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*Original article (Orijinal araştırma)*

# Susceptibility of *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae) to some commonly used insecticides

*Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae)'un yaygın olarak kullanılan insektisitlere duyarlılığı

**Tange Denis ACHIRI[1]** **Ekrem ATAKAN[1]** **Serkan PEHLİVAN[1*]**

## Abstract

*Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae), a destructive pest of maize, was recently recorded in the Mediterranean Region of Turkey. The pest is considered to be an invasive species, displacing indigenous stem borers in many parts of the world. The aim of the study was to determine the effect of commonly used insecticides in a maize production system in Turkey on egg hatch and mortality of first instar larvae of *C. partellus* under laboratory conditions (27±2ºC, 70% RH and 14:10 h L:D photoperiod) at the Plant Protection Department, Faculty of Agriculture, University of Çukurova. Eight insecticides registered for indigenous lepidopteran pests were used at the recommended rates. The percentage of hatched egg masses were significantly different. The smallest percentage was 30.6% with deltamethrin. The percentages of hatched egg masses were greater than 80% for all other insecticides. Mortality of hatched larvae was significantly different. The highest and lowest mortalities of hatched larvae were 84.5% and 38.2% with emamectin-benzoate and lambda-cyhalothrin, respectively. Seventy-two h after exposure of the first instar larvae to sprayed maize leaf disks, the lowest and highest mortalities were 62.6% and 96.7% with indoxacarb and emamectin-benzoate, respectively. Survival analyses revealed that hazard ratios ranged from 4.91 (95% CI: 1.66-14.6) to 15.6 (95% CI: 5.33-45.6) with chlorpyrifos-ethyl and emamectin-benzoate, respectively. The mortality of first instar larvae was about 16, 10 and 9 times that of the control with emamectin-benzoate, lambda-cyhalothrin and deltamethrin, respectively. Feeding activity of larval stage was reduced by all treatments. The implications of this study are discussed.

**Keywords:** Black-head stage, *Chilo partellus*, hatch, mortality, survival, Turkey

## Öz

*Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae), Türkiye'de Akdeniz Bölgesi'nde son zamanlarda saptanan oldukça zararlı bir mısır zararlısıdır. İstilacı bir tür olarak değerlendirilen *C. partellus*, dünyanın birçok bölgesinde diğer yerli mısırkurtlarının yerini almaktadır. Bu amaçla, Türkiye'de mısır üretim alanlarında diğer Lepidopter türlerine karşı yaygın olarak kullanılan 8 insektisidin önerilen dozlarının *C. partellus*'un yumurta ve birinci dönem larvalarına etkisi, Çukurova Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü laboratuvarında 27±2ºC, %70 orantılı nem, 14:10 (A: K) koşullarda araştırılmıştır. Bu çalışmayla insektisitlerin yumurtaların açılma oranları ve larva ölüm oranları üzerinde etkili olduğu bulunmuştur. En düşük açılma oranı deltamethrinde %30.6 olarak belirlenmiştir. Diğer tüm insektisit uygulamalarında yumurta açılma oranları %80'nin üzerinde saptanmıştır. Larvaların ölüm oranlarında ise en yüksek oran %84.5 ile emamectin-benzoat'ta belirlenirken, en düşük oran %38.2 ile lambda-cyhalothrinde belirlenmiştir. İnsektisite daldırılmış mısır yaprak disklerinde ise birinci dönem larvaların en düşük ve en yüksek ölüm oranları, 72 saat sonra indoxacarb ve emamectin-benzoat için sırasıyla %62.6 ve % 96.7 olmuştur. Hayatta kalma analizleri, risk oranlarının chlorpyrifos-ethyl ve emamectin-benzoat için sırasıyla 4.9 (%95 CI: 1.66-14.6) ile 15.6 (%95 CI: 5.33-45.6) arasında değiştiğini göstermiştir. Birinci dönem larvalarının ölüm oranlarının kontrol ile kıyaslandığında emamectin-benzoate, lambda-cyhalothrin ve deltamethrinde sırasıyla 16, 10 ve 9 kat yüksek olduğu belirlenmiştir. Ayrıca tüm uygulamalarda larvaların davranışlarında azalma gözlenmiştir.

**Anahtar sözcükler:** Siyah-baş dönemi, *Chilo partellus*, yumurta açılma, ölüm oranı, yaşam, Türkiye

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## Introduction

The maize spotted stem borer, *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae), originally from the Indian subcontinent (Harris, 1989) has been reported in many parts of the world especially in Asia, eastern and southern parts of Africa (Ndema et al., 2001; Kfir et al., 2002; Melaku et al., 2006; Mutyambi et al., 2014), and recently in the Mediterranean Basin (Yonow et al., 2017). Owing to its ability to survive in low and high elevations (Kfir, 1993, 1997; Guofa et al., 2001), and on a broad temperature spectrum (Kfir, 1997; Mutamiswa et al., 2017), and higher potential rate of increase and shorter life cycle (Kioko et al., 1995; Kfir, 1997; Ofomata et al., 2000), *C. partellus* gains competitive advantage over other maize stem borers. *Chilo partellus* is described as an invasive species displacing indigenous maize stem borers and becoming the predominant borer pest (Overholt et al., 1994; Kfir, 1997; Ofomata et al., 2000).

*Chilo partellus* is known to cause severe damage to maize and sorghum wherever it is found (Kfir et al., 2002; Arabjafari & Jalali, 2007). Not only does it damage the vegetative parts of the plant, but it also damages the reproductive parts, causing losses between 24 and 75% (Kumar, 2002), and 80 and 100% in severe infestation in Asia and Africa (Overholt et al., 2000; Arabjafari & Jalali, 2007). The normal damage patterns characteristic of maize stem borers includes bored holes on stems and destroyed internal stem tissue resulting into tunnels (Slabbert & Van den Berg, 2009) and a mixture of rotten, pungent insect and plant tissue debris within the stems. Consequently, the damaged plant is prone to toppling and lodging from any form of disturbance such as heavy rains, storms and strong winds. In addition, *C. partellus* like many other stem borers feed and destroy the growing apex of the maize plant resulting into a condition called deadheart; the growing central leaves die, the plant become stunted and/or dies (Rauf et al., 2017).

Several control and management methods have been employed in an attempt to reduce the damage caused by *C. partellus*. Murenga et al. (2011) presented some control strategies for *C. partellus* and discussed their shortcomings. Resistant maize cultivars have been tried with some success (Ajala et al., 1995; Ahmed et al., 2003; Murenga et al., 2011). Parasitoids such as *Cotesia* spp. (Hymenoptera: Braconidae) and *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) have also been used for control of *C. partellus* with relative success (Kfir et al., 2002; Ahmed et al., 2003). Recently, push-pull technology has gained prominence with a huge adoptability rate especially in the eastern parts of Africa. With regards to push-pull technology, brachiara and Napier grass are among the most commonly used grasses (Van den Berg, 2006; Cheruiyot et al., 2018). The use of pheromones (Beevor et al., 1990), essential oils (Sing et al., 2009) and microorganism-based products (Odindo, 1990; Poinar & Polaszek, 1998) are also gaining grounds. Intercropping is also successfully used for reduction of infestation (Ampong-Nyarko et al., 1994; Pats et al., 1997) up to 30% in maize/sorghum/cowpea-intercropping systems. Other cultural practices such as manipulating planting dates (Van Hamburg, 1979), management of crop residue (Pats et al., 1997), and fertilizer application are also being investigated and used (Van den Berg et al., 1991). However, insecticides applied in foliar and granular formulations are also widely used as preferred solution by farmers since they remain the most convenient control method (Rauf et al., 2017). The insecticides are most effective if applied in the initial stages of an infestation in order to prevent egg hatch and eliminate first and second instar larvae before burrowing in the maize stems (Kfir et al., 2002; Kumar, 2002). In Pakistan, foliar application of fenvalerate, endosulfan, cypermethrin, monocrotophos, quinalphos, and granular application of chlorpyrifos-ethyl and carbofuran are commonly recommended for management of maize stem borers (Mathur & Satyadev, 1992; Katole & Mundiwate, 1995; Bhat & Baba, 2007). Entomopathogens (Odindo, 1991; Gardeze et al., 1998) and novel insecticides such as novaluron, spinosad, emamectin-benzoate have also shown promising results against *C. partellus* (Rameash et al., 2012). Cypermethrin, carbofuran and methamidophos are commercially available in India against the maize stem borers (Khan & Amjad, 2000). According to Van den Berg & Van den Westhuizen (1995), endosulfan and deltamethrin are also used against *Chilo partellus* in South Africa.

Recently, the spotted stem borer was recorded in the Mediterranean Region of Turkey in 2014 (Sertkaya et al., 2014) causing a stir among maize farmers, who have been battling with the voracious indigenous lepidopteran maize stem borer pests such as *Sesamia nonagrioides* (Lefebvre, 1827) (Lepidoptera: Noctuidae) *Ostrinia nubilalis* Hübner, 1796 (Lepidoptera: Crambidae), and *Spodoptera* sp. (Lepidoptera: Noctuidae) especially in the second maize growing season, July-October (Okyar & Kornoşor, 1997). Farmers in Turkey rely almost entirely on insecticides to control maize stem borers. While research is ongoing on the population dynamics and other aspects of *C. partellus*, preliminary results reveal that it is present in maize in Turkey in both the first (April-June) and second (July-October) maize growing seasons, with an infestation rate of 5 to 55% and 20 to 90%, respectively (unpublished data). There are currently no prescribed insecticides in the Turkish market registered for *C. partellus* control. This study was designed to evaluate the potential of some insecticides used against other stem borers for control of *C. partellus*. As such, eight commonly used insecticides in maize production systems used against stem borers in Turkey were screened on eggs and first instar larvae of the spotted stem borers under laboratory conditions.

## Materials and Methods

### Stem borer colony

The stem borer colony used in this study was the F1 generations of field-collected larvae of *C. partellus*. In June 2018, larvae were collected from a maize field (not sprayed with insecticides) in the Research and Implementation Area of Çukurova University (39°01’50.5" N; 35°21’06.7" E). The larvae were reared on insecticide-free maize stalks (Pioneer Hybrid 1/2013) in the entomology laboratory of Plant Protection Department, Faculty of Agriculture, University of Çukurova. The maize stalks were cut (10 cm) and packed in plastic cups (10 x 10 x 10 cm) covered with a muslin and fitted with a rubber band. The maize stalks were replaced every 5-6 d until pupation. Upon pupation, the pupae were kept in new plastic jars (10 x 10 x 10 cm) lined with a Whatman filter paper for oviposition and covered with a muslin. A cotton ball soaked in water was added in the plastic jar for the adults. Egg masses were laid on the filter paper and these were collected daily for bioassays. The eggs and larvae from these F1 generations were used in the study.

### Insecticides used in the bioassay

Table 1 shows the list of selected insecticides commonly used in maize production systems or registered for other lepidopteran stem borers in Turkey. The insecticides which have different mode of actions such as nerve action, chloride channel activator and insect growth regulators were used. Pesticides were used at the manufacturer’s recommendation for other lepidopteran stem borer pests of maize, diluted in distilled water. Topical and leaf-dip bioassay were used for the hatching and mortality studies, respectively.

### Bioassay

### Effect of insecticides on egg hatch

A drop (5µl) of insecticide was applied with a micropipette to egg masses (~40 eggs) when these were at the black-head stage (4-5 d old), after which the eggs usually hatch within 24 h. The egg mass was placed on a maize leaf disk (5 cm), and then placed on a water-soaked cotton in a cup (5 cm diam. x 2.5 cm high). A hole (1 cm), covered with muslin, was perforated on the lid of the cup. There were three replicates per treatment. The setup was kept in a rearing chamber (27±2ºC, 70% RH and 14:10 h L:D photoperiod). The number of eggs hatched was counted 24 h after insecticide application.

Table 1. Tested Insecticides; description, formulations and uses

| Active ingredient (ai) / Trade name / Firm name / Country | Con. / Form. | Field rate (ai%) | Rates (ppm) | Chemical family | Mode of action | Crops | Target |
|---|---|---|---|---|---|---|---|
| Spinosad / LaserTM / Dow AgroSciences / United Kingdom | 480 g $L^{-1}$/ SC | 200 ml $ha^{-1}$ | 200 | Spinosyn | Ingestion and contact. Nicotinic acetylcholine (nAChR) receptor agonist. Nerve action. | Tomato, eggplant, pepper, potato, strawberry, legumes, pome fruits, vegetables, ornamental plants, grass | Lepidoptera, Coleoptera, Diptera |
| Deltamethrin / Decis® / Bayer CropScience / France | 25 g $L^{-1}$/ EC | 500 ml $ha^{-1}$ | 5000 | Pyrethroid | Ingestion and contact. Paralyses nervous system; knockdown effect. | Pear, vegetables, grapes, pistachio, lentil, chickpea, maize, beet, cereals, sunflower, hazelnut | Mites, thrips, aphids, Lepidoptera, Coleoptera |
| Lambda-cyhalothrin / Karate® / Syngenta Chemicals / Belgium | 50 g $L^{-1}$/ CS | 300 ml $ha^{-1}$ | 3000 | Pyrethroid | Ingestion and contact. Paralyses nervous system; knockdown effect. | Maize, cotton, apple, grapes, potatoes, beet, tomatoes, nuts, cabbage, cereals, olive | Lepidoptera, mites, aphids |
| Chlorantraniliprole / Coragen® / DuPont / France | 200 g $L^{-1}$/ SC | 150 ml $ha^{-1}$ | 1500 | Anthanilic diamide | Ingestion. Ryanodine receptor modulator. | Tomato, eggplant, pepper, cucurbit crops, leafy vegetables, maize, peach, quince, plum | Lepidoptera |
| Indoxacarb / Tunchii® / Astranova / Turkey | 150 g $L^{-1}$/ SC | 300 ml $ha^{-1}$ | 3000 | Oxadiazine | Ingestion and contact. Voltage-dependent sodium channel blocker. | Tomato (field and greenhouse), hazelnut, maize | Lepidoptera |
| Chlorpyrifos-ethyl / Dursban®4 / Dow AgroSciences / United Kingdom | 480 g $L^{-1}$/ EC | 1800 ml $ha^{-1}$ | 1800 | Organophosphate | Ingestion and contact. Inhibits cholinesterase. Nerve action. | Pear, vegetables, grapes, pistachio, lentil, chickpea, maize, beet, cereals, sunflower, hazelnut, cotton | Lepidoptera |
| Emamectin-benzoate / Pancart® / Platin Kimya / Turkey | 5%/ SG | 300 g $ha^{-1}$ | 3000 | Avermectin | Ingestion. Chloride channel activator. | Pepper, tomato (field and greenhouse) | Lepidoptera |
| Novaluron / RIMON SUPRA® / Adama / Israel | 100 g $L^{-1}$/ SC | 400 g $ha^{-1}$ | 4000 | Benzoylphenyl urea | Ingestion and contact. Insect Growth Regulator-inhibits chitin synthesis. | Tomato (field and greenhouse), pepper (greenhouse), cotton, soya, cucumber | Lepidoptera |

Con.: concentration; Form.: formulation; SC: suspension concentrate; SG: soluble granules; EC: emulsifiable concentrate; CS: capsule suspension.

**Effect of insecticides on survival of emerging larvae**

The first instar larvae hatched from insecticide-treated eggs were kept separately on fresh insecticide-free maize leaf disks. The leaf disks were kept on moist cotton in plastic cups (5 cm diam. x 2.5 cm high), and then covered with a perforated lid, sealed with a muslin for ventilation. There were three replicates per treatment. The setup was kept in the temperature chamber (27±2ºC, 70% RH and 14:10 h L:D photoperiod). Mortality of the emerging larvae was assessed after 48 h.

**Effect of insecticides on first instar larvae**

Leaf-dip bioassay was used to evaluate the effect of insecticides on first instar larvae of *C. partellu*s. Fresh maize leaf disks (2 cm) were immersed in insecticide solution for 5 s, and then allowed to dry for 1 h under laboratory conditions (25±2ºC). The leaf disks were then placed on moist cotton in cups and covered with a perforated lid, sealed with a muslin for ventilation. Ten first instar larvae of *C. partellus*, collected from F1 generation of laboratory reared field-collected larvae were placed on the leaf disk with the help of a fine camel hair brush. There were three replicates per treatment. The setup was kept in a climatic chamber set to 27±2ºC, 70% RH and 14:10 h L:D photoperiod. Mortality of the larvae was assessed every 12 h for 3 d (72 h). A larva was considered dead if they did not move after being touched by a fine camel hair brush.

**Data analysis**

Normality and homogeneity of variance tests for the data were done using Kolmogorov-Smirnov and Levene's test, respectively. Percentage hatch of black-head stage eggs after 24 h was subjected to one-way analysis of variance (ANOVA) and means were separated by post hoc Duncan's multiple range test (DMRT) procedure at a significance level of 0.05. Mortality of emerged larvae was assessed48 h after hatching. Mortality of hatched larvae after 48 h and first instar larval mortality on insecticide-treated leaves after 72 h was analyzed (one-way ANOVA, DMRT, $P<0.05$). The mortality was also assessed every 12 h for survival analysis.

Survival analysis, which is generally a set of methods for analyzing data where the outcome variable is the time until the occurrence of an event of interest (mortality), was conducted. Kaplan-Meier survival curves were used to estimate median survival times ($LT_{50}$; median survival time is the time at which 50% of the first instar larvae survive) 72 h after exposure to the insecticides and their respective 95% confidence intervals (CI) were determined. Hazard ratios after 72 h were estimated using Cox regression to estimate the probability of mortality of first instar larvae occurring in the insecticide-treated leaves compared to the water-treated (control) leaves at any given time. All analyses were done using Statistical Package for Social Sciences (Ver. 23, 2015).

## Results and Discussion

**Effect of insecticides on hatch of black-head stage egg masses**

The effect of various insecticides on hatch of black-head stage egg masses of *Chilo partellus* are shown in Table 2. There was a significant difference ($F=41.2$, $df=8,18$, $P<0.05$) in the mean percentage of hatched egg mass exposed to different insecticides. The smallest percentage hatch (30.6%) was with deltamethrin, followed by t chlorpyrifos-ethyl (86.3%) and lambda-cyhalothrin (89.9%). The percentage of eggs that hatched from the other insecticides was greater than 90%. It is known that the chorion layer of lepidopteran egg is not particularly permeable to ovicidal and toxic chemicals; nevertheless, some chemicals can pass through. In such events, these toxic chemicals can negatively affect embryonic development and/or result in death (Trisyono et al., 2000; Galvan et al., 2005). Of all the insecticides, deltamethrin significantly prevented hatch of the black-head stage egg masses (Table 2). On average, hatch was about three times lower in deltamethrin relative to the other insecticides. It is not clear why there

was inconsistency in the percentage hatch between deltamethrin and lambda-cyhalothrin as they are members of the same chemical class.

Pineda et al. (2004) reported that spinosad diluted in water did not exhibit any ovicidal activity in *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) eggs. However, when diluted in acetone, the percentage hatch was significantly reduced from 80-89% to 36.0% in water and acetone solvents, respectively. The effect of insecticide on hatching and other biological events in insects is affected by the solvent used. According to Adan et al. (1996) the penetration and deposition of insecticide into insect cuticle is facilitated by organic solvents. In another study, the number of *O. nubilalis* eggs hatching after treatment with indoxacarb, novaluron, spinosad and water (control) at the black-head stage was not significantly different (Boiteau & Noronha, 2007). However, when the insecticides were sprayed 2 d before the black-head stage, the number of eggs that hatched was significantly reduced compared to application at the black-head stage by 60, 80 and 8% for indoxacarb, novaluron and spinosad, respectively. Mahmoudvan et al. (2014) also reported that indoxacarb SC (300 mg $L^{-1}$) and spinosad (480 mg $L^{-1}$) caused ovicidal control of 86 and 100%, respectively on the egg masses of *Plutella xylostella* (L.,1758) (Lepidoptera: Plutellidae). Hexaflumuron (200 mg $L^{-1}$), and lufenuron (1000 mg $L^{-1}$) which are in the same group as novaluron, also gave ovicidal control of 100 and 50%, respectively, in the same study. However, it should be noted that in the experiments of Mahmoudvan et al. (2014), the egg masses used were no more than 10 h old. This suggests that the effect of insecticides on egg hatch also depends on the age of the eggs; the younger the eggs, the more effective the insecticide. High ovicidal effect of novaluron on other lepidopteran has been reported in other studies (Assal et al., 1983; Chockalingam & Noorjahan, 1984).

