Technique (SIT) and the Inherited Sterility Technique (IST) are relatively sustainable and environment friendly control methods against some major pests (Vreysen *et al*., 2016). In addition, research on mosquitoes has shown that compared with chemical control, the cost of using SIT are more acceptable in terms of monetary expenditures and efficacy on long-term (Khater, 2018). Recent studies reported that, there is growing evidence that applying SIT and F_1 sterility (SIT/F1) (F_1 progeny of partially sterile male parents) or IST to suppress these pest populations has advantageous over chemical control (Blomefield *et al*., 2011; Eyidozehi *et al*., 2015). Thus, IST applications is considered an important approach to suppress population of codling moth in large areas. Moreover, IST was more effective than SIT in reducing *C. pomonella* population (Vreysen *et al*., 2016; Idris *et al*., 2019a). However, there are still some obstacles to both SIT and IST, such as the continued need to release sterilized males. Hence, SIT and IST methods can be used together with other methods (such as biological pesticides) as the main component of an integrated pest control plan (Eyidozehi *et al*., 2015). Moreover, the use of microbial insecticides, especially insecticidal toxins, has been efficient for the control of codling moth (Ammouneh *et al*., 2011). Com-

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bination of commercial BT preparations with other pest control methods has shown good results (Peralta and Palma, 2017). The main purpose of this study was to compare the response of F_1 progeny of partially sterile male parents (SIT/F_1) to the larval protein produced by *B. thuringiensis* (BT) and the F_1 progeny of unirradiated male parents, for the evaluation of these practices to suppress *C. pomonella* population.

Materials and Methods

General experiment procedures

All insects used in this study were obtained from our laboratory stock culture. The codling moth colony has been maintained in our laboratory for several years according to Makee *et al*. (2012).

Newly hatched larvae were placed on artificial diet consisted of the following ingredients: Agar-agar, maize, wheat germ, casein, yeast, Wesson salts, benzoic acid, fumidil, ascorbic acid, vitamins and nipagine at a constant temperature of 25 ± 1˚C with 70 ± 5% RH, and a photoperiod of 16:8 h (L: D) as outlined by Idris *et al*. (2019a).

Bioassays were conducted with Dipel® 2X, a formulation of *BT* subsp. *kurstaki* containing 32000 IU/mg.

Irradiation and reproduction of male parents

Newly emerged (age less than 24 h) adult males of *C. pomonella* (n = 50) were irradiated with 150 Gy gamma radiation. All irradiated males in this study were treated with Co_{60} source (Issledov Gamma Irradiator, Techsnabexport Co. Ltd., Moscow, Russia, http://www.tenex.ru). The average dose rate at the time of irradiation was approximately 44.24 Gy /min. A group of newly emerged unirradiated adult males (n = 50) was used as control.

Unirradiated and irradiated males were paired individually with newly emerged unirradiated females in Petri dishes (9 cm diameter). A wet cotton wool was placed in each Petri dish as drinking water source and the mated pairs were kept together until death (Idris *et al*., 2019b). The Petri dishes which had eggs were removed daily and eggs were left to hatch. Newly hatched larvae from unirradiated and irradiated males were taken for the following experiments.

Application of BT to F_1 progeny

The commercial biopesticide powder containing BT (Dipel® 2X) was suspended in water at a concentration of 1 g/1000 ml (1000 ppm). Serial dilutions were prepared from a 1000 ppm solution to provide nine other concentrations: 500, 250, 150, 100, 50, 25, 15, 10, and 5 ppm. Solutions of the BT concentrations and distilled water (control) were added to the artificial diet surface in the Petri dishes (1 ml/dish, 5 g artificial diet/ Petri dish, 5 Petri dishes/BT concentration). Then newly hatched F_1 larvae (55 larvae/BT concentration) of unirradiated and irradiated parents were placed on the artificial diet in the Petri dishes. The dishes were kept under the same environmental conditions as mentioned above until adult emergence.

Effect of BT concentrations on mortality of F_1 progeny

The number of emerged F_1 adults was documented for each BT concentration. The percentage of F_1 larval mortality of unirradiated and irradiated male parents was determined by calculating adults emerging from tested larvae according to the formula: Percentage of mortality = (number of tested larvae - number of adults emerging)/ number of tested larvae X 100.

The toxicity of BT concentrations were calculated by determining the lethal concentration to kill 50% (LC_{50}) of the F_1 larvae of unirradiated and irradiated male parents.

Effect of BT concentrations on fecundity and egg hatching of F_1 progeny

Newly emerged F_1 adults of irradiated and unirradiated male parents at each concentration were singly crossed as follows: F_1 male of irradiated or unirradiated male parents X unirradiated female, F_1 female of irradiated or unirradiated male parents X

unirradiated male. Twenty five pairs were examined at each BT concentration in each tested cross (75 combinations/BT concentration) (Figure 1). The egg number (fecundity) and hatching (%) were determined for each pair at each BT concentration.

Effect of BT and IST combination on mortality, fecundity, fertility and mating of F_1 progeny

Three groups of larvae (unirradiated larvae F_1, irradiated larvae F_1, treated larvae F_1) were used for five treatments (55 larvae/ treatment) as follows: treatment 1: newly hatched unirradiated larvae on untreated artificial diet (control); treatment 2: newly hatched unirradiated larvae on artificial diet treated with 13 ppm BT (BT); treatment 3: newly hatched larvae, generated from irradiated male parent, on untreated artificial diet (IST); treatment 4: newly hatched larvae, generated from irradiated male parent, on artificial diet treated with 13 ppm (IST + BT); treatment 5: newly hatched larvae on artificial diet treated with 13 ppm BT and emerging males irradiated with 150 Gy (BT + IST) (Figure 2).

Emerged adult males from each experiment were paired individually with newly unirradiated females (30 pairs/ each experiment). Then newly hatched larvae were placed on artificial media as mentioned above. The percentage of larvae mortality, the fecundity, fertility and mating of F_1 progeny were determined.

Statistical analysis

Mortality was calculated based on the numbers of emerged adults. Data is corrected by Abbott (1925) formula. The lethal concentration (LC_{50}) of F_1 offspring mortality data is analyzed by probit analysis. The resistance factor is obtained by dividing the lethal concentration (LC_{50}) of the offspring of unirradiated male parents by the lethal concentration (LC_{50}) of the irradiated offspring according to Makee *et al.* (2007). The statistical analysis was done using the STATISTIC program version 6 (Statsoft, Inc. 2003) at 5% significance level (P = 0.05). According to the ANOVA-Tukey HSD test, analysis of variance was performed on the data of fertility, mortality, mating and egg hatching to determine the statistical significance of the mean and percentage difference.

Results

Effect of BT concentrations on mortality of F_1 progeny

Table 1 shows that the mortality percentage of F_1 progeny of irradiated parents was significantly higher than unirradiated parents (P<0.001). Compared with the