At the commercial recommended application rate, the percentage hatch of lepidopteran egg masses can be strongly influenced by the solvent (medium) of the insecticide and the age of egg mass. De Smedt et al. (2015) indicated that hydrophobic compounds are more likely to absorb the intermediate and nonpolar poly (*p*-phenylene) PPPs, and therefore can easily attach to the hydrophobic egg surface, causing desiccation of the eggs. For this reason, perhaps, a decreased hatching percentage might have been observed had a hydrophobic solvent like acetone and young egg masses (hours old) been used.

Table 2. Mean percentage hatched black-head stage eggs of *Chilo partellus* 24 h after application of insecticides

| Treatment | Replicates | Egg/mass | Mean (%)±SEM | 95% confidence interval (CI) |
|---|---|---|---|---|
| Control | 3 | 35-40 | 98.2±0.65 c | 97.3-99.2 |
| Spinosad | 3 | 30-34 | 91.6±2.07 bc | 88.2-95.0 |
| Deltamethrin | 3 | 32-40 | 30.6±5.76 a | 21.7-39.6 |
| Lambda-cyhalothrin | 3 | 30-40 | 89.9±1.20 bc | 88.0-91.9 |
| Emamectin-benzoate | 3 | 30-36 | 95.8±0.88 bc | 94.5-97.1 |
| Indoxacarb | 3 | 30-32 | 99.0±0.50 c | 97.9-99.0 |
| Chlorpyrifos-ethyl | 3 | 30-37 | 86.3±1.90 b | 83.4-89.3 |
| Chlorantraniliprole | 3 | 32-40 | 95.1±1.85 bc | 92.2-97.9 |
| Novaluron | 3 | 30-40 | 97.8±1.02 c | 95.6-97.8 |

Number of eggs refers to the number of eggs per egg mass on which topical application of insecticide was made. Sem: standard error of means. 95% confidence interval of percentage hatched eggs of means. The SEM (standard error of means) and the 95% CI was Bootstrapped 1000 times using the bias corrected acceleration. Means in the same column followed by the same letter are not statistically significantly different by DMRT at $p<0.05$. Each replicate had 10 insects.

### Mortality of hatched larvae 48 h after hatching

The mortality rate of larvae 48 h post hatching was also compared across the different insecticides (Figure 1). The percentage mortality was significantly different (F=34.3, df=8,18, P<0.05) across the various insecticides. The highest percentage mortality was observed from emamectin-benzoate (84.5%), and followed by chlorpyrifos-ethyl (84.4%). The lowest mortality was recorded from indoxacarb (23.2%), followed by novaluron (30.5%) and lambda-cyhalothrin (38.2%). The survival of larvae hatched from the insecticide-treated egg masses was impacted by all insecticides. Emamectin-benzoate, chlorpyrifos-ethyl, deltamethrin, spinosad and chlorantraniliprole had higher larvicidal percentages. The dead larvae were probably contaminated by these insecticides as they chew their way out of the egg masses through their mouth or by contact with contaminated surfaces. Indoxacarb is reported to have ovi-larvicidal properties against some Lepidoptera pest such as codling moth and *O. nubilalis* (Boiteau & Noronha, 2007), however, this was not the case in the current study as this chemical had lowest number of dead neonate larvae (Figure 1).

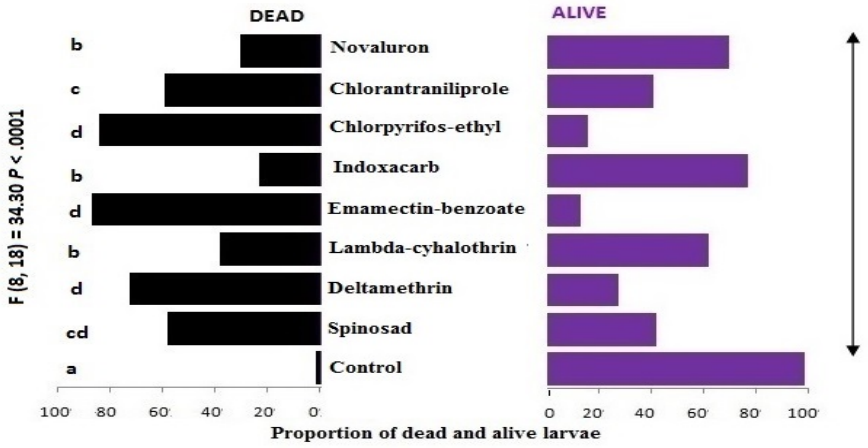


Figure 1. Mean percentage mortality of larvae that hatched from insecticide-treated eggs after 48 h: comparing percentages of dead and alive across different treatments. Bars with the same letter are not statistically different (DMRT, α=0.05). Arrow shows direction of mean comparison.

### Effect of insecticides on first instar larvae

The effect of the various insecticides on the first instar larvae 72 h post application is given in Figure 2. The insecticides significantly influenced (F=8.26, df=8,18, P<0.05) the percentage mortality of first instar larvae. The highest percentage mortality was with emamectin-benzoate (96.7%), and lambda-cyhalothrin (96.3%). The smallest percentage mortality was with indoxacarb (62.2%) and chlorpyrifos-ethyl (62.6%). The percentage mortalities recorded in this study were greater than 60% for all insecticides. The effect of insecticides on the first instar larvae revealed that the first instar larvae were very susceptible to many insecticides. Emamectin-benzoate, pyrethroids and spinosad are known to adversely affect larval stages of lepidopterans such as *O. nubilalis, S. nonagrioides, Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), *P. xylostella* (Pineda et al., 2004; Boiteau & Noronha 2007; Kurt & Kayis, 2014; Mahmoudvan et al., 2014) including *C. partellus* (Tanwar et al., 2017; Kumar & Alam, 2017). Intoxication of the larvae possibly came from contamination from the leaves as they moved around and during feeding.

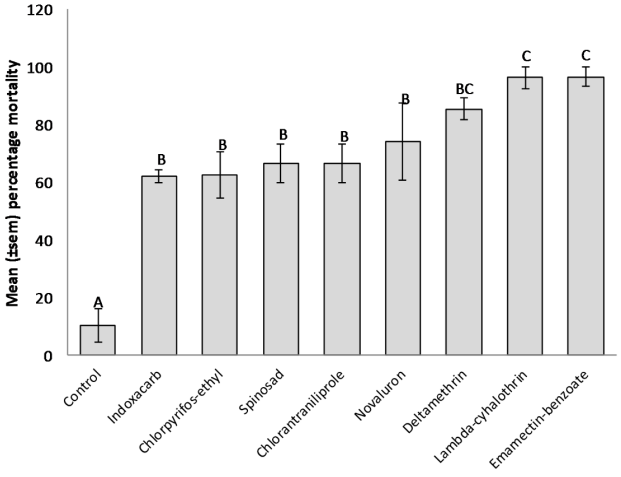


Figure 2. Mean percentage mortality of first instar larvae on insecticide-treated eggs 72 h post exposure to insecticide-infested leaf disks (percentage mortality was transformed using arcsine transformation before analysis). Bars with the same letter are not statistically different (DMRT, α=0.05).

### Feeding behavior

On completion of the bioassays (72 h), the number of feeding scars made by the larvae on the pesticide-treated leaves were counted and scored. The values were subjected to Kruskal-Wallis ANOVA. The amount of leaves consumed; indicated by the mean rank was significantly different ($\chi^2$=18.8, df=8, P<0.05). The highest mean rank was obtained with the control and novaluron treatments. The smallest mean ranks were recorded with deltamethrin, lambda-cyhalothrin, emamectin-benzoate and chlorantraniliprole (Figure 3). The feeding capability experiment revealed that these insecticides reduced the feeding activity of first instar larvae, thereby ensuring adequate photosynthesis for the plants. In this study, deltamethrin, lambda-cyhalothrin, emamectin-benzoate exerted the greatest negative effect on the feeding capability of the first instar larvae. In a related study, Hannig et al. (2009) investigated the effect of chlorantraniliprole and seven other commercial insecticides on the feeding behavior of four lepidopteran species, *P. xylostella*, *Trichoplusia ni* (Hübner, 1803) (Lepidoptera: Noctuidae), *S. exigua* and *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae). For time to feeding cessation and reduction in feeding, chlorantraniliprole was the fastest-acting insecticide followed of emamectin-benzoate, indoxacarb, lambda-cyhalothrin, esfenvalerate and methomyl.

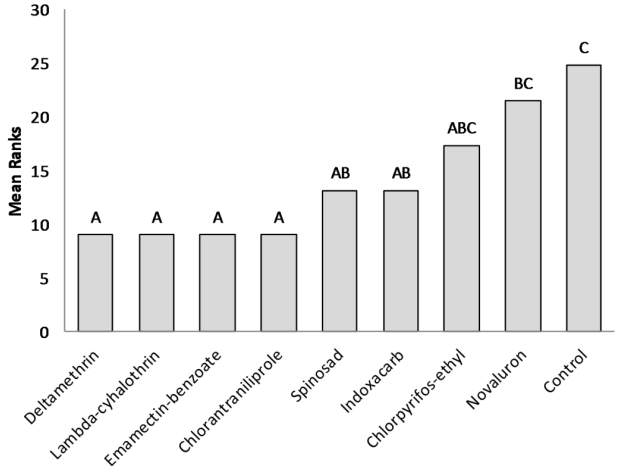


Figure 3. Insect feeding behavior. Mean ranks with the same letter are not significantly different (Duncan's test, α=0.05).

**Survival analysis**

The mortality of the first instar larvae was assessed every 12 h. Figure 4 shows the Kaplan-Meier curve and Table 3 shows the median time (LT50) and the hazard ratios with their corresponding 95% CI for each insecticide.

The steepness of the slope started from 48 h for deltamethrin, with 4 larvae surviving to the end of the study. The steepness for lambda-cyhalothrin is similar to that of deltamethrin; however, only two larvae survived to the end of the study. The steepness began from 24 h for emamectin-benzoate with one larva surviving to the end of the study. The steepness of the slope for indoxacarb started in the 12 h with 11 larvae surviving to the end. The steepness of the slopes for chlorpyrifos-ethyl and chlorantraniliprole started from 36 h with 11 and 10 larvae surviving to the end, respectively. Novaluron also had a steep slope beginning from 36 h with seven larvae surviving to the end (Figure 4). Survival analysis is widely used in scientific research to evaluate the rate of a certain outcome (mortality) over time. The study revealed that emamectin-benzoate had the lowest median time and the highest hazard ratio.

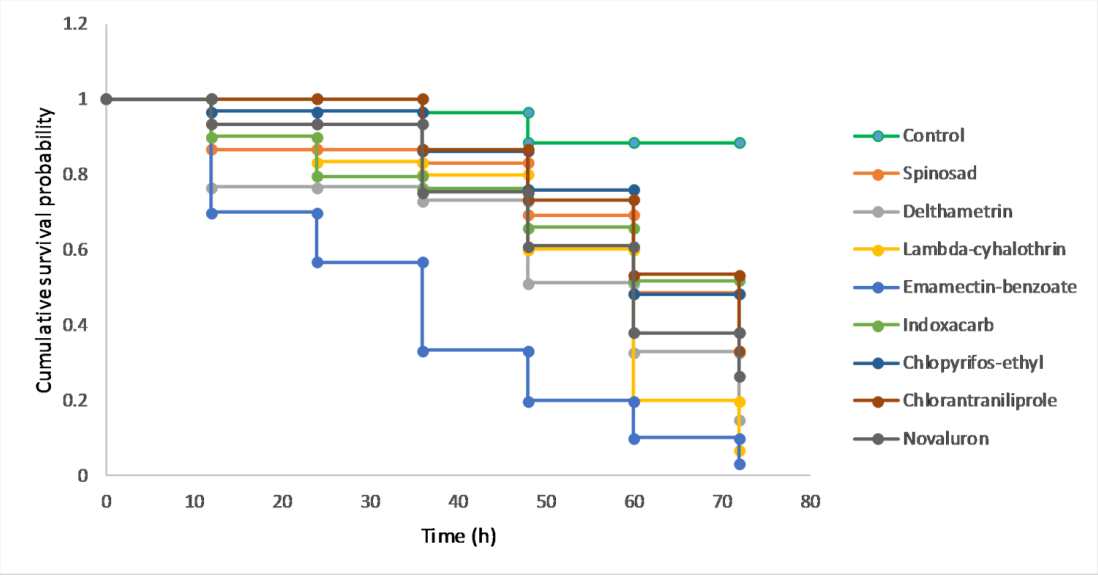


Figure 4. Kaplan-Meier survival curves for first instar larvae of *Chilo partellus* exposed to different insecticide-treated leaf disks after 72 h.

The reference treatment in this study was the control. Since the mortality in the reference treatment never reached 50% in any replicate, it is impossible to calculate median time values for the control. The media time ranged from 36 to 72 h. The shortest median time was recorded with emamectin-benzoate (36h, 95% CI: 27.3-44.7). The highest median time was recorded with indoxacarb (72 h, 95% CI: 56.7-87.3) and chlorantraniliprole (72 h, 95% CI: 61.9-82.1). The median time for all other insecticide was 60 h. For spinosad, the mortality slope was gentle, with 11 larvae surviving to the end of the study. The hazard ratios in this study ranged from 4.91 (95% CI: 1.66-14.6, P<0.0001) with chlorpyrifos-ethyl to 15.6 (95% CI: 5.33-45.6), P<0.0001) with emamectin-benzoate. The mortality of first instar larvae was about 16, 10 and 9 times that of the control with emamectin-benzoate, lambda-cyhalothrin and deltamethrin, respectively.

This study was conducted as an initial attempt to evaluate the effectiveness of insecticides used in maize production system in Turkey in order to develop baseline data against *C. partellus*, a newly recorded pest of maize in Turkey. The findings of this study present the short-term efficacy (acute toxicity) of these insecticides. A better understanding of these insecticides requires a long-term (indirect and subtler effect) study on the physiology and behavior of the target pests and their natural enemies (Biondi et al., 2013; Guedes et al., 2016).

Table 3. Hazard ratios and median time ($LT_{50}$s)

| Treatment | Hazard Ratio | 95% CI | $LT_{50}$ (h) | 95% CI | *P* values |
|---|---|---|---|---|---|
| Spinosad | 5.13 | 1.73-15.18 | 60.0 | 47.15-72.8 | .003 |
| Deltamethrin | 8.82 | 3.04-25.62 | 60.0 | 49.56-70.44 | .0001 |
| Lambda-cyhalothrim | 10.17 | 3.50-29.52 | 60.0 | 55.05-64.95 | .0001 |
| Emamectin-benzoate | 15.58 | 5.33-48.56 | 36.0 | 27.33-44.68 | .0001 |
| Indoxacarb | 5.38 | 1.82-15.93 | 72.0 | 56.67-87.33 | .002 |
| Chlorpyrifos-ethyl | 4.92 | 1.66-14.58 | 60.0 | 50.09 -69.91 | .004 |
| Chlorantraniliprole | 5.39 | 1.82-15.80 | 60.0 | 50.38-69.62 | .002 |
| Novaluron | 6.70 | 2.28-19.72 | 60.0 | 56.32-63.68 | .001 |

Omnibus test for the hazard ratios (chi square $\chi^2$=65.6, df=8, P<0.0001), log rank for $LT_{50}$ (chi square $\chi^2$=80.6, df=8, P<0.0001). CI: confidence interval.

Additional experiments are therefore recommended to determine the ecological effects of these insecticides in field trials. Points of focus could be on the rate and time of application. There are numerous advantages of using insecticides with ovicidal and larvicidal activity, targeting eggs, newly hatched larvae and old larva than insecticides that are only larvicidal. Thus, the role of novaluron and chlorantraniliprole should be considered alongside emamectin-benzoate, lambda-cyhalothrin and deltamethrin as part of an integrated pest management plan.

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*Original article (Orijinal araştırma)*

# Chemical composition and insecticidal potential of different *Origanum* spp. (Lamiaceae) essential oils against four stored product pests

Farklı *Origanum* spp. (Lamiaceae) uçucu yağlarının kimyasal kompozisyonu ve dört depolanmış ürün zararlısına karşı insektisidal potansiyeli

**Mustafa ALKAN[1*]**

## Abstract

This study was conducted to determine the contact and fumigant toxicity of plant essential oils extracted from four *Origanum* spp. against four stored product pests, *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae), *Tribolium confusum* Jacquelin Du Val, 1863 (Coleoptera: Tenebrionidae), *Sitophilus granarius* (L., 1875) and *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae). Chemical composition of the essential oils was determined using GC-MS. The trials were conducted under laboratory conditions in 2019 at Plant Protection Central Research Institute. Essential oils extracted from *Origanum onites* L. and *Origanum vulgare* L. var. *hirtum* caused 100% mortality of *R. dominica* and *T. confusum*. The computed $LD_{50}$ value for *O. vulgare* var. *verticium* against *R. dominica* 24 h after application was 0.046 µl/insect. Single concentration fumigant study indicated that *O. onites* and *O. vulgare* var. *hirtum* essential oils cause high mortality (91 and 70%, respectively) of *R. dominica* within 24 h. Essential oils of *O. vulgare* showed the highest activity against *R. dominica* with $LC_{50}$ and $LC_{90}$ values of 0.0052 and 0.0144 µl/ml, respectively. The main components of *O. onites* essential oil were thymol (22.9%), γ-terpinene (13.0%), p-cymene (12.9%) and carvacrol (7.2%). Similarly, the essential oils of *O. vulgare* var. *hirtum* were composed of carvacrol (32.5%), thymol (16.1%), p-cymene (12.2%) and γ-terpinene (7.9%). Likewise, the essential oil of *O. vulgare* var. *verticium* had carvacrol (35.0%), p-cymene (11.6%), γ-terpinene (10.3%) and thymol (9.1%). Nonetheless, *O. vulgare* x *O. onites* essential oil had carvacrol (15.2%), cis-sabinene hydrate (14.6%), terpinen-4-ol (14.6%) and γ-terpinene (8.7%).

**Keywords:** Contact activity, essential oils, fumigant activity, GC-MS, Lamiaceae

## Öz

Bu çalışmanın amacı dört *Origanum* türünden elde edilen uçucu yağların kontakt ve fumigant etkinliklerini *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae)*, Tribolium confusum* Jacquelin Du Val, 1863 (Coleoptera: Tenebrionidae)*, Sitophilus granarius* (L., 1875) ve *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae) erginlerine karşı belirlemektir. Uçucu yağlarının kimyasal kompozisyonu GC-MS cihazı kullanılarak belirlenmiştir. Denemeler laboratuvar koşullarında 2019 yılında Zirai Mücadele Merkez Araştırma Enstitüsü'nde yürütülmüştür. *Rhyzopertha dominica* ve *T. confusum* erginlerinde *Origanum onites* L. ve *Origanum vulgare* L. var. *hirtum* uçucu yağları %100 ölüme neden olmuştur. Yirmi dört saat sonunda *O. vulgare* var. *verticium* bitki uçucu yağının *R. dominica* için $LD_{50}$ değeri 0.046 µl/böcek olarak hesaplanmıştır. Tek konsantrasyon fumigant etki denemeleri sonucunda *O. onites* ve *O. vulgare hirtum* uçucu yağları 24 saat sonunda *R. dominica*'ya karşı yüksek aktivite (sırasıyla %91 ve %70) göstermiştir. Fumigant konsantrasyon etki denemeleri sonucunda bitki uçucu yağlarından *O. vulgare* uçucu yağı *R. dominica* için en yüksek etkinliği göstermiş ve 24 saat sonunda $LC_{50}$ ve $LC_{90}$ değerleri sırasıyla 0.0052 µl/ml ve 0.0144 µl/ml olarak hesaplanmıştır. *Origanum onites*'in ana bileşenleri, thymol %22.9; γ-terpinene %13.0; p-cymene %12.9; carvacrol %7.2, *O. vulgare* var. *hitsitum* ana bileşenleri carvacrol %32.5; thymol %16.1; p-cymene %12.2; γ-terpinene %7.9, *O. vulgare* var. *verticium* ana bileşenleri carvacrol %35.0; p-cymene %11.6; γ-terpinene %10.3; thymol %9.1, *O. vulgare* x *O. onites'*in ana bileşenleri carvacrol %15.2; cis-sabinene hydrate %14.6; terpinen-4-ol %14.6 ve γ-terpinene %8.7 olarak belirlenmiştir.