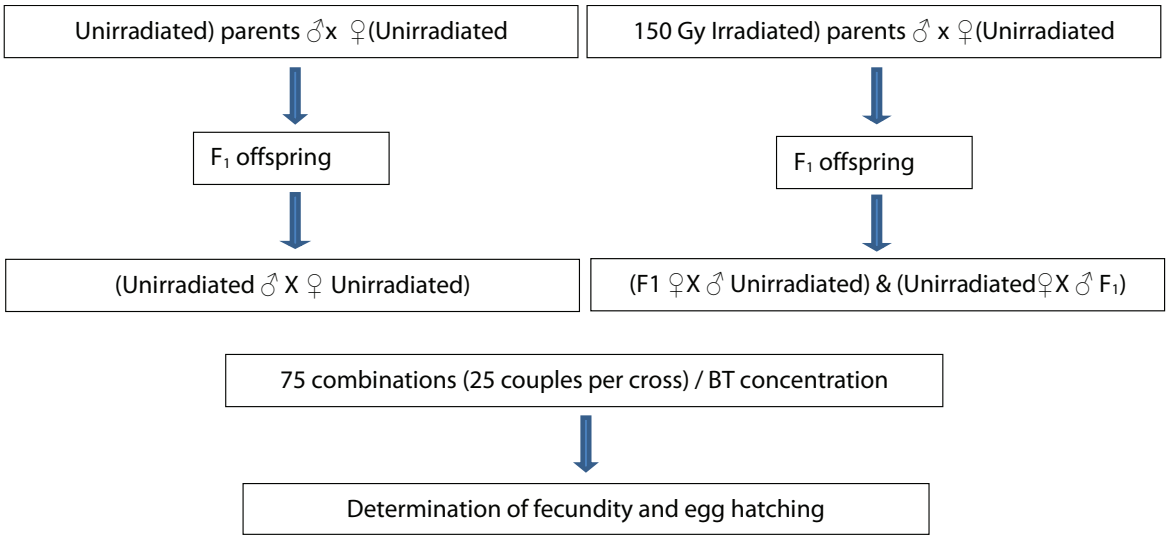

Figure 1. Experimental design I.

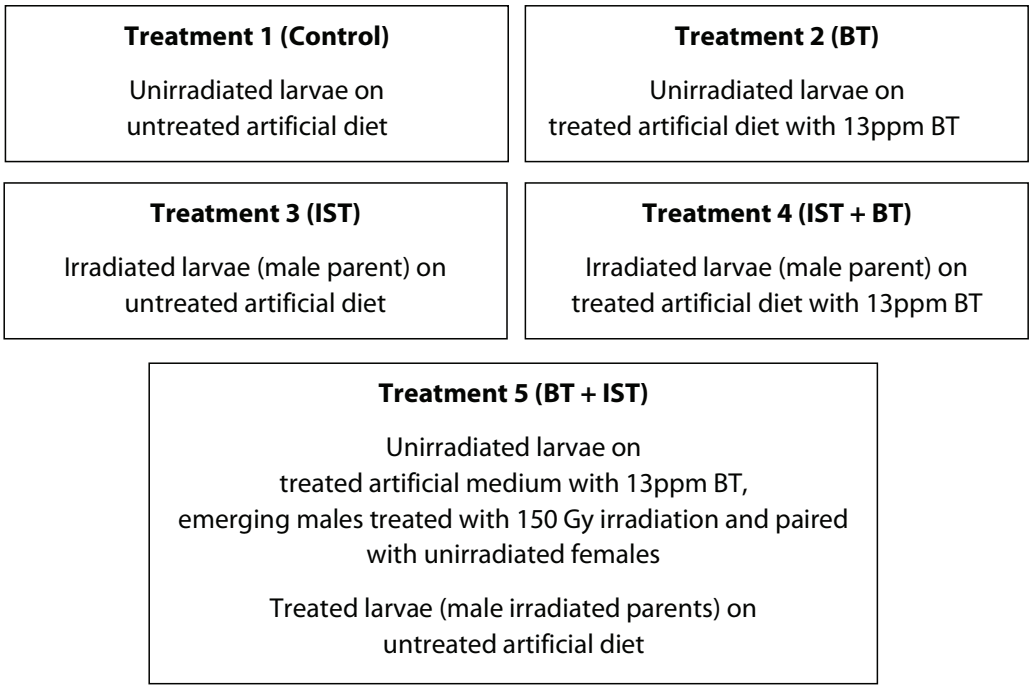

Figure 2. Experimental design II.

control group, by increasing the BT concentration, mortality of F_1 progeny of unirradiated and irradiated parents increased significantly (df=10, f=87893.189, P<0.01, df=10, f= 116073.796, P<0.01, respectively). When BT concentration was higher than 25 ppm (P<0.001), the mortalities of F_1 progeny of both parents were not significantly different (Table1). LC_{50} of F_1 progeny of irradiated parents (2.9 ppm) was significantly lower than unirradiated (13 ppm), and the resistance factor was 4.48.

Effect of BT concentrations on fecundity and egg hatching of F_1 progeny

Table 2 shows the fecundity of F_1 (female or male) of unirradiated and irradiated parents. When they are crossed with their unirradiated counterparts of the opposite sex, their F_1 fecundity is significantly reduced higher than that of the control group (without BT treatment) (df=6, f= 13160, P<0.01; df=6, f= 4580, P<0.01, df=6, f= 14802, P<0.01, df=6, f= 20790, P<0.01, respectively). The fecundity of F_1 females and males of irradiated parents was significantly lower than unirradiated parents, regardless of concentrations (P<0.001). When BT was applied, the fecundity of F_1 females and males of irradiated parents (F_1 females and males of irradiated parents which were crossed with unirradiated counterparts of the opposite sex) was significantly lower than that of F_1 females and males of unirradiated parents (P<0.001).

The egg hatching percentage was significantly reduced at the concentrations higher than 5 ppm, when F_1 females and males of unirradiated parents were crossed with the counterparts of the opposite sex (df=6, f= 10308, P<0. 01; df=6, f= 427; P<0. 01, respectively) (Table 3). However, the increase in BT concentration has no significant effect on the hatchability of F_1 females and males of irradiated parents (df=6, f= 1552, P<0.01, df=6, f= 20790, P<0.01, respectively), but it was lower than that of F_1 females of unirradiated parents at all concentrations (P<0.001).

Effect of BT and IST combination on mortality, fecundity, fertility and mating number of F_1 progeny

Results in Table 4 indicate that when BT and IST treatments were combined, percentage of the mortality was significantly higher comparing to each one separately. The mortality in F_1 progeny of irradiated male par-

Table 1. Percentage mortality of the codling moth, *Cydia polomonella,* when F_1 larvae of irradiated and unirradiated male parents were reared on artificial diet treated with different concentrations of *B. thuringiensis*.

BT concentration (ppm)	% mortality in F_1 progeny[a]				
	Unirradiated parents			Irradiated parents	
	n[b]	Observed	Corrected	Observed	Corrected
0	55	47.7	f 0 B	61.8	d 27.6 A
5	55	72.7	e 48.3 A	78.0	c 58.6 A
10	55	70.9	d 44.8 B	83.0	b 68.9.1 A
15	55	76.3	c 55.2 B	96.6	a 93.1 A
25	55	87.3	b 76 B	100.0	a 100 A
50	55	97.0	a 93.5 A	100.0	a 100 A
100	55	100	a 100 A	100.0	a 100 A
150	55	97.0	a 93.5 A	100.0	a 100 A
250	55	100	a 100 A	100.0	a 100 A
500	55	100	a 100 A	100.0	a 100 A
1000	55	100	a 100 A	100.0	a 100 A

Percentages preceded by different small letters (columns) and followed by different capital letters (rows) are significantly different at $P < 0.05$ (Tukey HSD test).
[a] Observed mortalities calculated from the numbers of adults that emerged at each *B. thuringiensis* concentration were corrected for mortality in water-treated control (Abbott, 1925).
[b] n= Number of tested larvae/ concentration.

Table 2. Effect of different concentrations of *B. thuringiensis* and irradiation upon fecundity (Mean ± SE) of the codling moth, *Cydia pomonella*, when F_1 females and males were mated with unirradiated partners.

BT concentration (ppm)	No. of egg laid/ F_1 female		No. of egg laid/F_1 male	
	Unirradited parents[a]	Irradiated parents[a]	Unirradited parents[a]	Irradiated parents[a]
0	183.8 ± 0.7 A	81.7 ± 0.4 A	169.0 ± 0.7 A	69.7 ± 0.4 A
5	138.6 ± 0.7 B	78.4 ± 0.3 A	146.2 ± 0.8 B	50.7 ± 0.4 B
10	135.7 ± 0.6 B	45.6 ± 0.7 B	134.1 ± 0.8 B	41.7 ± 0.4 C
15	96.7 ± 1.1 C	21.7 ± 0.6 C	112.0 ± 0.9 C	18.7 ± 0.4 D
25	68.0 ± 1.2 D	0.0 D	72.7 ± 1.0 D	0.0 E
50	0.0 E	0.0 D	66.6 ± 0.8 D	0.0 E
100	0.0 E	0.0 D	65.0 ± 1.4 D	0.0 E

Means followed by different capital letters are significantly different at $P < 0.05$ (Tukey HSD test).
[a] Number of F_1 adults tested at each concentration was 25.

ents treated with BT was not significantly different from the mortality of those progeny whose male parents had been treated with BT first and then emerged F_1 was irradiated at 150 Gy separately (df=4, f= 3227, P<0.01). The fecundity, fertility and mating frequency of F_1 progeny were significantly lower in progeny treated with both BT and gamma radiation compared to those of separate treatments. In addition, all parameters in treatment 5 was significantly lower than in treatment 4 (df=4, f= 818, P<0.01; df=4, f= 500, P<0.01, df=4, f= 1331, P<0.01, respectively) (Table 4).