**Anahtar sözcükler:** Kontakt etkinlik, uçucu yağ, fumigant etkinlik, GC-MS, Lamiaceae

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## Introduction

Cereals can be infested by many pests after harvest if not kept under appropriate storage conditions. Qualitative and quantitative losses occur in the stored products due to these pests. Different cultural, physicomechanical and chemical control methods are used to reduce the damage caused by stored product pests. Chemical control is the most widely and extensively used method to manage these pests globally. The most commonly used synthetic chemicals to control these pests are methyl bromide and aluminum phosphide (Bond, 1984; Taylor, 1994; Mutungi et al., 2014). The use of these chemicals is being prohibited in the scope of Montreal protocol due to their toxicity against warm-blooded organisms and damage to ozone layer but phosphide is the major fumigant in current use.

Plants employ various defense mechanisms to protect themselves from enemies. Various secondary metabolites synthesized within the plant cells occupy an important place among these mechanisms. These compounds having insecticidal and behavioral activities against various pests (Güncan & Durmuşoğlu, 2004) can be classified as alkaloids, glycosides, phenols, terpenoids, tannins and saponins (Shanker & Solanki, 2000). The plant essential oils contain terpenic or non-terpenic volatile compounds that are hydrocarbons and their derivatives (Başer, 2009).

*Origanum* (Lamiaceae) includes important medicinal aromatic plants and many studies have been conducted on their biological activities. Different activities of *Origanum* spp. such as antioxidant (Dutra et al., 2019), cytotoxic (Coccimiglio et al., 2016), antimicrobial (Lesjak et al., 2016; Reyes-Jurado et al., 2019), anti-acetylcholinesterase (Abou-Taleb et al., 2016; Hajlaoui et al., 2016; López et al., 2018), antibacterial (da Cunha et al., 2018; Wijesundara & Rupasinghe, 2018), repellent (Govindarajan et al., 2016; La Pergola et al., 2017; Giatropoulos et al., 2018), antifungal (Vinciguerra et al., 2018), allelopathic (Boukaew et al., 2017), phytotoxic (Ibáñez & Blázquez, 2018; Grul'ová et al., 2019) insecticidal (Kim et al., 2016; Szczepanik et al., 2018; Benelli et al., 2019) have been determined in a number of studies.

The studies conducted to the determine the essential oil composition of *Origanum* spp. have reported that the main are carvacrol (Martucci et al., 2015; Lesjak et al., 2016), thymol (Mechergui et al., 2016), γ - terpinene (Hajlaoui et al., 2016; Lesjak et al., 2016), p-cymene (Martucci et al., 2015; Hajlaoui et al., 2016; Mechergui et al., 2016), terpinen-4-ol (Hajlaoui et al., 2016), linalool (Aligiannis et al., 2001), sabinene (Hajlaoui et al., 2016), α-terpinene (Hajlaoui et al., 2016), cis-sabinene hydrate (Hajlaoui et al., 2016), terpinene , α-pinene (Martucci et al., 2015) and 4-terpineol (Couto et al., 2015).

Coleoptera is the largest insect order and includes the most common and important stored product pests. The pests belonging to this order live in a wide variety of habitats. Stored product pests have different behavior patterns; thus, some of them are regarded as primary pests, while others are defined as secondary pests. The Curculionidae family includes some of the stored product pests. *Sitophilus* spp. belong to this family and considered as primary pests. The Tenebrionidae family comprises of >10,000 species, of which 100 are stored product pests, and are regarded as secondary pests. Pests belonging to *Tribolium* are in this family.

The contact and fumigant toxicity of essential oils extracted from four *Origanum* spp., *Origanum onites* L., *Origanum vulgare* L. var *hirtum*, *Origanum vulgare* L. var *verticium* and *O. vulgare* x *O. onites* (Lamiaceae) were determined against four important stored product pests, *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae), *Tribolium confusum* Jacquelin Du Val, 1863 (Coleoptera: Tenebrionidae), *Sitophilus granarius* (L., 1875) and *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae). In addition, the essential oil components of these species were determined by GC-MS. Many studies have been conducted on the effect of *Origanum* spp. on storage pests, but the insecticidal activity of the essential oil of *O. vulgare* x *O. onites* was studied for the first time. The results of the study will help to devise alternative and environmentally safe management strategies for control of stored product pests.

## Materials and Methods

### Plant material

Shoots of *O. onites*, *O. vulgare* var. *hirtum*, *O. vulgare* var. *verticium* and *O. vulgare* x *O. onites* were collected during the flowering period July 2018 from the production area of Field Crops Central Research Institute, Ankara, Turkey. All vegetative parts of the plant were used in the production of essential oils. The species were identified by PhD Reyhan Bağdat Bahtiyarca. The herbaria of these species were prepared and deposited at the Directorate of Plant Protection Central Research Institute, Ankara, Turkey.

### Extraction of essential oils

The aerial parts (100 g each) of the air-dried plant samples of all the species were separately hydro-distilled for 4 h using a Clavenger apparatus. Oils yields were 2.2, 4.6, 2.8 and 3.1% for *O. onites*, *O. vulgare* var *hirtum*, *O. vulgare* var *verticium*, and *O. vulgare* x *O. onites*, respectively. The extracted oils were stored at -20°C until analyzed.

### Analysis of essential oils

The GC-MS analysis was performed with an Agilent 5975C InertXL EI/CI MSD system. In the preparation of essential oil samples for analysis GC-MS 20 ml of essential oil and 180 ml of hexane was added to vials. The GC-MS analysis was conducted using an Innowax FSC column (60 m x 0.25 mm) containing helium carrier gas (1 ml/min) with temperature program. The oven temperature was kept at 60°C for 10 min and then raised to 220°C at 4°C/min. The oven was kept at this temperature for 10 min and then temperature was raised to 240°C at 1°C/min. Mass spectra were recorded in the 70 eVita mass range/load ratio of 35-450. GC/FID analysis was performed simultaneously in the same column where GC-MS analysis was conducted with same gas, gas flow and temperature used in GC-MS analysis. RRI (relative retention index) values of the essential oil components were compared with those previously reported in the literature (Başer et al., 1998, 2000, 2001, 2002a, b, 2009; Kirimer et al., 2000; Demirci et al., 2003, 2004, 2006; Jiang & Kubota, 2004; Lourens et al., 2004; Kürkçüoğlu et al., 2006; Tabanca et al., 2006; Özkan et al., 2008; Bardakci et al., 2012; Maggio et al., 2012; Polatoğlu et al., 2012a, b, c, 2013, 2017).

### Insect rearing

The insect cultures were obtained from the stock cultures of the Plant Protection Central Research Institute, Ankara, Turkey. A mixture of ground soft bread wheat and dry yeast [*Saccharomyces cerevisiae* Meyen ex E. C. Hansen, 1883 (Saccharomycetales: Saccharomycetaceae)] was used to rear *T. confusum* and *R. dominica*. The wheat was crushed to coarse size in feed crushing machine and kept in freezer at -18°C for 72 h to eliminate possible contamination by insect and mites. Dry yeast was ground in a grinding mill, sieved through 100 mesh sieves and added to wheat at 5% w/w. Whole wheat grains were used for rearing *S. granarius* and *S. oryzae*. In order to obtain the adults of desired age, adult emergence was recorded daily about 3 weeks after the eggs were taken into jars. The adults emerging between 7 and 28 d after first emergence were used in the study.

### Contact toxicity assay

In single-dose contact activity assays, essential oils were prepared with acetone at a concentration of 0.15% v/v and applied to the ventral of each insect abdomen (1 µl/insect) with micro applicator (Hamilton, Bonaduz, GR, Switzerland). The same amount of acetone was applied to the insects in control treatment of the study. Twenty adult individuals were used in each replication, which were transferred to Petri dishes (6 cm diameter) containing food, and mortality was recorded after 24 and 48 h. The insects unable to move synchronously upon touching with a sable brush were considered as to be dead. The Petri dishes were kept in an incubator at 25±2°C and 65% RH (Polatoğlu et al., 2013). The experiment was laid out according

in a completely randomized design with five replicates. The plant essential oils showing 70% or higher mortality were included in the dose-response assays. The essential oils of all *Origanum* spp. were applied against *R. dominica*, *T. confusum, S. granarius* and *S. oryzae* at different doses ranging from 0.025 to 0.2% v/v and $LD_{50}$ and $LD_{90}$ values were calculated.

**Fumigant toxicity assay**

Glass tubes (10 ml) with airtight caps were used in single concentration fumigant activity assays. Five adult individuals were released in each tube. Discs of 10 mm diameter were cut from Whatman No1 filter paper and attached to the caps of the glass tubes with a needle. Concentrations of essential oils 0.1% v/v were prepared with acetone and 10 µl was applied to each filter paper disc with a micropipette. The same amount of acetone was applied to the insects in a control treatment. The tubes were kept under fume hood for 5 min to allow the acetone to evaporate. The silicon septic caps of the tubes were then closed with a motor creeper. The tubes were incubated in a temperature controlled climatic chamber at 25±2°C and dying insects were recorded after 24 and 48 h of exposures (Polatoğlu et al., 2013). The experiment was laid out in a completely randomized design with 18 replicates. The plant essential oils showing 70% or higher mortality were included in dose-response assays. The essential oils of *O. onites*, *O. vulgare* var *hirtum* and *O. vulgare* var *verticium* were applied against *R. dominica* and *S. oryzae* at different doses ranging from 0.025 to 0.2% v/v and $LC_{50}$ and $LC_{90}$ values were calculated.

**Statistical analysis**

The mortality data recorded in single-dose assays were converted to percent mortality and then transformed by arcsine transformation. One-way analysis of variance was used to test the significance, and treatment means were separated by Tukey's multiple comparison test. The statistical analyses were carried out on MINITAB (Release 16) computer program. The data recorded form dose-response assays were analyzed by Polo-PC probit package program and $LC/LD_{50}$ and $LC/LD_{90}$ values and confidence intervals were computed. Principle component analysis (PCA) was performed with GenStat statistical software.

## Results and Discussion

**Composition of essential oils**

A total of 54 compounds were identified from the essential oil of *O. onites*, which represented 99.1% of the essential oil. Similarly, 50 compounds were recognized from *O. vulgare* var. *hirtum* essential oil, which constituted 97.9% of the oil. The GC-MS analysis identified 43 compounds in the essential oil of *O. vulgare* var. *verticium*, and the identified compounds represented 98.7% of the total essential oil. Likewise, 57 essential oil components of *O. vulgare x O. onites* were identified and represented 97.0% of the oil (Table 1).

The major components of *O. onites* essential oil were thymol (22.9%), γ-terpinene (13.0%), p-cymene (12.9%) and carvacrol (7.2%). Similarly, the main components of *O. vulgare* var. *hirtum* essential oil were carvacrol (32.5%), thymol (16.1%), p-cymene (12.2%) and γ-terpinene (7.9%). Likewise, the major components identified from the essential oil of *O. vulgare* var. *verticium* were carvacrol (35.0%), p-cymene (11.6%), γ-terpinene (10.3%) and thymol (9.1%). Nonetheless, the major essential oil components of *O. vulgare* x *O. onites* were carvacrol (15.2%), cis-sabinene hydrate (14.6%), terpinen-4-ol (14.6%) and γ-terpinene (8.7%). PCA divided the species in two groups based on their essential oil components. The PCA indicated that *O. vulgare* var. *hirtum* and *O. vulgare* var. *verticium* had similar essential oils, but *O. onites* and *O. vulgare* x *O. onites* are different (Figure 1).

Table 1. Essential oil composition of *Origanum onites* (*Ao*), *O. vulgare* var. *verticium* (*Ovv*), *O. vulgare* var. *hirtum* (*Ovh*) and *O. vulgare* x *Origanum onites* (*Ovo*) (All components were identified by mass spectrometry database matches and comparison of relative retention index from the literature)

| Compound | RRI | RRI L. | *Ao* (%) | *Ovv* (%) | *Ovh* (%) | *Ovo* (%) |
|---|---|---|---|---|---|---|
| α-Pinene | 1024 | 1026 | 0.80 | 1.75 | 1.52 | 0.89 |
| α-thujene | 1028 | 1028 | 2.16 | 0.46 | 2.54 | 1.38 |
| Camphene | 1070 | 1069 | 0.25 | 0.32 | 0.28 | 0.06 |
| Hexanal | 1090 | 1087 | - | - | - | 0.01 |
| β-Pinene | 1115 | 1114 | 0.19 | 0.19 | 0.29 | 0.38 |
| Sabinene | 1129 | 1126 | - | - | - | 4.03 |
| δ-3-carene | 1156 | 1159 | 0.15 | 0.20 | 0.20 | 0.03 |
| Myrcene | 1171 | 1168 | 2.83 | 3.18 | 3.06 | 1.69 |
| p-Mentha-1(7).8-diene | 1177 | 1183 | - | - | - | 0.04 |
| α-Terpinene | 1187 | 1183 | 4.95 | 2.27 | 2.21 | 6.12 |
| Dehydro 1.8-cineole | 1197 | 1194 | - | - | - | 0.03 |
| Limonene | 1206 | 1202 | 0.64 | 0.51 | 0.50 | 0.70 |
| 1.8-Cineole (=Eucalyptol) | 1214 | 1212 | - | 0.06 | - | 0.06 |
| β-Phellandrene | 1216 | 1218 | 0.39 | 0.38 | 0.42 | 0.98 |
| (E)-2-Hexanal | 1229 | 1232 | 0.18 | 0.07 | - | 0.14 |
| β-Z-ocimene | 1244 | 1246 | 0.12 | - | 0.07 | 0.57 |
| γ-Terpinene | 1257 | 1251 | 13.00 | 7.93 | 10.33 | 8.69 |
| β-E-ocimene | 1261 | 1265 | 0.10 | 0.08 | 0.08 | 0.10 |
| 5-Methyl-3-heptanone | 1263 | 1265 | - | 0.29 | 0.38 | - |
| p-cymene | 1281 | 1277 | 12.94 | 12.17 | 11.62 | 3.54 |
| α-Terpinolene | 1292 | 1290 | 0.35 | 0.23 | 0.11 | 2.24 |
| 1-Octenyl acetate | 1387 | 1386 | - | 0.07 | - | 0.16 |
| 3-Octanol | 1397 | 1393 | 0.25 | 0.09 | 0.14 | - |
| α. p-Dimethylstyrene | 1451 | 1452 | - | 0.09 | - | - |
| 1-Octen-3-ol | 1456 | 1457 | 2.01 | 0.75 | 1.23 | 0.16 |
| trans-Sabinene hydrate | 1473 | 1469 | 2.12 | 0.43 | 0.71 | 4.40 |
| α-Campholene aldehyde | 1505 | 1500 | 0.14 | - | - | 0.03 |
| Linalool | 1555 | 1552 | 0.68 | 1.24 | 0.09 | 1.95 |
| cis-Sabinene hydrate | 1557 | 1554 | 1.34 | 0.36 | 0.40 | 14.58 |
| Linalyl acetate | 1568 | 1565 | 0.85 | 0.34 | - | 0.28 |
| trans-p-Menth-2-en-1-ol | 1575 | 1570 | 0.30 | 0.10 | - | 2.24 |
| Bornyl acetate | 1596 | 1593 | - | 0.08 | - | 0.12 |
| trans-β-bergamotene | 1598 | 1594 | 0.11 | - | - | - |
| β-Caryophyllene | 1616 | 1609 | 6.82 | 5.77 | 8.71 | - |
| Carvacrol methyl ether | 1619 | 1614 | - | 0.36 | 0.17 | - |
| Terpinen-4-ol | 1620 | 1611 | - | - | - | 14.57 |
| Aromadendrene | 1625 | 1628 | 0.21 | - | 0.06 | - |
| cis-Dihydrocarvone | 1627 | 1624 | | 0.18 | 0.06 | 0.41 |
| p-Menth-3-en-1-ol (=Terpinen-1-ol) | 1639 | 1638 | 0.13 | 0.07 | - | - |
| Terpinen-1-ol | 1640 | 1628 | - | - | - | 1.37 |
| trans-Dihydrocarvone | 1647 | 1645 | - | - | 0.03 | - |
| cis-Isodihydrocarvone | 1649 | 1645 | - | - | - | 0.48 |
| trans-Pinocarveol | 1672 | 1667 | 0.08 | - | - | 0.06 |
| α-Humulene (=α-Caryophyllene) | 1690 | 1685 | 0.19 | 0.35 | 0.83 | 0.16 |
| trans-Piperitol | 1693 | 1688 | - | - | - | 0.59 |
| γ-Muurolene | 1706 | 1702 | - | 0.14 | 0.08 | - |
| α-Terpineol | 1710 | 1706 | 0.67 | 0.62 | 0.25 | 4.37 |
| Borneol | 1717 | 1717 | 1.57 | 0.95 | 0.65 | 0.16 |
| Germacrene D | 1730 | 1726 | - | - | - | 0.05 |
| β-Bisabolene | 1742 | 1741 | 5.58 | 0.71 | 1.49 | 0.31 |
| Bicyclogermacrene | 1756 | 1755 | - | - | - | 0.56 |

Table 1. Continued

| Compound | RRI | RRI L. | *Ao* (%) | *Ovv* (%) | *Ovh* (%) | *Ovo* (%) |
|---|---|---|---|---|---|---|
| cis-Piperitol | 1759 | 1756 | 0.16 | - | - | 0.89 |
| Carvone | 1760 | 1755 | - | 0.11 | - | - |
| Geranyl acetate | 1769 | 1765 | 0.22 | 0.12 | - | 0.06 |
| γ-Cadinene | 1781 | 1774 | 0.23 | 0.09 | 0.08 | - |
| β-Sesquiphellandrene | 1787 | 1783 | 0.22 | - | - | - |
| trans-Carveol | 1850 | 1845 | - | - | - | 0.10 |
| Geraniol | 1856 | 1852 | 0.21 | 0.11 | - | 0.08 |
| p-Cymen-8-ol | 1865 | 1860 | 0.09 | 0.08 | 0.08 | 0.06 |
| Thymyl acetate | 1870 | 1868 | 0.34 | - | - | - |
| Carvacryl acetate | 1894 | 1890 | - | 0.32 | 0.48 | 0.16 |
| Piperitenone oxide | 1987 | 1983 | 0.13 | - | 0.17 | - |
| Isocaryophyllene oxide | 2007 | 2001 | 0.13 | 0.41 | 0.52 | - |
| Caryophyllene oxide | 2022 | 2007 | 1.15 | 4.02 | 3.56 | 0.07 |
| (E)-Nerolidol | 2052 | 2045 | 0.13 | - | - | - |
| Humulene epoxide-III | 2080 | 2081 | - | 0.26 | 0.25 | - |
| Elemol | 2101 | 2096 | 0.31 | - | - | - |
| Globulol | 2102 | 2098 | - | - | - | 0.07 |
| Cumin alcohol | 2124 | 2113 | - | - | 0.07 | - |
| Spathulenol | 2154 | 2142 | 0.58 | 0.15 | 0.31 | 0.71 |
| Isothymol | 2185 | 2180 | 0.12 | 0.39 | 0.25 | - |
| Eugenol | 2194 | 2187 | 0.08 | - | - | - |
| Thymol | 2203 | 2198 | 22.94 | 16.05 | 9.13 | 0.46 |
| 4-Isopropyl-2-methylphenol | 2223 | 2219 | 0.12 | 0.43 | 0.32 | - |
| Carvacrol | 2236 | 2239 | 7.22 | 32.49 | 35.00 | 15.23 |
| α-Eudesmol | 2252 | 2242 | 0.54 | - | - | - |
| α-Cadinol | 2260 | 2255 | - | - | - | 0.04 |
| β-Eudesmol | 2262 | 2250 | 0.50 | - | - | - |
| Caryophylla-2(12).6(13)-dien-5β-ol caryophylladienol-I) | 2325 | 2317 | - | - | - | 0.06 |
| 14-Hydroxy-β-caryophyllene | 2362 | 2357 | 1.23 | 0.07 | - | - |
| Manoyl oxide | 2384 | 2375 | 0.35 | - | - | - |
| Caryophylla-2(12).6-dien-5β-ol (=caryophyllenol-II) | 2404 | 2392 | - | - | - | 0.04 |
| Aromadendren oxide | 2406 | 2399 | - | - | - | 0.11 |
| Pseudo phytol | 2550 | 2551 | - | - | - | 0.15 |
| Monoterpene hydrocarbons | | | 38,87 | 29,76 | 33,23 | 31,44 |
| Oxygenated monoterpenes | | | 47,35 | 56,51 | 50,65 | 63,98 |
| Sesquiterpene hydrocarbons | | | 7,78 | 6,35 | 9,83 | 0,16 |
| Oxygenated sesquiterpene | | | 4,57 | 4,91 | 4,64 | 1,10 |
| Oxygenated diterpenes | | | 0,35 | - | - | 0,15 |
| Others | | | 0,18 | 0,36 | 0,38 | 0,15 |
| Total | | | 99.10 | 97.89 | 98.73 | 96.98 |

RRI, relative retention index; RRI L., RRI of the compound at same GC column and similar GC condition.