Table 3. Effects of different concentrations of *B. thuringiensis* upon % hatchability of eggs laid by the codling moth, *Cydia pomonella*, when F_1 females and males were mated with unirradiated partners.

BT concentration (ppm)	% eggs hatched/ F_1 female		% eggs hatched/ F_1 male	
	Unirradiated parents[a]	Irradiated parents[a]	Unirradiated parents[a]	Irradiated parents[a]
0	88.0 a	24.3 a	87.2 a	21.2 a
5	85.2 a	23.6 a	86.0 a	19.6 a
10	76.6 b	21.8 a	84.6 ab	20.4 a
15	76.0 b	21.2 a	84.3 ab	18.3 a
25	73.5 b	0.0 b	84.2 ab	0.0 b
50	0.0 c	0.0 b	82.2 b	0.0 b
100	0.0 c	0.0 b	76.9 c	0.0 b

Percentages followed by different small letters are significantly different at P< 0.05 (Tukey HSD test).
[a] Number of F_1 adults tested at each concentration was 25.

Table 4. Effect of *B. thuringiensis* and Inherited Sterility Technique (IST) combination on mortality, fecundity, fertility and mating of the codling moth, *Cydia pomonella*, when F_1 males were mated with unirradiated females.

Treatment[a]	Mortality %	Mean fecundity (±SE)	Mean egg hatch (±SE)	Mean no. of mating (±SE)
1 Control	41.0 d	133.8 ± 15.2 a	94.5 ± 12.9 a	1.3 ± 0.08 a
2 BT	67.0 c	91.3 ± 7.2 b	59.1 ± 5.5 b	0.86 ± 0.03 b
3 IST	61.0b	83.4 ± 7.1 b	43.4 ± 5.6 b	0.73 ± 0.08 c
4 BT + IST	98.0 a	39.8 ± 3.8 c	13.6 ± 1.4 c	0.53 ± 0.14 d
5 IST + BT	97.5 a	21.7 ± 2.8 d	7.7 ± 1.2 d	0.42 ± 0.01 e

Means and percentages followed by different small letters are significantly different at P< 0.05 (Tukey HSD test).
[a] Number of males tested at each treatment was 30.

Discussion

Males irradiated with low doses of gamma ray (150Gy) compete more efficiently with wild males than the completely sterile males irradiated with high doses (450Gy) of gamma radiation (Vreysen, 2016). The successful production of sterile F_1 progeny of Lepidoptera insects by IST requires two conditions: 1) the male parents need to be partially sterilized and, 2) the female parents should be fully sterilized (Vreysen *et al*., 2016; Idris *et al*. 2019a, b). Many field experiments using partially sterilized males by 150 Gy dose of gamma radiation have proved that the competitiveness of the sterile moths was weak in the spring generation (flight period) (Thistlewood and Judd, 2019). This research combines the application of IST and BT to increase the efficacy of insect suppression under such conditions.

Previously, our studies showed that gamma irradiation increases the mortality of F_1 progeny significantly (Idris *et al*., 2019b). It also affects other reproduction parameters, i.e. reduction of fecundity and fertility, prolongation of development time, distortion of the sex ratio of F_1 progeny in favor of males (Idris *et al*., 2019a). The results of this study showed that BT had a clear effect on the mortality of *C. pomonella* F_1 progeny, independently of whether they were from unirradiated or irradiated parents. This is probably because BT causes serious damage to the gut and mouth parts of the larvae, and cessation of digestive processes,

which eventually lead to its death (Ignoffo and Gregory 1972). Similar results were obtained on *Cadra cautella* and *Ephestia calidella*, where larval mortality increased with increasing the BT concentrations (Farukiand Khan, 1993; Hazaa *et al*., 2013).