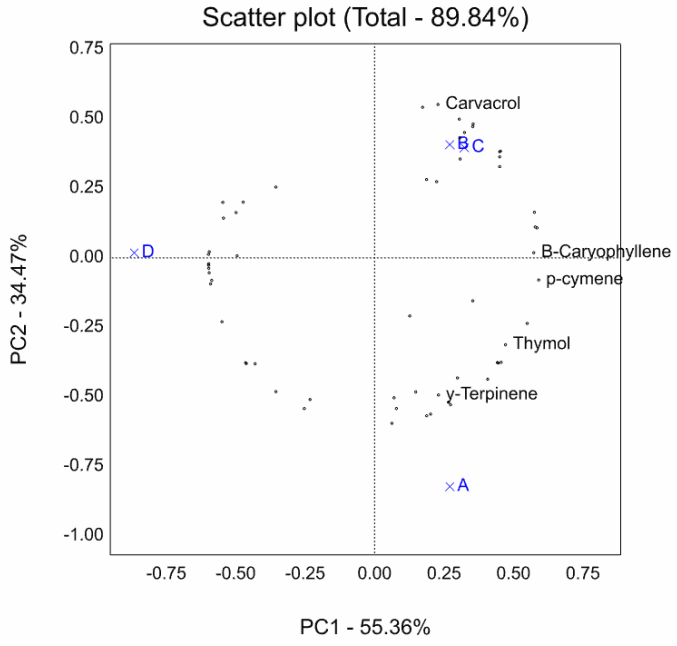


Figure 1. Principal component analysis of essential oil composition of *Origanum* spp. A: *O. onites*; B: *O. vulgare* var. *hirtum*; C: *O. vulgare* var. *verticium*; D: *O. vulgare* x O*riganum onites.*

The chemical composition of the essential oils obtained from *O. onites*, *O. vulgare* var. *verticium*, *O. vulgare* var. *hirtum* and *O. vulgare* x *O. onites* was in line with the findings of previous studies (Aligiannis et al., 2001; Hajlaoui et al., 2016; Mechergui et al., 2016). However, the percentage of different compounds in total oil varied. In a previous study, *Tanacetum chiliophyllum* (Fisch. & C. A. Mey.) Sch.Bip. var. *chiliophyllum* (Asteraceae) was collected from the same region at different times. The essential oil components of the specie as well as biological activities varied with respect to collection time (Polatoğlu et al., 2012c). On the other hand, in a previous study it was reported that the essential oil of *O. vulgare*, contains pulegone, menthone, cis-isopulegone, piperitone and β-myrcene (Abdelgaleil et al., 2016). The current study used subspecies of *O. vulgare*, i.e., *O. vulgare* var. *hirtum* and *O. vulgare* var. *verticium* and the main components of essential oils were carvacrol, thymol, p-cymene and γ-terpinene. Numerous studies have suggested that plant essential oils and their main components have considerable potential to be used in the management of different pests (Isman, 2000; Koul et al., 2008; Lopez et al., 2008; Tripathi et al., 2009).

**Contact toxicity of essential oils**

Single-dose assay indicated that essential oils of all *Origanum* spp. exhibited >70% contact activity against *R. dominica* after 24 h ($F=289$; $df=4,24$; $P < 0.001$). The essential oils of *O. onites* and *O. vulgare* var. *hirtum* caused 100% mortality of *R. dominica*. Similarly, the essential oils of *O. onites* and *O. vulgare* var. *hirtum* caused 100% mortality in *T. confusum*, whereas 18.3% and 7.7% mortality were recorded with essential oils of *O. vulgare* var. *verticium* and *O. vulgare* x *O. onites*, respectively ($F=58.3$; $df=4,24$; $P < 0.001$). *S. oryzae* showed high sensitivity to applied essential oils as >90% mortality was recorded with the essential oils of all species except *O. vulgare* x *O. onites* ($F=150$; $df=4,24$; $P < 0.001$). The essential oils included in the study indicated high contact activity against *S. granarius* as >99.2% mortality was recorded with all essential oils after 24 h except *O. vulgare* x *O. onites* which caused 21.5% mortality ($F=537$; $df=4,24$; $P < 0.001$). The activity of plant essential oils was linearly increased with time after 48 h (Table 2).

Table 2. Single-dose (0.15% v/v) contact activities of different *Origanum* spp. essential oils against test insect species

| | | Mortality±SE (%) | | | | |
|---|---|---|---|---|---|---|
| | | Control | *Ao* | *Ovh* | *Ovv* | *Ovo* |
| 24 ETH | *Rd* | 0.2±0.45 c[1] | 100.0±0.00 a | 100.0±0.00 a | 99.8±0.45 a | 75.7±0.76 b |
| | *Tc* | 0.2±0.45 c | 99.6±0.92 a | 99.2±0.68 a | 18.3±5.44 b | 7.7±1.45 bc |
| | *So* | 1.7±1.37 d | 100.0±0.00 a | 100.0±0.00 a | 92.8±1.08 b | 48.0±0.38 c |
| | *Sg* | 0.0±0.00 c | 100.0±0.00 a | 100.0±0.00 a | 99.2±0.68 a | 21.5±0.43 b |
| 48 ETH | *Rd* | 0.2±0.45 c | 100.0±0.00 a | 100.0±0.00 a | 99.8±0.45 a | 82.4±0.35 b |
| | *Tc* | 0.2±0.45 c | 99.6±0.92 a | 99.8±0.45 a | 19.9±5.83 b | 8.6±1.56 bc |
| | *So* | 1.7±1.37 c | 100.0±0.00 a | 100.0±0.00 a | 98.8±1.05 a | 73.3±0.38 b |
| | *Sg* | 0.0±0.00 c | 100.0±0.00 a | 100.0±0.00 a | 100.0±0.00 a | 25.7±0.34 b |

[1] Values followed by the same letter within a row are not statistically different (ANOVA $P < 0.05$, Tukey test). *Ao*, *Origanum onites*; *Ovh*, *O. vulgare* var. *hirtum*; *Ovv*, *O. vulgare* var. *verticium*; *Ovo*, *O. vulgare* x O. *onites;* ETH, exposure time (h); *Rd, Rhyzopertha dominica*; *Tc*, *Tribolium confusum*; *So*, *Sitophilus oryzae*; and *Sg*, *S. granarius*.

The $LD_{50}$ and $LD_{90}$ values of essential oils included in dose-response assays were computed. The essential oils exhibited varying activity against *R. dominica*. Essential oils of *O. vulgare* var. *verticium* exhibited the highest contact activity against *R. dominica* with $LD_{50}$ value of 0.046 µl/insect, which was followed by the essential oils of *O. vulgare* var. *hirtum* and *O. onites* with $LD_{50}$ values of 0.068 and 0.070 µl/insect, respectively. The highest contact activity against *T. confusum* was recorded for the essential oils of *O. onites* with $LD_{50}$ value of 0.083 µl/insect 24 h after application, which was followed by the essential oils of *O. vulgare* var. *verticium* and *O. vulgare* var. *hirtum* with $LD_{50}$ values of 0.095 and 0.103 µl/insect, respectively. The lowest $LD_{50}$ value of 0.061 µl/insect against *S. oryzae* was recorded for the essential oil of *O. vulgare* var. *verticium*. The highest activity against *S. granarius* after 24 h was determined for the essential oil of *O. vulgare* var. *verticium* with $LD_{50}$ value of 0.066 µl/insect. Keeping in view the $LD_{90}$ values, the highest activity against *S. granarius* was recorded with the essential oil of *O. vulgare* var. *hirtum* having $LD_{90}$ value of 0.092 µl/insect (Table 3).

The biological activity of *Origanum* spp. has been tested by different researchers against various storage pests in earlier studies (Kim et al., 2010; Qari & Abdel-Fattah, 2017; Benelli et al., 2019). Furthermore, some of the main components of the essential oils of this genus have been tested for their biological activities under laboratory conditions against storage pests (Ertürk et al., 2017; Shahriari et al., 2018). The highest contact activity against *R. dominica* was recorded with the essential oil of *O. vulgare* var. *verticium* after 24 h ($LD_{50}$ 0.046 µl/insect) in the current study. Two of the main constituents of the essential oil of *O. vulgare* var. *verticium* were carvacrol and thymol. The main components of essential of *Satureja* spp. are carvacrol and thymol, displayed contact activity against *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) with $LD_{50}$ value of 20.1-40.6 µg/adult (Taban et al., 2017). The four storage pests included in the study exhibited varying response to the essential oil of the same species. This might be explained with the chemical composition of the essential oil, as well as the physiological and biochemical differences of different pest species. Previous studies have shown that insects of the same genus or different species to which the same plant essential oil or extract were applied showed varying response (Negehban et al., 2007; Guo et al., 2017; Liang et al., 2017). These results are consistent with the results of the current study.

Table 3. The results of dose-response assays used to determine the contact activity of different essential oils against test insect species

| Essential oil | Insect | ETH | Slope±SE | $LD_{50}$ (µl/insect) (95% fiducial Limit) | $LD_{90}$ (µl/insect) (95% fiducial Limit) |
|---|---|---|---|---|---|
| *Ao* | *Rd* | 24 | 5.97±0.55 | 0.070 (0.065-0.075) | 0.115 (0.105-0.129) |
| | | 48 | 5.51±0.53 | 0.067 (0.062-0.073) | 0.115 (0.104-0.131) |
| | *Tc* | 24 | 4.33±0.43 | 0.083 (0.073-0.092) | 0.165 (0.143-0.202) |
| | | 48 | 4.15±0.42 | 0.080 (0.070-0.090) | 0.164 (0.141-0.204) |
| | *So* | 24 | 5.87±0.63 | 0.075 (0.067-0.082) | 0.124 (0.115-0.138) |
| | | 48 | 5.26±0.48 | 0.054 (0.047-0.060) | 0.094 (0.085-0.108) |
| | *Sg* | 24 | 9.37±0.72 | 0.075 (0.072-0.079) | 0.103 (0.098-0.110) |
| | | 48 | 9.44±0.83 | 0.072 (0.069-0.075) | 0.099 (0.093-0.106) |
| *Ovh* | *Rd* | 24 | 4.40±0.47 | 0.068 (0.058-0.076) | 0.132 (0.113-0.171) |
| | | 48 | 4.26±0.46 | 0.065 (0.054-0.074) | 0.130 (0.110-0.172) |
| | *Tc* | 24 | 9.54±0.97 | 0.103 (0.099-0.108) | 0.141 (0.133-0.152) |
| | | 48 | 9.15±0.99 | 0.102 (0.097-0.106) | 0.140 (0.1032-0.152) |
| | *So* | 24 | 7.64±0.70 | 0.069 (0.065-0.073) | 0.102 (0.096-0.111) |
| | | 48 | 6.91±0.64 | 0.065 (0.060-0.069) | 0.100 (0.093-0.108) |
| | *Sg* | 24 | 10.19±0.88 | 0.068 (0.065-0.071) | 0.092 (0.087-0.098) |
| | | 48 | 10.51±0.92 | 0.067 (0.064-0.070) | 0.089 (0.084-0.095) |
| *Ovv* | *Rd* | 24 | 3.78±0.53 | 0.046 (0.028-0.057) | 0.100 (0.081-0.153) |
| | | 48 | 3.32±0.54 | 0.038 (0.017-0.051) | 0.093 (0.074-0.150) |
| | *Tc* | 24 | 9.55±0.83 | 0.095 (0.091-0.099) | 0.130 (0.123-0.139) |
| | | 48 | 9.93±0.86 | 0.093 (0.089-0.097) | 0.125 (0.119-0.133) |
| | *So* | 24 | 3.15±0.46 | 0.061 (0.050-0.070) | 0.156 (0.131-0.207) |
| | | 48 | 3.33±0.70 | 0.032 (0.018-0.041) | 0.077 (0.065-0.096) |
| | *Sg* | 24 | 7.19±0.64 | 0.066 (0.062-0.071) | 0.100 (0.092-0.112) |
| | | 48 | 8.25±0.74 | 0.065 (0.061-0.068) | 0.092 (0.086-0.101) |
| *Ovo* | *Rd* | 24 | 3.46±0.43 | 0.100 (0.090-0.111) | 0.234 (0.192-0.317) |
| | | 48 | 3.75±0.42 | 0.091 (0.083-0.101) | 0.200 (0.170-0.256) |

*Ao*, *Origanum onites*; *Ovh*, *O. vulgare* var. *hirtum*; *Ovv*, *O. vulgare* var. *verticium*; *Ovo*, *O. vulgare* x O. *onites;* ETH, exposure time (h); *Rd, Rhyzopertha dominica*; *Tc*, *Tribolium confusum*; *So*, *Sitophilus oryzae*; and *Sg*, *S. granarius*.

**Fumigant toxicity of essential oils**

Single-dose (0.1 v/v) fumigant assays exhibited a varying degree of fumigant activity according to insect species and exposure time (Table 4). The plant essential oils of *O. onites* and *O. vulgare* var. *hirtum* showed 90.95% and 70.42% activity against *R. dominica* after 24 h ($F=55.0$; $df=4,89$; $P < 0.001$). The other essential oils did not exhibit a significant activity against this pest. Among different essential oils tested, only *O. onites* essential oil gave 52.7% mortality of *T. confusum*, which was statistically different from the control treatment ($F=48.8$; $df=4,89$; $P < 0.001$). When essential oils were evaluated for fumigant activity

against *S. oryzae*, essential oil of *O. vulgare* var. *verticium* gave 75.5% mortality after 24 h, followed by *O. onites* essential oil which caused 70.3% mortality (F=30.9; df=4,89; P < 0.001). None the tested essential oils had significant toxicity to *S. granarius.*

Table 4. Single-dose fumigant activities of different essential oils against test insect species

| | | Mortality±SE (%) | | | | |
|---|---|---|---|---|---|---|
| | Insect | Control | *Ao* | *Ovh* | *Ovv* | *Ovo* |
| 24 ETH | *Rd* | 0.0±0.00 d[1] | 91.0±2.08 a | 70.4±2.90 b | 44.0±0.93 c | 26.7±1.90 c |
| | *Tc* | 0.0±0.00 b | 52.7±2.05 a | 0.8±1.03 b | 1.1±0.91 b | 0.4±0.84 b |
| | *So* | 1.7±1.06 c | 70.3±2.50 a | 64.3±1.87 a | 75.5±2.09 a | 32.1±2.31b |
| | *Sg* | 0.0±0.00 b | 0.07±0.28 b | 16.4±2.82 a | 3.6±1.88 b | 0.0±0.00 b |
| 48 ETH | *Rd* | 0.0±0.00 c | 91.1±2.87 b | 99.9±0.61 a | 99.7±0.53 a | 78.9±2.64 b |
| | *Tc* | 0.0±0.00 c | 84.7±3.22 a | 17.5±1.75 b | 3.7±1.48 c | 0.1±0.28 c |
| | *So* | 1.7±1.06 d | 99.4±0.74 a | 87.2±2.86 b | 92.9±1.48 ab | 38.0±1.48 c |
| | *Sg* | 0.0±0.00 c | 23.7±1.60 b | 63.4±2.06 a | 26.5±2.66 b | 1.0±1.31 c |

[1] Values followed by the same letter within a row are not statistically different (ANOVA P < 0.05, Tukey test). *Ao*, *Origanum onites*; *Ovh*, *O. vulgare* var. *hirtum*; *Ovv*, *O. vulgare* var. *verticium*; *Ovo*, *O. vulgare* x O. *onites;* ETH, exposure time (h); *Rd, Rhyzopertha dominica*; *Tc*, *Tribolium confusum*; *So*, *Sitophilus oryzae*; and *Sg*, *S. granarius*.

In dose-response assays, essential oil of *O. onites* showed the highest activity against *R. dominica* and $LC_{50}$ and $LC_{90}$ values after 24 h were 0.0052 and 0.0144 µl/ml air, respectively (Table 5). These values were 0.0047 and 0.0124 µl/ml air, respectively after 48 h. The essential oil of *O. onites* showed a significant fumigant activity against *S. oryzae* after 24 h with $LC_{50}$ and $LC_{90}$ values of 0.0135 and 0.0653 µl/ml air, respectively. These $LC_{50}$ and $LC_{90}$ values after 48 h were 0.0101 and 0.0512 µl/ml air, respectively. The $LC_{50}$ and $LC_{90}$ values of *O. vulgare* var. *hirtum* against *R. dominica* after 24 h were 0.0080 and 0.0144 µl/ml air, respectively. The essential oil of *O. vulgare* var. *verticium* was evaluated for fumigant activity only against *S. oryzae*, and $LC_{50}$ and $LC_{90}$ values at the end of 24 h were 0.0104 and 0.0262 µl/ml air, respectively.

Table 5. The results of dose-response assays used to determine the fumigant activity of different essential oils against test insect species

| Essential oil | Insect | ETH | Slope±SE | $LC_{50}$ (µl/ml) (95% fiducial Limit) | $LC_{90}$ (µl/ml) (95% fiducial Limit) |
|---|---|---|---|---|---|
| *Ao* | *Rd* | 24 | 2.91±0.27 | 0.0052 (0.0046-0.0058) | 0.0144 (0.0122-0.0180) |
| | | 48 | 3.03±0.28 | 0.0047 (0.0041-0.0052) | 0.0124 (0.0107-0.0151) |
| | *So* | 24 | 1.87±0.38 | 0.0135 (0.0111-0.0200) | 0.0653 (0.0345-0.0745) |
| | | 48 | 1.81±0.36 | 0.0101 (0.0080-0.0136) | 0.0512 (0.0272-0.0654) |
| *Ovh* | *Rd* | 24 | 5.01±0.58 | 0.0080 (0.0070-0.0087) | 0.0144 (0.0132-0.0164) |
| | | 48 | 5.37±0.76 | 0.0065 (0.0051-0.0074) | 0.0112 (0.0103-0.0127) |
| *Ovv* | *So* | 24 | 3.19±0.40 | 0.0104 (0.0092-0.0119) | 0.0262 (0.0201-0.0435) |
| | | 48 | 2.76±0.38 | 0.0087 (0.0074-0.0099) | 0.0252 (0.0190-0.0446) |

Ao*, Origanum onites;* Ovh*, O. vulgare var. hirtum;* Ovv, *O. vulgare* var. *verticium;* Ovo, *O. vulgare x O. onites*; ETH, exposure time (h); Rd, *Rhyzopertha dominica;* Tc*, Tribolium confusum;* So*, Sitophilus oryzae*; and Sg*, S. granarius.*

*Origanum* spp. used in the study showed significant fumigant activity against *S. oryzae* and *R. dominica*. Several earlier studies determined the fumigant of plant essential oils against *S. oryzae* (Kim et al., 2003; Kim & Park 2008; Cardiet et al., 2012) and *R. dominica* (Shaaya et al., 1991; Lee et al., 2004). The current study indicated that the essential oil of *O. onites* var. *hirtum* had the strongest fumigant activity against *R. dominica*, while *O. vulgare* had the strongest fumigant activity against *S. oryzae*. Lee et al., (2001) indicated that essential oil of eucalyptus exhibited fumigant activity with $LD_{50}$ of 28.9 µl/ml air against tested insect species. Previously, several studies have determined the insecticidal activity of essential oils of the Lamiaceae family against storage pests (Chu et al., 2011; Conti et al., 2011; Kim et al., 2016). The main components of the essential oils exhibiting the highest fumigant activity in the current study are thymol and carvacrol. Previous studies with these two essential oil components or essential oils containing high percentage of these components have found a high fumigant activity against different storage pests (Erler, 2005; Kim & Park, 2008).

In this study, insecticidal effects of essential oils obtained from *Origanum* spp. against four important stored product pests that cause significant damage in warehouses were tested under laboratory conditions and main components of plant essential oils were determined. As a result of the study, it was determined that these plant essential oils have both contact and fumigant activity. It was also concluded that the activity varies depending on the chemical composition of plant essential oils and the insect species applied. This study is a basic study and its future applicability will be demonstrated with the studies to be done.