In addition, while the F_1 progeny of both unirradiated and irradiated male parents had similar susceptibility at higher BT concentrations, the F_1 progeny of irradiated male parents were more susceptible at low concentrations of BT. This could be attributed to the synergistic effects of the inherited damaged DNA caused by gamma radiation and the inhibitory effects of BT to DNA dependent on RNA-polymerase, and the subsequently cell mitosis blocking (Idris and Shoaib., 2019). The irradiated Lepidoptera moths have a wide range of susceptibility to BT treatments (Hazaa, 2013). This change in sensitivity can be resolved by resistance factor (RF) values. The resistance factor (RF) value is 4.48 for *C. pomonella,* and 2.04 for the potato tuber moth *Phthorimaea operculella* (Makee *et al*., 2007). Based on resistance factor, F_1 progeny of the codling moth produced from irradiated male parents was more sensitive to BT than the potato tuber moth.

BT application significantly reduced the fecundity of F_1 progeny of unirradiated and irradiated parents, especially at high concentrations. This reduction is more significant for F_1 progeny of irradiated male parents. Previous studies have shown that the larvae of *Pectinophora gossypiella* and *Spodoptera littoralis* fed withbacteria- treated food are smaller than control insects. Besides, when BT is used in combination with gamma radiation, the weight of insects F_1 progeny is significantly reduced (Makee *et al*., 2007; Makee *et al*., 2012; Hazaa 2013; Ismail and Albittar, 2016). Thus, the reduction in the fecundity of F1 progeny mentioned above is related to the decrease in size and weight of the insects.

At high BT concentrations, the hatching percentage of eggs laid by F_1 progeny of unirradiated parents was significantly reduced for both males and females. This decrease in egg hatching may be due to a sharp drop in sperm transfer and egg fertilization, as reported in other economic insects such as *Phthorimaea operculella* (Makee *et al*., 2007), *Cadra cautella* (Faruki and Khan, 2001) and *Spodoptera littoralis* (Hazaa, 2013). The egg hatching percentage of F_1 progeny of irradiated male parents was also significantly lower than that of unirradiated male parents, and there was no change regardless of the BT concentration. Many studies have reported that this finding is related to the high sterility of F_1 progeny inherited from the irradiated male parents (Vreysen *et al*., 2016; Idris *et al*., 2019 a,b; Idris and Shoaib, 2019).

The application of IST or combined use of BT leads to a deleterious effect on the reproduction of F_1 progeny. Although, the two treatments have no significant difference in the mortality of F_1 progeny, the synergistic effects of this combination, independently of which treatment is applied first, reduces the fecundity, egg hatching percentage and the number of F_1 mated moths.

The synergistic effect of bacterial compound on IST is consistent with previous results, that showed the increasing efficiency of IST application on many economical insect pests such as *Galleria mollenela* (Jafriand Sabiha, 1947; Mohamed *et al*., 2006)*, Cadra cautella* (Faruki and Khan, 2001), *Spodoptera littoralis* (Hazaa, 2013), *Phythorimaea Opereulella* (Makee *et al*., 2007), *Artogeia rapae* and *Agrotis ipsilon* (Hazaa, 2013).

Conclusion

Overall, this study strengthens the idea that the applying BT treatment in combination with IST increases the suppression efficiency of *C. pomonella* populations. Moreover, the second major finding is that the adults from the surviving offspring from the BT treatment, lay fewer eggs, and have significantly lower fertility than the untreated. These findings indicate that applying BT and releasing irradiated insects has positive synergistic effect against the reproduction of codling moth. For example, in the wild, ap-

ple trees are treated with a low concentration of *B. thuringiensis* (13 ppm). Then, releasing males exposed to low-dose radiation (150 Gy) can reduce the *C. pomonella* population, and can increase its inhibitory effect on insects by delaying development of resistance to BT. Therefore, the combining IST with BT is a promising effective method that can be used to control codling moth.