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*Original article (Orijinal araştırma)*

# Chlorpyrifos and deltamethrin degradation potentials of two *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) strains

## İki *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) suşunun chlorpyrifos ve deltamethrini parçalama potansiyelleri

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## Abstract

Many soil bacteria can degrade the synthetic insecticides chlorpyrifos and deltamethrin by their esterase enzymes and/or by using them as carbon and energy sources. The hypothesis tested was that similar degradation potential could be found in *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) which is used in food fermentations. This study was conducted in-vitro in Bursa Uludağ University laboratories during 2017-2018 to demonstrate the two degradation mechanisms of *L. plantarum* strains LB-1 and LB-2, 4 d after inoculation. Significant growth in LB-1 found in mineral salt (MS) medium containing chlorpyrifos and deltamethrin compared with MS medium without insecticide and any carbon source. This strain also exhibited significantly enhanced hydrolysis activity. These capacities were found lower in LB-2 than LB-1. Based on periodically GC-MS analysis, degradation of chlorpyrifos and deltamethrin in MS medium proceeded by strains LB-1 and LB-2 reached the values of 96 and 90% and 24 and 53% after 3 d, respectively. Significant degradation of deltamethrin with both strains (86-82%) determined after 10 d. The study demonstrated that some *L. plantarum* strains could degrade chlorpyrifos and deltamethrin. Further studies should be conducted to show their effectiveness in the fermentation process of some fruits and vegetables and different bacteria inoculation rates.

**Keywords:** Degradation, esterase, insecticides, lactic acid bacteria, organophosphates, synthetic pyrethroids

## Öz

Birçok toprak bakterisi chlorpyrifos ve deltamethrin gibi sentetik insektisitleri esteraz enzimleriyle ve/veya bunları karbon ve enerji kaynağı olarak kullanarak parçalayabilmektedir. Bizim bu çalışmadaki hipotezimiz gıda fermantasyonu aşamalarında kullanılan *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae)'un benzer bir insektisit parçalama potansiyelinin gösterilmesidir. Bu çalışma, *L. plantarum*'un iki farklı suşunun (LB-1 ve LB-2) aşılamadan sonraki 4 gün içinde, iki farklı insektisit parçalama mekanizmasını göstermek amacıyla 2017-2018 yıllarında, Bursa Uludağ Üniversitesi laboratuvarlarında, in-vitro koşullarda gerçekleştirilmiştir. Herhangi bir karbon ve enerji kaynağı içermeyen mineral tuz (MS) ortamı ile karşılaştırıldığında, chlorpyrifos ve deltamethrin içeren MS ortamında önemli düzeyde LB-1 gelişimi saptanmıştır. Ayrıca, bu suş için önemli düzeyde artan hidroliz aktivitesi de gözlemlenmiştir. Bu özellikler LB-2'de bir miktar daha düşük bulunmuştur. GC-MS cihazı ile yapılan periyodik analizler sonucunda, LB-1 ve LB-2 inoküle edilmiş MS ortamı içinde chlorpyrifos ve deltamethrin'in parçalanma oranları, 3 gün sonra chlorpyrifos için sırasıyla %96 ve 90, deltamethrin için %24 ve 53 olarak belirlenmiştir. Deltamethrin için önemli düzeyde bir parçalanma (%86-82) inkübasyondan 10 gün sonra gerçekleşmiştir. Bu çalışma, denemede kullanılan *L. plantarum* suşlarının chlorpyrifos ve deltamethrin parçalanma potansiyellerinin olduğunu göstermiştir. İleride bazı meyve sebzelerin fermentasyon süreçlerinde kullanımı ve bu suşların farklı inokülasyon oranlarında etkinliğinin belirlenmesi amacıyla daha fazla çalışma yapılması gerekmektedir.

**Anahtar sözcükler:** Parçalanma, esteraz, insektisitler, laktik asit bakterileri, organik fosforlular, sentetik piretroitler

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## Introduction

Although the use of environmentally-friendly and target-specific pesticides have become widespread around the developed and the developing world in recent years, the broad-spectrum synthetic insecticides, organophosphorus and synthetic pyrethroids, are the most commonly used compounds for the control of the critical pests of many cultivated plants with 13 and 4% usage rates, respectively, among all insecticides (FAO, 2019). Also, chlorpyrifos and deltamethrin are the most commonly-used insecticides around the world (Maya et al., 2011; Cycon et al., 2014). Although a number of organophosphorus insecticides including chlorpyrifos have been banned due to hazard on the human, environment and non-target organisms in European Union Countries and Turkey, the use of these compounds has continued in a large part of the world (Anonymous, 2019; EC, 2019). Chlorpyrifos has been commercially used since the 1960s for the control of many insect pests in agriculture areas, is a moderately toxic compound having an acute oral $LD_{50}$ of 135-163 mg/kg for rats and have a relative risk for lung cancer in human (Cho et al., 2009). Although the synthetic pyrethroids are much less toxic to mammals than organophosphorus, they have adverse health effects to human such as lymph node and splenic damage, carcinogenesis, and hormonal activity. The half-life ($DT_{50}$) of chlorpyrifos is between 27 and 386 d, and 386 d under laboratory conditions at 20°C. In the presence of hydroxyl radicals, this can be as short as 6 h. Sunlight, water content and/or soil microorganisms effect chlorpyrifos degradation (Anonymous, 2020; Eaton et al., 2008). The key metabolites are 3,5,6-trichloro-2-pyridinol and 2,3,5-trichloro-6-methoxypyridine, other known metabolite is O-ethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioic acid.

Deltamethrin has been used widely since the 1980s on various crops including all cultivated plants and human-disease vectors. The acute toxicity of deltamethrin was calculated orally as $LD_{50}$ of 114-168 mg/kg for rats (Wu et al., 2006). Depending on the combined effects of some factors such as pH, temperature, microbial activity, metabolism and photolysis, approximately half-life of chlorpyrifos and deltamethrin is 36-46 d and 11-19 d, respectively (Roberts et al., 1998; Simon, 2014). The residues of the two compounds would be found on various foods depending on the degradation duration of the insecticide. The $DT_{50}$ of deltamethrin changes between 21-58 d, and 28 d under laboratory conditions at 20°C. The key metabolites are decamethrinic acid and 3-phenoxybenzoic acid, other known metabolites are 3-(4-hydroxyphenoxy) benzoic acid, 4-hydroxydeltamethrin, 3-phenoxybenzoic acid (Anonymous, 2020).

Previous studies have shown that many soil bacteria could metabolize broad-spectrum synthetic insecticides, i.e., organophosphorus and synthetic pyrethroids by their esterase enzymes and/or by using the insecticides as carbon and energy sources (Lu et al., 2006; Singh & Walker, 2006; Yang et al., 2006; Lakshmi et al., 2008; Chen et al., 2011a, b, c, d, 2012a, b; Fenner et al., 2013). Chlorpyrifos degradation in soil and water occurred with both chemical hydrolysis and microbial activity. In that case, some aerobic bacteria can transform the compound by hydrolysis to produce diethyl thiophosphoric acid and 3,5,6-trichloro-2-pyridinol (Lu et al., 2006; Yang et al., 2006). Similarly, deltamethrin is degraded via both hydrolysis and microbial activity in soil, but slower under anaerobic conditions (Cycon et al., 2014). Deltamethrin degraded firstly to a-hydroxy-3phenoxy-benzeneacetonitrile and 3-phenoxybenzaldehyde with various genera soil bacteria using carboxyl ester. The latter compound is oxidized to 2-hydroxy-4-methoxy benzophenone (Chen et al., 2011b).

Similarly, some lactic acid bacteria (LAB) in some genera such as *Lactobacillus* (Cho et al., 2009; Islam et al., 2010; Zhao & Wang, 2012; Dordevic et al., 2013) and *Leuconostoc* (Cho et al., 2009) can metabolize insecticides by their specific enzymes such as esterase and/or by using these compounds as carbon and energy sources (Choi et al., 2004; Cho et al., 2009; Islam et al., 2010; Kumral & Kumral, 2013). LAB have gained much interest for their health benefits and are widely used as probiotics and starter culture for fermented products because of their generally recognized as safe status (Maragkoudakis et al., 2006). Mainly, the bacteria in fermented product play various roles during fermentation and the primary benefit of

its use is the preservative effect, by suppressing harmful bacterial growth during fermentation or storage periods. Biodegradation of pesticides is a promising technology since its potential for the removal of residues from food and agricultural products (Maragkoudakis et al., 2006). Some previous studies showed that some LAB including *Lactobacillus* spp. are involved in the degradation of insecticides but there is limited information about their potential to be used for the decontamination of foodstuff.

This study aimed to monitor the insecticide degradation potential and mechanisms of two *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) strains isolated from fermented table olives. To demonstrate the use of insecticides as an energy/carbon source, the growth of the *L. plantarum* strains was monitored by plate counts in mineral salt (MS) medium supplemented with nitrogen, and containing chlorpyrifos or deltamethrin as the sole carbon sources. Metabolic capacities of the strains were determined by spectrophotometric esterase enzyme activity tests in the presence and absence of the insecticides. Insecticide degradation potentials of the strains were detected by GC-MS analysis in MS medium periodically.

## Materials and Methods

This study was conducted in-vitro in Bursa Uludağ University laboratories during 2017-2018.

### Test Microorganisms

*Lactobacillus plantarum* strains LB-1 and LB-2 used in this research were previously isolated from the fermentation brines of naturally fermented black olives of Gemlik variety in Bursa (Turkey). Strains were identified by 16s rRNA technique (Kumral et al., 2012) and differentiated from other group members according to Torriani et al. (2001). These strains were selected according to their survival potential in MS medium containing insecticides as sole carbon sources (Kumral & Kumral, 2014, 2016).

### Chemicals and Reagents

Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate) and deltamethrin ([(S)-cyano-(3-phenoxyphenyl) methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane-1-carboxylate) were purchased from Sigma-Aldrich Chemical Company. All other reagents were of analytical grade.

### Monitoring the Growth of Test Strains

The growth media used during the tests were De Man, Rogosa and Sharpe (MRS) broth, the optimum growth medium for LAB, and mineral salt (MS) medium containing no carbon source (Table 1). The growth of both test strains was monitored in; (i) MRS broth, (ii) MS medium, (iii) MRS broth supplemented with insecticides (chlorpyrifos and deltamethrin), (iv) MS medium supplemented with insecticides (chlorpyrifos and deltamethrin). Sterilized MRS broth and MS media tubes supplemented with filter sterilized insecticides at 100 mg/L. All the trials were inoculated with 18-24 h old test strains at a concentration of $10^8$-$10^9$ colony forming units (CFU)/mL and left for incubation at 30ºC for 4 d (Cho et al., 2009). Microbial changes were monitored periodically using the spiral plating method.

### Esterase Activity

The esterase activity of the test strains was detected by using α-naphthyl acetate (α-NA) as the substrate using the method which adapted in part from Morichi et al. (1968). Esterase activity was detected in MS media in the presence and absence of insecticides. MS media without insecticides were used as controls for both test strains. After 16 h of incubation, bacteria cultures were harvested by centrifugation at 10 000 g for 10 min at 4ºC. Bacteria cells were washed twice with 0.1 M phosphate buffer (pH 7.0) and resuspended with the same buffer. The assay mixture contained 60 µl of 100 mM sodium phosphate buffer, pH 7.0, 20 µl of α-naphthyl acetate (10 mM in dimethyl sulphoxide), 100 µl of cell suspension and 20 µl of fast blue RR (1.5 g/L). The absorbance change was kinetically measured at 500 nm at 23ºC in a Bio-Tek

Kinetic Microplate Reader (Winooski, USA) for 60 min. To determine the enhanced esterase activity of the strains, the assays performed in the presence and absence of the insecticides as an activator. The amount of protein in the enzyme source was determined according to the original procedure of Bradford (1976). The formation of the 1-naphthol-Fast Blue RR dye complex was measured at 500 nm and converted to specific activity using a standard curve of 1-naphthol and Fast Blue RR. All of the analysis was done in triplicate and the results were given as µmol p-nitrofenol per minute per mg protein.

Table. 1. Chemical composition of media (de Man et al., 1960; Cho et al., 2009)

| Ingredients | Concentration (g/L) | |
|---|---|---|
| | MS Medium | MRS broth |
| Potassium dihydrogen phosphate | 2.27 | 2.0 |
| Sodium dihydrogen phosphate dodecahydrate | 5.97 | - |
| Sodium chloride | 1 | - |
| Magnesium sulfate heptahydrate | 0.5 | 0.2 |
| Calcium chloride dihydrate | 0.01 | - |
| Manganese sulfate tetrahydrate | 0.02 | 0.05 |
| Ferrous sulfate heptahydrate | 0.05 | - |
| Pepton from casein | 0.01 | 10 |
| Meat extract | - | 8.0 |
| Yeast extract | - | 4.0 |
| Glucose | - | 20.0 |
| Sodium acetate | - | 5.0 |
| Di-potassium hydrogen phosphate | | 2.0 |
| Di-ammonium hydrogen citrate | | 2.0 |
| Tween 80 | | 1.0 |

**Insecticide Residue Analysis**

Extraction and purification of chlorpyrifos and deltamethrin were carried out based on the method of Aksu (2007). Liquid MS medium samples (5 mL) added with anhydrous $MgSO_4$ (2 g) and NaCl (0.25 g) were extracted with 5 mL of acetonitrile-dichloromethane (1:1, v/v) by vortexing for 2 min, and centrifuged at 7000 g for 5 min. The supernatant (2 mL) and $MgSO_4$ (0.15 g) were transferred to a new tube, and vortexed for 2 min, and centrifuged at 7000 g for 5 min. The liquid phase was used for further GC-MS analysis. Concentrations of chlorpyrifos and deltamethrin were measured using Perkin Elmer Clarus 680 Gas Chromatography-Clarus SQ8T Mass Spectrometry (Ohio, USA). Analyses were performed with a capillary column (PerkinElmer Elite-5MS, 30 m, 0.25 mm ID, film thickness 0.25 µm) and using Helium (1 mL/min) as a carrier gas. The injection temperature was 220ºC, and the injection volume was 1 µL. The oven temperature was increased linearly from 70 to 150ºC by 25ºC/min, and 150 to 200ºC by 2.7ºC/min and 200 to 285ºC by 6ºC/min. Retention time for chlorpyrifos and deltamethrin under these chromatographic conditions was 20.8 and 39.5 min, respectively (Figure 1). Five concentration levels from 0.01 to 100 mg/L were analyzed in the GC to show the linear range of detection of the insecticides or to calculate the standard curve by linear regression analysis.

The instrument was operated in SIR mode, and three selected ions for each compound for identification and quantification were monitored (chlorpyrifos, 97, 197 and 199 m/z; deltamethrin 181, 253 and 77 m/z). These ions were selected following the criteria of highest relative abundance, characteristic fragment ions and no interferences with the nearby peaks. Typical GC-MS peaks and selected ions of chlorpyrifos and deltamethrin standards are given in Figure1.

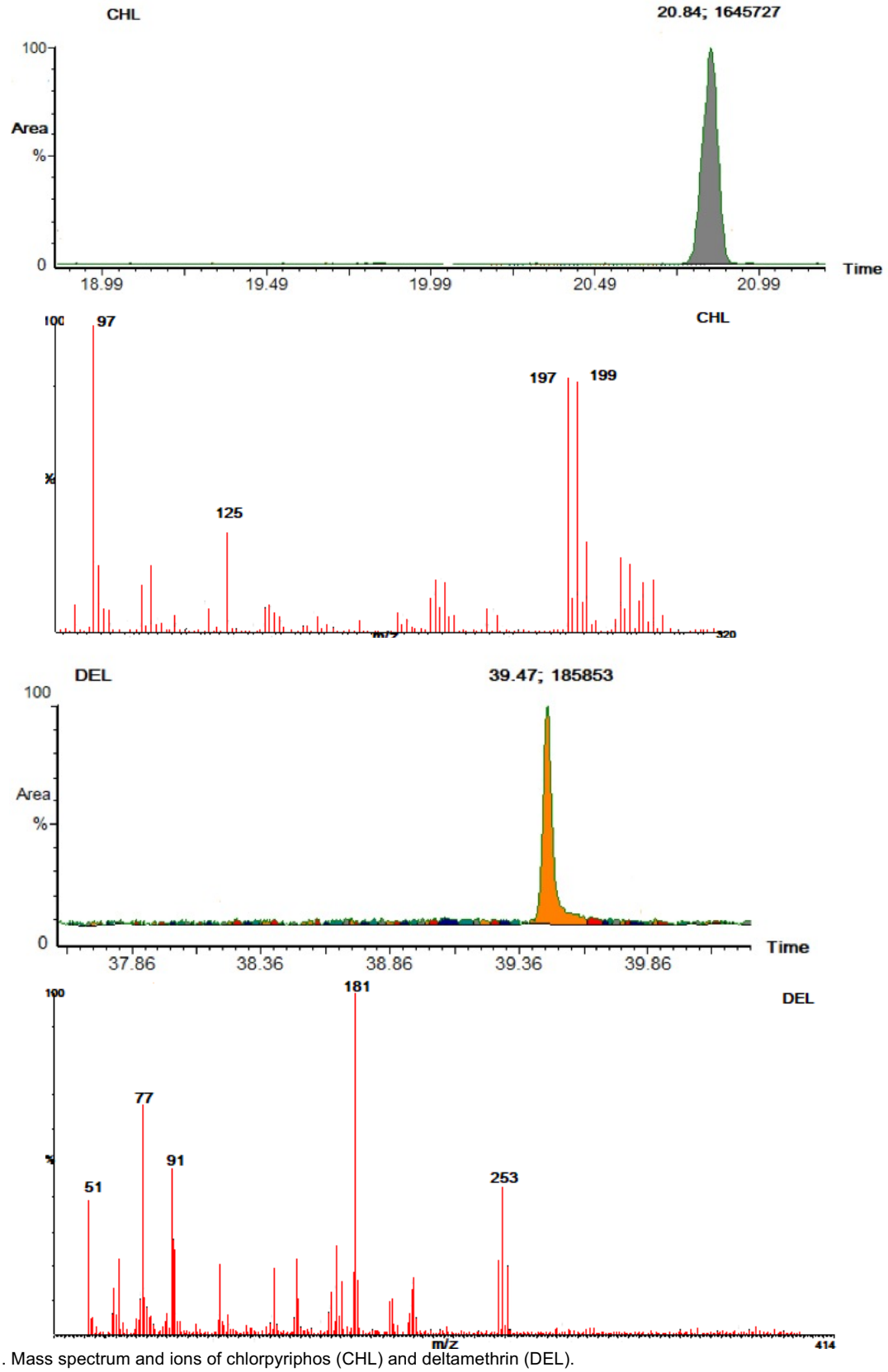


Figure 1. Mass spectrum and ions of chlorpyriphos (CHL) and deltamethrin (DEL).

### Statistical Analysis

Two-way analysis of variance (ANOVA) (two-way) was performed on mean values for each detection time. The effects of time, LB strains, medium on bacteria growth were analysis fit model of SAS. Then post hoc testing ($p<0.05$) of multiple comparisons was performed by Tukey test (SAS 2007).

## Results and Discussion

### Growth of bacteria

LAB is used as starter cultures during industrial food processing to control the overall fermentation process, to reduce the risk of fermentation failure and fermentation period, to improve end product quality and to standardize the process (Kavitake et al., 2018). Especially they are of great industrial significance in that they play a vital role in the manufacturing, flavor, and texture development of fermented dairy foods. Furthermore, additional interest in starter bacteria has been generated because of the data accumulating on the potential health benefits of these organisms (Cogan et al., 2007). The effects of different groups of soil bacteria on pesticide degradation has reported previously (Lu et al., 2006; Singh & Walker, 2006; Yang et al., 2006; Lakshmi et al., 2008; Chen et al. 2011a, b, c, d, 2012a, b; Fenner et al., 2013), but there is scarce information about the potential of LAB. The results of this study showed that, the growth of bacterial strains (LB-1 and LB-2) in the presence and absence of chlorpyrifos and deltamethrin (at 100 mg/L) in MS and MRS medium are shown in Figure 2. In the MS medium containing chlorpyrifos and deltamethrin as the only carbon sources, after 2 d, an increase in the cell numbers of LB-1 was detected compared with that of control without insecticides (Figure 2) ($F_{5,11=}$40.3, $P$<0.01). In MRS medium containing alternative carbon and energy sources, in both of the insecticide containing treatments, a decline was observed in the cell growth of LB-1 in all treatments and control, probably as a result of decreasing energy sources. However, there is no significant difference between MS and MRS media containing insecticides with LB-1 after 4 d ($F_{5,11=}$13.6, $P$<0.01). This result may be evidence for the use of both chlorpyrifos and deltamethrin as a carbon and energy source by LB-1, in the absence of main nutrient sources (Figure 2).