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Literature cited

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal* of *Economic Entomology,* 18(2): 265-267.

Ammouneh, H. Harba., M. Idris., E. and Makee, H. 2011. Isolation and characterization of native *Bacillus thuringiensis* isolates from Syrian soil and testing of their insecticidal activities against some insect pests. *Turkish Journal of Agriculture and Forestry*, 35(4): 421-431.

Basheer, A.M., Alhaj, S.I., and Asslan, L.H. 2016. Parasitoids on codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) in apple and walnut orchards in Syria. *EPPO Bulletin*, 46(2): 295-297.

Blomefield, T., Carpenter, J.E., and Vreysen, M.J. 2011. Quality of mass-reared codling moth (Lepidoptera: Tortricidae) after long-distance transportation: 1. Logistics of shipping procedures and quality parameters as measured in the laboratory. *Journal of Economic Entomology*, 104(3): 814-822.

Carpenter, J.E., Bloem, S. and Taggart, S. 2010. Effect of rearing strategy and gamma radiation on fecundity and fertility of codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae). *Journal of Applied Entomology*, 134(3): 221-226.

Eyidozehi, K., Fanooj, M.A., and Mokhtari, A. 2015. The sterile insect technique and inherited sterility in Lepidoptera. *In Biological Forum*: Vol, 7, No. 1, p. 1871. Research Trend.

Faruki, S.I., and Khan, A.R.1993. The effect of *Bacillus thuringiensis* var. *kurstaki* on the fecundity and fertility of *Cadra cautella* (Walker) (Lepidoptera: Pyralidae). *Annals of Entomology (India).*

Faruki, S.I. and Khan, A.R. 2001. The pathogenicity of *Bacillus thuringiensis* ssp. kurstaki to γ-irradiated *Cadra cautella* (Walker) (Lepidoptera: Phycitidae). *Journal of Invertebrate Pathology*, 77(2): 92-98.

Harba, M. and Idris, I. 2018. The effect of host density and viability on superparasitism behavior of *Trichogramma cacoeciae* and *T. principium* females. *Agriculture and Forestry Fish,* 7(1): 11-18.

Hazaa, M.A. 2013. The Pathogenicity of Bio-Insecticide Protecto to Gamma Irradiated Cotton Leaf Worm *Spodoptera littoralis* (Boisd.). *Journal of Radiation Research and Applied Sciences*, 6(1): 1-18.

Idris, I., Hussian, K., Alali, N. and Ikhtiar, A. 2019a. irreversible fertility of irradiated *Phthorimaea operculella* (Lepidoptera: gelechiidae) females. *Journal of Bio Innovation*, 8(4): 517-531. ISSN 2277-8330 (Electronic).

Idris, I., Hussian, K., Haroon, H. and Alali N. 2019b. The possibility of applying inherited sterility against codling moth *Cydia pomonella* l. (lepidoptera: tortricidae). *Journal of Bio Innovation*, 8 (3): 349-359. ISSN 2277-8330 (Electronic).

Idris, I., and Shoaib, A. 2019. Efficiency of AFLP markers to detect genetic variation in *Phthorimaea operculella* (Lepidoptera: Gelechiidae) offspring irradiated males. *Advances in Horticultural Science*, 33(3): 321- 326.

Ignoffo, C.M. and Gregory, B. 1972. Effects of *Bacillus thuringiensis* β-exotoxin on larval maturation, adult longevity, fecundity, and egg viability in several species of Lepidoptera. *Environmental Entomology*, 1(3): 269-272.

Ismail, M. and Albittar, L. 2016. Mortality factors affecting immature stages of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), and the impact of parasitoid complex. *Biocontrol Science and Technology*, 26(1):72-85.

Jafri, R.H., and Sabiha, H. 1947. Development of *Bacillus thuringiensis* in *Galleria mellonella* larvae exposed to gamma radiation. *Journal of Invertebrate Pathology*, 23(1): 76-84.

Khater, H.F. 2018. Introductory chapter: the globalization of mosquito-borne diseases and their ecofriendly control. From local to global impact of mosquitoes. *IntechOpen*.