For LB-2 strain, in MS media both with presence and absence of chlorpyrifos and deltamethrin, significant declines were observed in cell numbers after 2 and 4 d compared with those of MRS media ($F_{5,11=}$690, $P$<0.01). In MS medium containing deltamethrin, a slower declining trend was observed between 2 and 4 d (Figure 2). In MRS media containing both insecticides, a significant decline was observed in the cell number of LB-2 compared with that of control (Figure 2) ($F_{5,11=}$125, $P$<0.01). Bacteria growth models for LB-1 and LB-2 were significant ($F_{17,35=}$53.4, $P$<0.01; $F_{17,35=}$321, $P$<0.01). According to the fit model of LB-1 and LB-2, while MRS media significantly increased the growth of the bacteria ($F_{1,1=}$98.3, $P$<0.01; $F_{1,1=}$1720, $P$<0.01), addition of deltamethrin to both media was enhanced only the growth of LB-1 compared with no deltamethrin containing media ($F_{2,2=}$9.6, $P$<0.01).

In the present study, significant cell growth in one of the *L. plantarum* strains (LB-1) was detected in MS media containing chlorpyrifos and deltamethrin as the only carbon source compared to control (MS media without insecticide and any carbon source). Additionally, the growth capacity of LB-2 strain of *L. plantarum* was found weaker compared with LB-1. These results confirm that some strains of *L. plantarum* are capable of using chlorpyrifos and deltamethrin as carbon and energy source. Cho et al. (2009) reported that four LAB [*Lactobacillus brevis* (Orla-Jensen, 1919)*, L. plantarum, Lactobacillus sakei* Katagiri et al., 1934 and *Leuconostoc mesenteroides* (Tsenkovskii, 1878) van Tieghem, 1878] that they isolated from kimchi fermentation, grew well in the first day, but decreased slowly by day 2, and then increased gradually at day 6 in media containing 30 mg/L of chlorpyrifos. In accordance with our results, the authors showed that chlorpyrifos could be utilized by the strains as the sole carbon source. Additionally, it was reported that some soil bacteria [e.g., *Bacillus pumilus* Meyer & Gottheil, 1901*, Pseudomonas* spp., *Serratia liquefaciens* (Grimes & Hennerty, 1931) Bascomb et al., 1971*, Serratia marcescens* Bizio, 1823] metabolized chlorpyriphos

or other organophosphorus insecticides even in the presence of adequate nutrients in environment and its degrading ability was positively influenced by the presence of the supplementary nutrient sources (Nawab et al., 2003; Anwar et al., 2009; Cycon et al., 2009). Similarly, Cycon et al. (2014) showed that culturing bacteria in MS media containing two strains of soil originated *S. marcescens* were capable of using deltamethrin as sources of carbon and energy. Furthermore, pyrethroid-degrading soil bacteria were reported in various genera such as *Bacillus, Micrococcus, Ochrobactrum, Pseudomonas, Sphingobium, Stenotrophomonas* and *Streptomyces* (Madiha et al., 2013; Cycon et al., 2014). Our results are similar to those of other bacteria studies on deltamethrin, the findings obtained from this study are the first attempt of degradation of deltamethrin with *L. plantarum* strains.

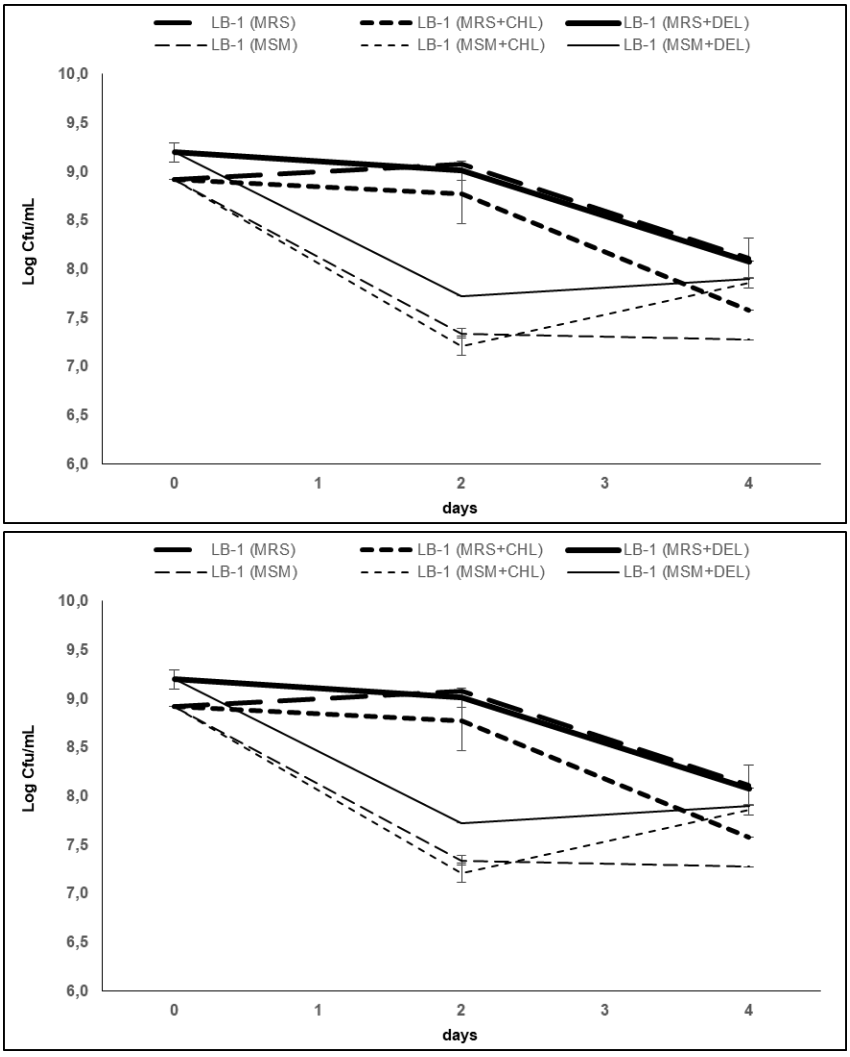


Figure 2. Mean bacterial growth of two *Lactobacillus plantarum* strains, LB-1 and LB-2, in the presence and absence of chlorpyrifos (CHL) and deltamethrin (DEL) in MS and MRS media (CFU, colony forming units).

**Esterase Activity**

The esterase activities in two *L. plantarum* strains at the presence and absence of chlorpyrifos and deltamethrin were given in Table 2. Based on the two-way ANOVA, the effects of strains and pesticide addition on the esterase activity were significant ($F_{5,17}$=17.6, $P$=0.01). There are no significant differences between esterase activities of both strains in the absence of the insecticides (Table 2). When chlorpyrifos or deltamethrin was added to the medium containing LB-1, the level of the esterase activity did not change

significantly ($F_{2,2}$=8.29, $P$=0.006). However, the esterase activity of LB-1 was inhibited 1.8% and 20% with deltamethrin and chlorpyrifos, respectively, and the effects were not significant. In LB-2, when deltamethrin added to the medium, the level of esterase activity decreased significantly. Similarly, the activity of LB-2 was affected by the addition of chlorpyrifos to the medium, but the effect (39.6%) was not found significant. The esterase activity of LB-1 was found significantly higher than that of LB-2 ($F_{1,1}$=53.4, $P$<0.01).

Table 2. The esterase activity in two *Lactobacillus plantarum* strains with and without chlorpyrifos (CHL) and deltamethrin (DEL) in MS medium

| Treatments | Esterase activity (µmol p-nitrofenol/min/mg protein)* | Inhibition rate (%)** |
|---|---|---|
| LB-1 | 20532±2002 a | - |
| LB-1 + DEL | 20165.4±3099 a | 1.78 |
| LB-1 + CHL | 16389±110 ab | 20.18 |
| LB-2 | 15667.8±1054 ab | - |
| LB-2 + DEL | 3002.7±481 c | 80.84 |
| LB-2 + CHL | 9470±851 bc | 39.56 |

* Means are not significantly different with same letters (Tukey, P<0.01);
** Inhibition rate was shown the inhibition of esterase activities of LB strains by insecticides as percentage.

In the present study, LB-1 grew faster and also had higher esterase activity. It is well known that some bacteria can metabolize insecticides by their specific enzymes such as esterase (Cycon et al., 2009). Enzymes initiate the significant mechanism for degradation, which depends upon the nature and type of substrates (Kumral & Kumral, 2013; Simon, 2014). Several studies have shown that many organophosphorus compounds, include esters of phosphoric acid, and can be hydrolyzed by carboxylesterase and phosphotriesterase (Simon, 2014). Cho et al. (2009) pointed out that some strains of *L. brevis, L. mesenteroides*, *L. plantarum* and *L. sakei* were capable of hydrolyzing five organophosphorus compounds, viz., chlorpyrifos, coumaphos, diazinon, parathion and methyl parathion. Similarly, Islam et al. (2010) demonstrated that one strain of *L. brevis* isolated from kimchi was capable of biodegrading chlorpyrifos. The researchers also pointed out that the *L. brevis* strain can hydrolyze chlorpyrifos with some enzymes including esterase and use the hydrolyzed products as their sole source of carbon. Accordance with our results, some investigators demonstrated that the purified recombinant esterase enzyme obtained from some bacteria species, viz., *Bacillus licheniformis* (Weigmann, 1898) Chester, 1901, *Bacillus stearothermophilus* Donk, 1920 and *Lactobacillus casei* (Orla-Jensen, 1916) Hansen & Lessel, 1971, can hydrolyze cypermethrin, permethrin, fenvalerate, deltamethrin, and malathion (Kim et al., 1998; Alvarez et al., 1999; Sogorb & Vilanova, 2002; Choi et al., 2004). Therefore, our results showed that some *L. plantarum* strains can utilize organophosphorus and synthetic pyrethroids through hydrolysis using bacterial enzyme-esterase.

**Insecticide Residues**

Changes in the chlorpyrifos levels of MS media inoculated with LB-1 and LB-2 strains detected with GC-MS analysis are given in Figure 3. During the first 3 d of incubation, the concentration of chlorpyrifos decreased quickly in media inoculated with LB-1 and LB-2 (96 and 90% reduction, respectively). Although there was no significant difference between the two test strains, the degradation rate in control was lower compared to test strains ($F_{2,9}$=13.3, $P$=0.002). After 7 d, a large portion of the chlorpyrifos (93-98%) was degraded in all media including control. Although the reduction rate (98%) in medium contains LB-2 strain was higher than the rates of LB-1 (97%) and control (93%), the differences were not significant ($F_{2,9}$=2.53, $P$=0.14). According to the fit model, LB strains have affected the degradation of chlorpyrifos ($F_{8,31}$=22.2; $P$<0.01). However, in these analysis time and time-treatment interactions were significant (time: $F_{2,2}$=88.7; $P$<0.01; time-treatment: $F_{4,4}$=0.55, $P$=0.69).

Changes in the deltamethrin levels of MS media inoculated with LB-1 and LB-2 strains detected with GC-MS analysis were given in Figure 3. Deltamethrin decomposed rapidly in medium containing LB-1 and LB-2 after 3 d (24 and 53% reduction, respectively). There is a significant difference among media inoculated with LB strains and control (no inoculation) ($F_{2,9}$=2.48, $P$=0.01). After 10 d, in media inoculated with the two strains, deltamethrin was degraded by a large proportion (86 and 82% for LB1 and LB2, respectively). The reduction (59%) in control was significantly lower compared to the media containing LB strains ($F_{2,9}$=11.7, $P$=0.003). Similarly, based on the fitted model, LB strains were affected to the degradation of deltamethrin ($F_{11,38}$=6.25, $P$<0.01). The effects of both time and treatment independently were found significant (time: $F_{2,2}$=16.2, $P$<0.01; treatment: $F_{2,2}$=5.59, $P$=0.007).

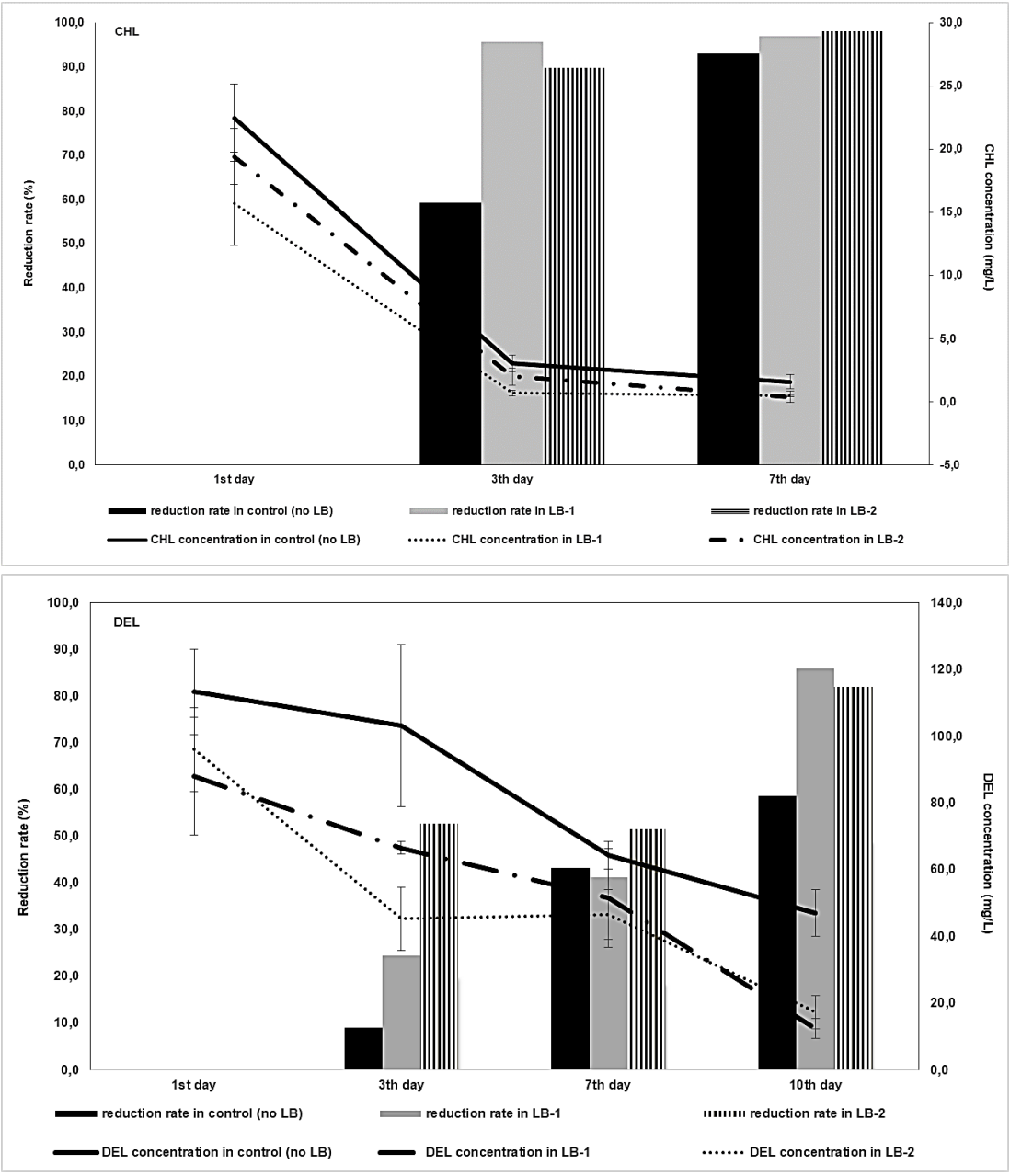


Figure 3. Effects of *Lactobacillus plantarum* strains LB-1 and LB-2 on degradation of chlorpyrifos (CHL) and deltamethrin (DEL) in MS media.

In the present study, degradation of chlorpyrifos and deltamethrin in MS media incubated with the LB-1 and LB-2 reached the values of 96 and 90% and 24 and 53% after 3 d, respectively, based on GC-MS analysis. Significant degradation of deltamethrin by LB-1 and LB-2 strains (86-82%, respectively) was determined after 10 d. This result was consistent with a previous study (Cho et al., 2009) reporting the effects of four LAB (*L. brevis, L. mesenteroides, L. plantarum* and *L. sakei*) on chlorpyrifos degradation. In that study, chlorpyrifos was degraded quickly within 3 d (83.3%), and then complete degradation occurred after 9 d during the fermentation of kimchi at 25ºC for 6 h. Additionally, Lakshmi et al. (2008) showed that some aerobic bacteria isolates obtained from soil samples [*Bacillius subtilis* (Ehrenberg, 1835) Cohn, 1872, *Brucella melitensis* (Hughes, 1893) Meyer & Shaw, 1920, *Pseudomonas aeroginosa* (Schroeter, 1872) Migula, 1900 and *Pseudomanas fluorescens* Migula, 1895] were capable of degrading chlorpyrifos (50 mg/mL) by an enrichment technique, significantly more (45, 50, 69 and 68%) compared with to control (5%) after 6 h of incubation and further increased to 69, 76, 100 and 93% after 12 h respectively. Dordevic et al., (2013), showed that the residue of bifenthrin, one of the synthetic pyrethroids, was reduced rate of 63% in wheat samples fermented with *L. plantarum* within 24 d at 30ºC. The findings obtained from this study are the first evidences about degradation rate of deltamethrin with *L. plantarum.*

## Conclusion

In conclusion, it was shown that some strains of *L. plantarum* can metabolize insecticides by the esterase enzyme and use these compounds as carbon and energy sources. The results are consistent with other publications on the role of bacteria in insecticide degradation in different food commodities (Sogorb & Vilanova, 2002; Wu et al., 2006; Cho et al., 2009; Islam et al., 2010; Zhao & Wang, 2012; Dordevic et al., 2013). Also, the results demonstrated that *L. plantarum* strains have potential for the degradation of the residues. According to our results, there are some differences between strains in terms of growth rate, esterase activity and degradation potential. This could be due to the preadaptation of the bacteria to insecticides, pesticide concentration or bacteria population (Boethling, 1993). In the future, additional LAB species and strains having higher biodegradation capacity should be examined. Also, the potentials of the test strains on the degradation of other insecticides should be studied. Considering the adverse effects of insecticides on human health, to decrease insecticide residues in contaminated foods, experimental studies on the degradation potential of these in some food fermentation processes should be conducted.

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*Original article (Orijinal araştırma)*

# Thrips species associated with medicinal and aromatic plants in Adana (Turkey) with first record of *Bregmatothrips bournieri* Pelikan, 1988 (Thysanoptera: Thripidae)[1]

## Adana (Türkiye)'da tıbbi ve aromatik bitkilerdeki thrips türleri ile *Bregmatothrips bournieri* Pelikan, 1988 (Thysanoptera: Thripidae)'nin ilk kaydı

**Naime Zülal ELEKCİOĞLU[2*]**

## Abstract

Medicinal and aromatic plants are used primarily in the medicine, food and cosmetic industries. Thrips are pests which feed on medicinal and aromatic plants, reducing the yield and commercial value of these crops. This study aimed to determine thrips species on medicinal and aromatic plants, and to contribute new species and hosts in Adana Province (Turkey). Thrips were collected by shaking of vegetative and generative plant parts on medicinal and aromatic plants. Samples were collected from 80 plant species belonging to 33 families in 2017 and 2018. A total of 32 thrips species belonging to four families; Thripidae (20 species), Aeolothripidae (5 species), Melanthripidae (2 species) and Phlaeothripidae (5 species) were identified. The most common and abundant species (with the number of samples and specimens found) were *Frankliniella occidentalis* (Pergande, 1895) (120 and 1379), *Thrips tabaci* Lindeman, 1889 (73 and 507) and *Thrips hawaiiensis* (Morgan, 1913) (42 and 503). Of the predatory thrips, *Aeolothrips collaris* Priesner, 1919 (48 and 144), was the most prevalent one. Most of the thrips species were obtained from plants of Lamiaceae (25 species) and Asteraceae families (15 species). *Bregmatothrips bournieri* Pelikan, 1988 collected on *Cymbopogon citratus* (DC.) Stapf (Poaceae) and *Lavandula angustifolia* Mill. (Lamiaceae) is a newly recorded species for fauna of Turkey.

**Keywords:** Aromatic plant, *Bregmatothrips bournieri*, *Frankliniella occidentalis*, Lamiaceae, medicinal plant

## Öz

Tıbbi ve aromatik bitkiler öncelikle ilaç, gıda ve kozmetik endüstrisinde kullanılmaktadır. Thripsler beslenmeleriyle bu bitkilerin verimini ve ticari değerini düşüren zararlılardır. Bu çalışmada Adana İli'nde (Türkiye) tıbbi ve aromatik bitkilerdeki thrips türlerini belirlemek ve yeni thrips ve konukçu türlerine katkıda bulunmak amaçlanmıştır. Thripsler tıbbi ve aromatik bitkilerin vejetatif ve generatif bitki kısımlarının silkelenmesi ile toplanmıştır. Örnekler 33 familyaya bağlı 80 bitki türünden 2017 ve 2018 yıllarında toplanmıştır. Dört familyaya bağlı toplam 32 thrips türü; Thripidae (20 tür), Aeolothripidae (5 tür), Melanthripidae (2 tür) ve Phlaeothripidae (5 tür) belirlenmiştir. En yaygın ve yoğun türler (örnekleme sayısı ve toplanan birey sayısı), *Frankliniella occidentalis* (Pergande, 1895) (120 ve 1379), *Thrips tabaci* Lindeman, 1889 (73 ve 507) ve *Thrips hawaiiensis* (Morgan, 1913) (42 ve 503) olmuştur. Avcı thripslerden *Aeolothrips collaris* Priesner, 1919 (48 ve 144), en yaygın tür olarak saptanmıştır. Thrips türlerinin çoğu Lamiaceae (25 tür) ve Asteraceae (15 tür) familyalarındaki bitkilerden elde edilmiştir. *Cymbopogon citratus* (DC.) Stapf (Poaceae) ve *Lavandula angustifolia* Mill. (Lamiaceae)'dan toplanan *Bregmatothrips bournieri* Pelikan, 1988 Türkiye faunası için yeni kaydedilen bir türdür.

**Anahtar sözcükler:** Aromatik bitki, *Bregmatothrips bournieri*, *Frankliniella occidentalis,* Lamiaceae, tıbbi bitki

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## Introduction

Thrips (Thysanoptera) are one of the most important insect groups which damage plants by sucking plant juices and scraping at fruits, flowers and leaves. Some are virus vectors and predators (Marullo & De Grazia, 2017). The order Thysanoptera currently consists of 6271 species in the world (ThripsWiki, 2019) of which about 200 species were recorded from Turkey (Tunç & Hastenpflug-Vesmanis, 2016).

Turkey, situated at the junction between Europe and Asia, is one of the richest countries for flora and fauna due to its diverse climatic and topographical conditions. Despite this richness and characteristic features, knowledge of thrips fauna of Turkey is still limited compared to neighboring countries located in the same zoogeographic region. For example, the thrips fauna of Bulgaria and Iran are known to consist of 155 and 270 species, respectively, despite these countries having lower floristic diversity than Turkey (Karadjova & Krumov, 2015; Mirab-Balou, 2018). Adana Province is located in the south part of Turkey, in the Mediterranean region. The region has a Mediterranean climate and known to have a high endemic floristic diversity. Of the nearly 12,000 plant taxa in Turkey, 1/3 of them are endemic (Arslan et al., 2015) and in Adana, 470 endemic species were recorded (Uygur et al., 2018). Turkey is one of the world's richest countries in terms of medicinal and aromatic plants; 30% of the plant species in the natural flora poses medicinal and aromatic properties (Bayram et al., 2010). These plants have been used as drugs to prevent disease, maintain health and wellness in traditional and modern medicine for many years (Demirci Kayıran & Kırıcı, 2019). Besides medicinal industry, medicinal and aromatic plants are used in the food, beverage, perfume and cosmetic industries (Yücer & Altıntaş, 2012). In order to evaluate sustainable production and market potential as competently in these plants, these products must be of the required quantity and quality. Studies on these plants in Turkey are mostly carried out on the cultivation of them. Pests are one of the major factors limiting the production (Simova-Tosia et al., 1997; Abro et al., 2016). They have adverse effects on the secondary metabolites in the plant parts used as drugs, as well as on the loss of productivity (Milek & Simala, 2010; Taşkın, 2015). Also, they cause direct damage such as shortening between nodes, color change in flower and other plant parts, decrease in number and diameter of flowers, leaf curling and leaf deformation. Thrips are one of the harmful pests feeding on the medicinal and aromatic plants (Popov, 1973; Czepiel, 2003; Pobożniak & Sobolewska, 2011).

This study aimed to determine thrips species on medicinal and aromatic plants in Adana Province, contribute new species and to understand their adaptations to different habitats. Such knowledge is of basic importance to explain the introduction and spread of species, particularly pest species. Also, to provide a detail of host plants, description of the new thrips records in order to support comprehensive and specific studies of thrips in Turkey.

## Materials and Methods

### Study sites

The studies were conducted in Karaisalı and Sarıçam districts located at Adana province, Turkey. Thrips species associated with medicinal and aromatic plants were collected from the experimental field of Çukurova University, Karaisalı Vocational School (37°15''12.96' N; 35°4''7.28' E) (Karaisalı) with a 1 ha sampling area and Ali Nihat Gökyiğit Botanical Garden (37°3''2.53' N; 35°21''15.49' E) (Sarıçam) with 0.85 ha sampling area. Thrips collections were carried out with non-periodical surveys during 2017 and April-July 2018.

### Sampling and laboratory processing of specimens

Thrips were collected by shaking of vegetative (leaves and branches) and generative (flowers) plant parts. Five to ten plants from each medicinal and aromatic plant species (according to plant density) were randomly selected every sampling date. Five branches with/without flowers from each of the plants were tapped together into a white container (37 x 28 x 7 cm) for 15 s. The samples collected by fine brush were

then placed separately in plastic vials containing 60% ethanol (Atakan & Uygur, 2005). 178 medicinal and aromatic plant species from Sarıçam and 30 species from Karaisalı were checked. A total of 2795 thrips adults were collected from both districts during the two years. At the laboratory, specimens were extracted, labeled with information regarding their locations, host plants and date of collections. Specimens were identified by Prof. Dr. Ekrem Atakan (Plant Protection Department, Faculty of Agriculture, University of Çukurova, Adana, Turkey).

## Results and Discussion

### Thrips species

Totally 32 thrips species belonging to Thripidae (20 species), Aeolothripidae (5 species), Melanthripidae (2 species) and Phlaeothripidae (5 species) families were identified (Table 1). Of these, *Bregmatothrips bournieri* Pelikan, 1988 is a newly recorded species for the Thysanoptera fauna of Turkey.

Collection data are presented on a plant species basis. After each semicolon comes the collection date and after each date comes the number of collected specimens in brackets. Species are given in separate paragraphs by districts and years. Data on taxonomic information, world distribution and hosts are provided only for the new record species. Locality, collection date and host plants for the determined thrips species are given below.

**Aeolothripidae**

***Aeolothrips albicinctus* Haliday, 1836**

Material examined. Sarıçam, 10.IV.2017 (7), *Glebionis coronaria* (L.) Tzvelev (Asteraceae).

***Aeolothrips collaris* Priesner, 1919**

Material examined. Sarıçam, 05.IV.2017 (1), *Mentha arvensis* L. (Lamiaceae); 10.IV.2017 (1), *Euryops pectinatus* Cass. (Asteraceae); 17.IV.2017 (1), *Dianthus* sp. L. (Caryophyllaceae); 03.V.2017 (1), *Echinacea purpurea* (L.) Moench (Asteraceae); 08.V.2017 (2), *Lavandula stoechas* L. (Lamiaceae); (3), *G. coronaria*; (5), *Nigella sativa* L. (Ranunculaceae); 15.V.2017 (1), *Matricaria chamomilla* L. (Asteraceae); (5), *Perovskia atriplicifolia* Benth. (Lamiaceae); (6), *Achillea asplenifolia* Vent. (Asteraceae); (4), *Dracocephalum moldavica* L. (Lamiaceae); (5), *Melissa officinalis* L. (Lamiaceae); (1), *Thymus serpyllum* L. (Lamiaceae); 17.V.2017 (1), *Trigonella foenum-graecum* L. (Fabaceae); 22.V.2017 (3), *D. moldavica*; (4), *Carthamus tinctorius* L. (Asteraceae); (5), *Dianthus* sp.; (2), *Santolina chamaecyparissus* L. (Asteraceae); 26.V.2017 (2), *Thymbra spicata* L. (Lamiaceae); (1), *Ocimum basilicum* L. (Lamiaceae); 29.V.2017 (1), *P. atriplicifolia*; (3), *Senecio* sp. L. (Asteraceae); (1), *D. moldavica*; 05.VI.2017 (2), *Salvia fruticosa* Mill. (Lamiaceae); (8), *M. officinalis*; (1), *Phytolacca americana* L. (Phytolaccaceae); (1), *S. chamaecyparissus*; (1), *Lavandula angustifolia* Mill.; 12.VI.2017 (1), *Cistus salviifolius* L. (Cistaceae); (7), *Hypericum perforatum* L. (Hypericaceae); (2), *Senna floribunda* (Cav.) H.S. Irwin & Barneby (Fabaceae); 19.VI.2017 (1), *E. purpurea*; (2), *T. serpyllum*; (1), *D. moldavica*; 22.VI.2017 (2), *L. angustifolia*; (5), *Origanum vulgare* L. (Lamiaceae); 03.VII.2017 (1), *Salvia sclarea* L. (Lamiaceae); 06.VII.2017 (1), *H. perforatum*; (1), *Calendula officinalis* L. (Asteraceae); (3), *Cistus creticus* L. (Cistaceae).

Karaisalı, 16.III.2017 (2), *Rosmarinus officinalis* L. (Lamiaceae); 23.VI.2017 (3), *E. pectinatus*; 06.VII.2017 (28), *Mentha spicata* L. (Lamiaceae).

Sarıçam, 03.V.2018 (1), *D. moldavica*; 10.V.2018 (2), *A. asplenifolia*; (2), *L. stoechas*.

Karaisalı, 20.IV.2018 (1), *Pimpinella anisum* L. (Apiaceae); 17.V.2018 (6), *M. spicata*.

***Aeolothrips ericae* Bagnall, 1920**

Material examined. Sarıçam, 10.IV.2017 (2), *Teucrium chamaedrys* L. (Lamiaceae); 01.V.2017 (8), *Nigella damascena* L.; 08.V.2017 (1), *E. pectinatus*; 22.V.2017 (4), *Salvia officinalis*; (1), *D. moldavica*; 12.VI.2017 (2), *Sedum album* L. (Crassulaceae); 21.VI.2017 (1), *Erysimum cheiri* Crantz (Brassicaceae).

Karaisalı, 16.III.2017 (1), *R. officinalis*.

***Aeolothrips gloriosus* Bagnall, 1914**

Material examined. Karaisalı, 11.IV.2017 (1), *S officinalis*.

***Aeolothrips intermedius* Bagnall, 1934**

Material examined. Sarıçam, 10.IV.2017 (1), *E. pectinatus*; 17.IV.2017 (2), *Dianthus* sp.; 21.IV.2017 (1), *Origanum majorana* L.; 24.IV.2017 (3), *Achillea millefolium* L.; 01.V.2017 (1), *Linum usitatissimum* L. (Linaceae); 08.V.2017 (1), *Ranunculus flammula* L. (Ranunculaceae); 22.V.2017 (1), *Artemisia dracunculus* L. (Asteraceae); 26.V.2017 (1), *O. basilicum*; 29.V.2017 (5), *E. purpurea*; (1), *D. moldavica*.

Karaisalı, 29.VI.2017 (1), *Tagetes erecta* L. (Asteraceae).

**Melanthripidae**

***Melanthrips fuscus* Sulzer, 1776**

Material examined. Sarıçam, 10.IV.2017 (2), *Brassica rapa* subsp. *nipposinica* (L. H. Bailey) Hanelt (Brassicaceae); 17.V.2017 (1), *T. foenum-graecum*.

***Melanthrips pallidor* Priesner, 1919**

Material examined. Sarıçam, 01.V.2017 (1), *L. usitatissimum*; 08.V.2017 (1), *Peganum harmala* L. (Nitrariaceae); 15.V.2017 (2), *M. chamomilla*; 10.VII.2017 (1), *Datura innoxia* Mill. (Solanaceae); 18.XII.2017 (1), *Hyssopus officinalis* L. (Lamiaceae).

Karaisalı, 08.V.2017 (2), *R. officinalis*; 05.VI.2017 (2), *S. fruticosa*.

**Thripidae**

***Bregmatothrips bournieri* Pelikan, 1988**

Material examined. Sarıçam, 21.VII.2017 (2♂♂), *Cymbopogon citratus* (DC.) Stapf (Poaceae).

Karaisalı, 03.VII.2017 (1♂), *L. angustifolia*.

The identification keys of the *Bregmatothrips* species in Turkey is recognized as follows (zur Strassen & van Harten, 2007; Elimem et al., 2012; Minaei, 2017):

– Body uniformly brown. Antennae seven segmented, segment I with two dorso-apical setae, III and IV each with forked sense cones. Forewing first vein with two setae on distal half, clavus with three or four veinal and one discal setae. Abdominal sternites with no pore plate.....*B. willcocksi* (Priesner, 1939)

– Body brown to dark brown. Antennae eight segmented, II and III more or less symmetric. Tergites II-VIII with a flange at the hind margin. Pronotum with two pairs of long postero-angular setae, and by the uniformly pale fore wings. Sense cones on antennal segments III and IV are simple not forked ..... .....*B. dimorphus* (Priesner, 1919)

**–** Body bicolored (Figures 1a, b). Antennae 8 segmented (Figure 1c), III and IV with simple sense cones ..... *B. bournieri*

Description. Female large-winged (Figure 1a). Body bicolored, head, prothorax and abdominal tergites (II-X) brown (sometimes prothorax and abdominal tergites (II-VI) yellowish brown), antennal segments (I-II brown, III-V yellow, VI-VIII shaded, fore wings pale (Figure 1c). Head longer than wide with three pairs of ocellar setae present and projecting in front of compound eyes (Figure 1d); maxillary palps two-segmented. Antennae eight segmented (Figure 1c), segment I with two dorso-apical setae, III and IV each with simple sense cones. Pronotum trapezoidal with two pairs of well-developed posteroangular setae (Figure 1e). Mesonotum with weakly transverse sculpture, with no campaniform sensilla (Figure 1f). Metanotum reticulate (Figure 1f), median setae close to the anterior margin; campaniform sensilla absent. Prosternal ferna complete medially. Forewing first vein with three setae on distal half, clavus with 3 or 4 veinal and one discal setae. Abdominal tergite I weakly striate, I-VIII with campaniform sensilla close to posterior margin (Figure 1g), IX with two pairs of campaniform sensilla; X with dorsal split incomplete. Sternites without discal setae. Male microptera, similar in color and structure to female but smaller (Figure 1b). Tergite IX posterior without stout thornlike setae (Figure 1h). Abdominal sternites with no pore plate (Figure 1).

Distribution. Iran, Tajikistan and Turkmenistan.

Comments. The genus *Bregmatothrips* (Hood, 1912) is a common genus in tropical and subtropical areas (Mound & Marullo, 1996; Mound, 2011; ThripsWiki, 2019). Most of the species belonging to the genus are from Asia. In the genus, head protruding considerably in front of the eyes. This genus is closely related to *Sorghothrips* by having antennal segment I with paired median dorsoapical setae, and the abdominal tergites with posteromarginal craspeda and the median campaniform sensilla close to the posterior margin (Masumoto & Okajima, 2006). The damage belonging to the genus is characterized by white and silvery marks on the leaves caused by the cell contents being sucked out (Lewis, 1973). The genus, *Bregmatothrips* consists of 11 species worldwide. Of these only *B. willcocksi* and *B. dimorphus* have been recorded in Turkey (Tunç & Hastenpflug-Vesmanis, 2016). *Bregmatothrips bournieri* is newly defined. This species was originally described from Iran and its neighboring country, Turkmenistan from flowers of *Cynodon dactylon* (L.) Pers. (Pelikan, 1988). Some specimens occasionally also were collected on *Sorghum halepense* (L.) Pers. (Poaceae) (Minaei, 2017). In this study pest adults were collected from leaves of *C. citratus* and flowers of *L. angustifolia.* There is limited data about this species in literature. Further studies are needed to understand its distribution and host plants in Turkey.

***Chirothrips aculeatus* Bagnall, 1927**

Material examined. Sarıçam, 22.V.2017 (2), *Thymus vulgaris* L. (Lamiaceae); 10.VII.2017 (1), *C. officinalis*.

***Chirothrips manicatus* Haliday, 1836**

Material examined. Sarıçam, 12.VI.2017 (1), *C. salviifolius*.

***Frankliniella intonsa* (Trybom, 1895)**

Material examined: Sarıçam, 08.V.2017 (2), *N. sativa*; 22.V.2017 (4), *T. vulgaris*; 29.V.2017 (8), *E. purpurea*; (4), *D. moldavica*.

Karaisalı, 16.III.2017 (4), *M. spicata*; (2), *P. anisum*; (8), *Foeniculum vulgare* Mill. (Apiaceae); 04.V.2017 (2), *S. fruticosa*.

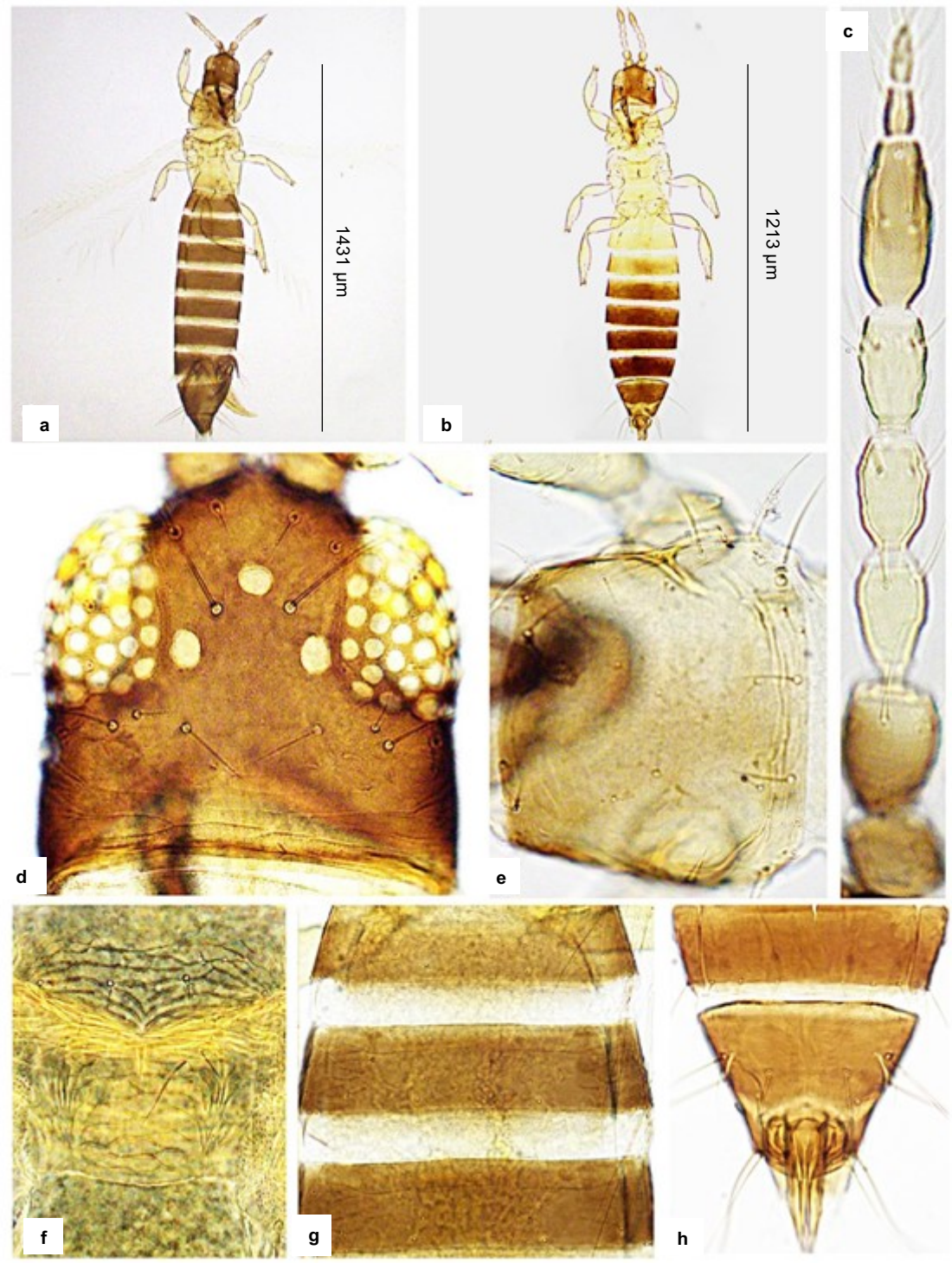


Figure 1. *Bregmatothrips bournieri*: a) female; b) male; c) antenna; d) head; e) pronotum; f) meso and metanotum; g) tergites II-IV; h) tergites VIII-X (male) (from Minaei, 2017).