Makee, H., Idris, I. and Hussian, K. 2012. Factors influencing mating incidence and reproduction in codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae). *Advances in Horticultural Science*, 180-186.

Makee, H., Tlas, M., Amer, S. and Abdulla, J. 2007. Effect of inherited sterility and *Bacillus thuringiensis* on mortality and reproduction of *Phthorimaea opercullela* Zeller (Lepidoptera: Gelechiidae). *Applied Entomology and Zoology*, 42(4): 557-562.

Mohamed, H.F., Mikhaiel, A.A. and Abul-Fadl, H.A. 2006. Histopathological effects of *Bacillus Thuringiensis* and gamma irradiation on F1 Larvae of the greater Wax Moth, *Galleria mellonella* L. *Isotope and Radiation Research*, 38(4): 1373.

Peralta, C. and Palma, L. 2017. Is the insect world overcoming the efficacy of *Bacillus thuringiensis*? *Toxins (Basel),* 9(1): 39.

Thistlewood, H. and Judd, G.J. 2019. Twenty-five years of research experience with the sterile insect technique and area-wide management of codling moth, *Cydia pomonella* (L.), in Canada. *Insects*, 10(9): 292.

Vreysen, M.J., Klassen, W. and Carpenter, J.E. 2016. Overview of technological advances toward greater efficiency and efficacy in sterile insect-inherited sterility programs against moth pests. *Florida Entomologist*, 99(1): 1-12.

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Επιδράσεις της ακτινοβολίας γ και του *Bacillus thuringiensis* σε απογόνους F_1 γενιάς της καρπόκαψας της μηλιάς (*Cydia pomonella*)

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Περίληψη Η καρπόκαψα της μηλιάς [*Cydia pomonella* L., (Lepidoptera: Tortricidae)] είναι ο κύριος εντομολογικός εχθρός στην πλειονότητα των μηλεώνων της Συρίας προκαλώντας απώλειες δισεκατομμυρίων δολαρίων στην ετήσια παραγωγή. Στην παρούσα εργασία εξετάστηκε η επίδραση της ακτινοβολίας γ και του βακτηρίου *Bacillus thuringiensis* (BT) σε απογόνους του *C. pomonella*. Ο πειραματικός σχεδιασμός βασίστηκε σε δύο παράγοντες, συγκεκριμένα απογόνους F_1 γενιάς από αρσενικά άτομα που είχαν λάβει ακτινοβολία γ στη δόση 150 Gy και από άτομα που είχαν αναπτυχθεί ως προνύμφες σε τεχνητό υπόστρωμα με BT. Οι πρώτοι απόγονοι (F_1) μη ακτινοβολημένων και ακτινοβολημένων αρσενικών ατόμων *C. pomonella*, προέρχονταν από γονείς στους οποίους έγινε εφαρμογή με εμπορικό σκεύασμα BT (Dipel® 2X). Οι απόγονοι F_1 χωρίς ακτινοβολία ήταν σημαντικά λιγότερο ευαίσθητοι στο BT από αυτούς των ακτινοβολημένων γονέων. Τα αποτελέσματα έδειξαν υψηλή θνησιμότητα στους απογόνους F_1 του *C. pomonella*, όταν έγινε συνδυασμένη εφαρμογή ακτινοβολίας γ και BT. Η μέση θανατηφόρα συγκέντρωση (LC_{50}) στους απογόνους F_1 ήταν 2,5 ppm όταν προέρχονταν από ακτινοβολημένους γονείς, ενώ 13 ppm από μη ακτινοβολημένους γονείς. Παρατηρήθηκε σημαντική μείωση της γονιμότητας και της εκκόλαψης των ωών των απογόνων F_1 από ακτινοβολημένους γονείς σε σύγκριση με τους μη ακτινοβολημένους. Η μελέτη έδειξε ότι για τους απόγονους F_1 ο συνδυασμός μεταξύ κληρονομούμενης στειρότητας (Inherited Sterility Technique) και BT είναι χρήσιμος για την επίτευξη ενός προγράμματος ολοκληρωμένης διαχείρισης της καρπόκαψας της μηλιάς.

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