***Frankliniella occidentalis* Pergande, 1895**

Material examined. Sarıçam, 08.III.2017 (1), *O. basilicum*; 16.III.2017 (1), *N. sativa*; 17.IV.2017 (15), *Dianthus* sp.; 24.IV.2017 (8), *Cynara scolymus* L. (Asteraceae); 01.V.2017 (10), *N. damascena*; 03.V.2017 (7), *E. purpurea*; 08.V.2017 (15), *R. flammula*; (6), *L. stoechas*; (14), *P. harmala*; (12), *Nicotiana tabacum* L. (Solanaceae); (1), *O. vulgare*; (30), *Senecio* sp.; (1), *N. sativa*; 13.V.2017 (2), *L. usitatissimum*; 15.V.2017 (1), *M. chamomilla*; (22), *P. atriplicifolia*; (24), *A. asplenifolia*; (9), *D. moldavica*; 22.V.2017 (1), *T. vulgaris*; (1), *N. sativa*; (25), *C. tinctorius*; (43), *D. moldavica*; (5), *S. officinalis*; (34), *A. dracunculus*; (7), *Tulbaghia violacea* Harv. (Amaryllidaceae); (1), *Helichrysum italicum* (Roth) G. Don (Asteraceae); (10), *Alcea rosea* L. (Malvaceae); (17), *Dianthus* sp.; (31), *S. chamaecyparissus*; 26.V.2017 (2), *O. basilicum*; (3), *Passiflora edulis* Sims. (Passifloraceae); (7), *Myrtus communis* L. (Myrtaceae); 29.V.2017 (12), *Pelargonium crispum* (P. J. Bergius) L'Hér. (Geraniaceae); (5), *Ricinus communis* L. (Euphorbiaceae); (36), *A. millefolium*; (31), *E. purpurea*; (64), *P. atriplicifolia*; (13), *Ecballium elaterium*. A. Rich. (Cucurbitaceae); (4), *P. harmala*; (12), *Momordica charantia* L. (Cucurbitaceae); (7), *L. stoechas*; (20), *T. erecta*; (26), *D. moldavica*; (16), *Senecio* sp.; 05.VI.2017 (1), *C. tinctorius*; (27), *M. officinalis*; (4), *T. serpyllum*; (11), *T. erecta*; (6), *P. americana*; (6), *S. fruticosa*; (11), *Silybum marianum* (L.) Gaertn. (Asteraceae); (3), *S. chamaecyparissus*; (18), *L. angustifolia*; 10.VI.2017 (2), *A. rosea*; 12.VI.2017 (20), *N. damascena*; (14), *H. perforatum*; (38), *Hemerocallis* sp. L. (Asphodelaceae); (18), *S. floribunda*; (5), *T. violacea*; (13), *S. album*; 17.VI.2017 (2), *S. chamaecyparissus*; 19.VI.2017 (11), *M. officinalis*; (43), *P. atriplicifolia*; (15), *E. purpurea*; (5), *Salvia transsylvanica* Schur (Lamiaceae); (1), *T. serpyllum*; (11), *D. moldavica*; 21.VI.2017 (39), *S. marianum*; (4), *Capparis spinosa* L. (Capparaceae); 22.VI.2017 (12), *O. vulgare*; 03.VII.2017 (3), *Leonurus cardiaca* L. (Lamiaceae); (1), *L. angustifolia*; 06.VII.2017 (12), *H. perforatum*; (17), *S. album*; (1), *C. creticus*; (3), *M. officinalis*; (15), *Eschscholzia californica* Cham. (Papaveraceae); (1), *P. crispum*; (32), *Oenothera biennis* L. (Onagraceae); 10.VII.2017 (3), *T. violacea*; (2), *C. officinalis*; (3), *Koelreuteria paniculata* Laxm. (Sapindaceae); (1), *Hemerocallis* sp.; (3), *R. flammula*; (6), *S. transsylvanica*; (5), *D. innoxia*; 17.VII.2017 (9), *S. officinalis*; (1), *D. moldavica*; 21.VII.2017 (1), *C. citratus*; 24.VII.2017 (3), *H. perforatum*; 10.VIII.2017 (4), *O. biennis*.

Karaisalı, 22.II.2017 (1), *Coriandrum sativum* L. (Apiaceae); 28.II.2017 (1), *C. sativum*; 16.III.2017 (1), *M. spicata*; (2), *F. vulgare*; 18.IV.2017 (1), *Origanum onites* L.; 02.V.2017 (5), *C. spinosa*; 04.V.2017 (12), *S. fruticosa*; 14.VI.2017 (56), *C. spinosa*; 22.VI.2017 (12), *M. arvensis*; (21), *M. spicata*; 23.VI.2017 (32), *E. pectinatus*; 29.VI.2017 (13), *T. erecta*; 06.VII.2017 (37), *M. spicata*; 12.VII.2017 (2), *P. anisum*.

Sarıçam, 28.III.2018 (3), *N. sativa*; 19.IV.2018 (1), *M. chamomilla*; 18.V.2018 (9), *A. asplenifolia*; 25.V.2018 (7), *A. millefolium*; (3), *A. dracunculus*; 17.VIII.2018 (1), *H. officinalis*.

Karaisalı, 19.IV.2018 (5), *S. officinalis*; 20.IV.2018 (7), *S. fruticosa*; 04.V.2018 (3), *F. vulgare*; 17.V.2018 (12), *C. spinosa*; 18.V.2018 (5), *C. tinctorius*; 22.V.2018 (3), *M. charantia*; 08.VI.2018 (2), *C. officinalis*; 04.VII.2018 (4), *M. spicata*; 06.VII.2018 (39), *C. officinalis*.

***Kakothrips pisivorus* (Westwood, 1880)**

Material examined. Karaisalı, 04.V.2017 (1), *S. fruticosa*.

***Limothrips cerealium* Haliday, 1836**

Material examined. Karaisalı, 30.V.2017 (2), *O. vulgare*.

***Limothrips denticornis* (Haliday, 1836)**

Material examined. Sarıçam, 29.V.2017 (1), *P. crispum*.

Karaisalı, 30.V.2017 (5), *O. vulgare*.

***Scolothrips longicornis* Priesner, 1926**

Material examined. Sarıçam, 17.IV.2017 (1), *Pelargonium graveolens*; 24.IV.2017 (1), *O. onites*.

***Taeniothrips inconsequens* (Uzel, 1895)**

Material examined. Karaisalı, 11.IV.2017 (3), *S. officinalis*.

***Tenothrips discolor* (Karny, 1907)**

Material examined. Sarıçam, 17.VII.2017 (1), *M. spicata*.

***Thrips atratus* Haliday, 1836**

Material examined. Karaisalı, 05.VI.2017 (2), *S. fruticosa*.

***Thrips australis* (Bagnall, 1915)**

Material examined. Sarıçam, 02.VII.2017 (1), *Origanum vogelii* Greuter & Burdet (Lamiaceae).

***Thrips hawaiiensis* (Morgan, 1913)**

Material examined. Sarıçam, 10.IV.2017 (1), *E. pectinatus*; 24.IV.2017 (6), *C. scolymus*; 03.V.2017 (1), *E. purpurea*; 08.V.2017 (2), *N. tabacum*; (1), *R. flammula*; (1), *N. sativa*; 15.V.2017 (60), *P. anisum*; 22.V.2017 (6), *A. dracunculus*; (1), *S. chamaecyparissus*; (1), *A. rosea*; (5), *D. moldavica*; 26.V.2017 (36), *M. communis*; (40), *P. edulis*; (2), *T. spicata*; (53), *R. communis*; 29.V.2017 (1), *E. elaterium*; (2), *A. millefolium*; (4), *P. harmala*; (29), *N. tabacum*; 05.VI.2017 (5), *M. officinalis*; (29), *P. americana*; (1), *S. chamaecyparissus*; (6), *T. erecta*; (1), *C. tinctorius*; 12.VI.2017 (8), *S. floribunda*; (20), *H. perforatum*; (1), *N. damascena*; 19.VI.2017 (6), *O. vulgare*; (38), *S. transsylvanica*; 21.VI.2017 (27), *C. spinosa*; 06.VII.2017 (7), *Plumeria alba* L. (Apocynaceae); 10.VII.2017 (1), *S. transsylvanica*; 10.VIII.2017 (12), *O. biennis*; 25.XII.2017 (62), *P. alba*.

Karaisalı, 02.V.2017 (1), *C. spinosa*; 08.V.2017 (13), *R. officinalis*; 14.VI.2017 (4), *C. spinosa*; 06.VII.2017 (1), *C. officinalis*.

Sarıçam, 18.V.2018 (2), *D. moldavica*; 01.VI.2018 (2), *T. erecta* ; 08.VI.2018 (2), *M. officinalis*.

Karaisalı, 07.V.2018 (3), *O. basilicum*.

***Thrips major* Uzel, 1895**

Material examined. Sarıçam, 21.IV.2017 (2), *O. majorana*; 10.VII.2017 (1), *Portulaca oleracea* L. (Portulacaceae); 17.VIII.2017 (1), *H. officinalis*.

Karaisalı, 16.III.2017 (1), *R. officinalis*.

***Thrips meridionalis* (Priesner, 1926)**

Material examined. Karaisalı, 28.II.2017 (5), *R. officinalis*.

***Thrips minutissimus* Linnaeus, 1758**

Material examined. Karaisalı, 16.III.2017 (3), *R. officinalis.*

***Thrips physapus* Linnaeus, 1758**

Material examined. Sarıçam, 05.VI.2017 (4), *S. marianum.*

***Thrips tabaci* Lindeman, 1889**

Material examined. Sarıçam, 08.III.2017 (1), *O. basilicum*; 05.IV.2017 (1), *M. arvensis*; 10.IV.2017 (8), *E. pectinatus*; (16), *T. chamaedrys*; (3), *B. rapa* subsp. *nipposinica*; (7), *G. coronaria*; 17.IV.2017 (12), *Dianthus* sp.; (5), *P. graveolens*; (10), *A. asplenifolia*; 24.IV.2017 (6), *A. millefolium*; 01.V.2017 (19), *L. usitatissimum*; (4), *N. damascena*; 08.V.2017 (6), *O. vulgare*; (1), *G. coronaria*; 13.V.2017 (2), *L.*

*usitatissimum*; 15.V.2017 (10), *M. chamomilla*; 17.V.2017 (10), *T. foenum-graecum*; 22.V.2017 (2), *S. officinalis*; (6), *A. rosea*; (23), *T. violacea*; (2), *S. chamaecyparissus*; (2), *H. italicum*; 26.V.2017 (2), *O. basilicum*; 29.V.2017 (2), *Cardiospermum halicacabum* L. (Sapindaceae); (1), *N. sativa*; (18), *M. charantia*; (2), *E. elaterium*; 05.VI.2017 (6), *L. angustifolia*; (2), *T. erecta*; (4), *C. tinctorius*; (2), *M. officinalis*; (16), *S. chamaecyparissus*; 10.VI.2017 (2), *Althaea officinalis* L. (Malvaceae); 12.VI.2017 (6), *S. album*; (14), *T. violacea*; 17.VI.2017 (19), *S. chamaecyparissus*; 21.VI.2017 (8), *E. cheiri*; (3), *S. marianum*; 03.VII.2017 (1), *L. cardiaca*; 06.VII.2017 (9), *C. creticus*; (1), *E. californica*; 10.VII.2017 (3), *C. officinalis*; (2), *D. innoxia*; (17), *T. violacea*; (16), *P. anisum*; (1), *R. flammula*; 17.VII.2017 (2), *D. moldavica*; (1), *Mentha piperita* L. (Lamiaceae); 02.VIII.2017 (2), *P. anisum*; 10.VIII.2017 (1), *O. biennis*.

Karaisalı, 02.II.2017 (7), *F. vulgare*; 22.II.2017 (17), *P. anisum*; (25), *C. sativum*; 28.II.2017 (24), *R. officinalis*; 16.III.2017 (8), *R. officinalis*; (10), *P. anisum*; 18.IV.2017 (2), *O. onites*; 02.V.2017 (3), *C. spinosa*; 30.V.2017 (4), *O. vulgare*; 22.VI.2017 (8), *C. tinctorius*; 12.VII.2017 (36), *P. anisum*.

Sarıçam, 20.IV.2018 (3), *T. erecta*; 10.V.2018 (3), *M. officinalis*; (4), *C. sativum*; 01.VI.2018 (2), *L. usitatissimum*; 08.VI.2018 (8), *S. chamaecyparissus*; 22.VI.2018 (6), *T. violacea*.

Karaisalı, 01.III.2018 (2), *N. sativa*; 13.IV.2018 (5), *R. officinalis*; 19.IV.2018 (2), *C. officinalis*; 20.IV.2018 (7), *P. anisum*; 27.IV.2018 (2), *F. vulgare*.

***Thrips vulgatissimus* Haliday, 1836**

Material examined. Sarıçam, 21.VI.2017 (3), *S. marianum.*

**Phlaeothripidae**

***Haplothrips aculeatus* (Fabricius, 1803)**

Material examined. Sarıçam, 29.V.2017 (2), *Senecio* sp.; 06.VII.2017 (1), *C. creticus*.

Karaisalı, 29.VI.2017 (1), *T. erecta*.

***Haplothrips distinguendus* Uzel, 1895**

Material examined. Sarıçam, 05.VI.2017 (1), *C. tinctorius*; 22.VI.2017 (1), *O. vulgare*.

***Haplothrips flavicinctus* (Karny, 1910)**

Material examined. Sarıçam, 05.IV.2017 (1), *M. arvensis*; 26.V.2017 (1), *P. edulis* ; 19.VI.2017 (1), *D. moldavica*; 17.VII.2017 (3), *M. piperita*.

Karaisalı, 30.V.2017 (3), *O. vulgare*.

***Haplothrips gowdeyi* (Franklin, 1908)**

Material examined. Sarıçam, 08.V.2017 (1), *N. tabacum*; 05.VI.2017 (15), *M. officinalis*; (14), *P. anisum*; (2), *P. americana*; 19.VI.2017 (3), *M. officinalis*; (1), *S. transsylvanica*; 10.VII.2017 (2), *K. paniculata*; 21.VII.2017 (1), *P. oleracea*.

***Haplothrips reuteri* (Karny, 1907)**

Material examined. Sarıçam, 10.IV.2017 (1), *G. coronaria*; (1), *T. chamaedrys*; 15.V.2017 (1), *A. asplenifolia*; 22.V.2017 (4), *C. tinctorius*; 26.V.2017 (5), *T. spicata*; 29.V.2017 (1), *E. purpurea*; 05.VI.2017 (1), *T. serpyllum*; (1), *M. officinalis*; (7), *S. marianum*; (1), *C. tinctorius*; 21.VI.2017 (24), *S. marianum*; 06.VII.2017 (1), *E. californica*.

Karaisalı, 11.IV.2017 (1), *S. officinalis*; 17.IV.2017 (1), *Dianthus* sp.; 04.V.2017 (9), *S. fruticosa*; 26.V.2017 (6), *Chamaedrys polium* (L.) Raf. (Lamiaceae); 30.V.2017 (1), *O. vulgare*; 05.VI.2017 (2), *S. fruticosa*; 23.VI.2017 (1), *E. pectinatus*; 06.VII.2017 (1), *C. officinalis*.

The most common and abundant species (with the number of samples-specimens found) were *F. occidentalis* (120 and 1379), *T. tabaci* (73 and 507) and *T. hawaiiensis* (42 and 503). Of the predatory species, *A. collaris* (48 and 144), was the most common and abundant one (Table 1).

Table 1. Frequency and abundance of thrips species found in Adana Province of Turkey in 2017 and 2018

| Thysanoptera species | f[1] | a[2] | a (%) |
|---|---|---|---|
| Aelothripidae | | | |
| *Aeolothrips albicinctus* | 1 | 7 | 0.25 |
| *Aeolothrips collaris** | 48 | 144 | 5.15 |
| *Aeolothrips ericae* | 8 | 20 | 0.71 |
| *Aeolothrips gloriosus* | 1 | 1 | 0.04 |
| *Aeolothrips intermedius** | 11 | 18 | 0.64 |
| Melanthripidae | | | |
| *Melanthrips fuscus*** | 2 | 3 | 0.11 |
| *Melanthrips pallidior*** | 7 | 10 | 0.36 |
| Thripidae | | | |
| *Bregmatothrips bournieri* | 2 | 3 | 0.11 |
| *Chirothrips aculeatus* | 2 | 3 | 0.11 |
| *Chirothrips manicatus* | 1 | 1 | 0.04 |
| *Frankliniella intonsa* | 8 | 34 | 1.21 |
| *Frankliniella occidentalis* | 120 | 1379 | 49.34 |
| *Kakothrips pisivorus* | 1 | 1 | 0.04 |
| *Limothrips cerealium* | 1 | 2 | 0.07 |
| *Limothrips denticornis* | 2 | 6 | 0.21 |
| *Scolothrips longicornis** | 2 | 2 | 0.07 |
| *Taeniothrips inconsequens* | 1 | 3 | 0.11 |
| *Tenothrips discolor* | 1 | 1 | 0.04 |
| *Thrips atratus* | 1 | 2 | 0.07 |
| *Thrips australis* | 1 | 1 | 0.04 |
| *Thrips hawaiiensis* | 42 | 503 | 18.00 |
| *Thrips major* | 4 | 5 | 0.18 |
| *Thrips meridionalis* | 1 | 5 | 0.18 |
| *Thrips minutissimus* | 1 | 3 | 0.11 |
| *Thrips physapus* | 1 | 4 | 0.14 |
| *Thrips tabaci* | 73 | 507 | 18.14 |
| *Thrips vulgatissimus* | 1 | 3 | 0.11 |
| Phlaeothripidae | | | |
| *Haplothrips aculeatus* | 3 | 4 | 0.14 |
| *Haplothrips distinguendus* | 2 | 2 | 0.07 |
| *Haplothrips flavicinctus* | 5 | 9 | 0.32 |
| *Haplothrips gowdeyi* | 8 | 39 | 1.39 |
| *Haplothrips reuteri* | 20 | 70 | 2.50 |
| TOTAL | | 2795 | 100.00 |

[1] Frequency - number of samples in which the species was found;
[2] abundance - total number of individuals of the species collected; a (%): percentage of total individuals;
* predatory thrips species;
** pollen feeder species.

As shown in Table 1, nearly half of the total specimens (49%) were *F. occidentalis* individuals. *Frankliniella occidentalis* was collected from 61 medicinal and aromatic plants and the highest number was collected on *P. atriplicifolia* (129 individuals), *D. moldavica* (90 individuals) and *C. spinosa* (77 individuals). The second abundant species, *T. tabaci* (18%) was collected from 47 medicinal and aromatic plant species, which was frequently found on *P. anisum* (88 individuals), *T. violacea* (60 individuals) and *S. chamaecyparissus* (45 individuals). *Thrips hawaiiensis* was collected from 33 species of medicinal and aromatic plants and was 18% of the specimens. It was mostly collected from *P. alba* (69 individuals), *P. anisum* (60 individuals) and *R. communis* (53 individuals). *Aeolothrips collaris* was collected from 32 medicinal and aromatic plant species, visiting them to feed upon various arthropods. The highest number of specimens was collected on *M. spicata* (34 individuals) feeding on *F. occidentalis*.

The total numbers of four common thrips species on some medicinal and aromatic plants in Adana Province is shown in Table 2. A few thrips specimens were collected from Turkish oregano (*O. onites*) which is one of the most cultured and exported medicinal and aromatic plants in Turkey. Another one anise, (*P. anisum*) was mostly infected by *T. tabaci* followed by *T. hawaiiensis*. Rosemary (*R. officinalis*), which is mostly obtained from nature was infected mostly by *T. tabaci* and *F. occidentalis*. English lavender, *L. angustifolia*, which the Ministry of Agriculture encouraged farmers to produce, is mostly infected by *F. occidentalis*. *Plumeria alba* was only infected with *T. hawaiiensis.* Thrips damage on the leaves of *S. officinalis*, *S. fruticosa*, *O. basilicum*, *M. officinalis*, *M. spicata*, *P. anisum, L. usitatissimum* and *N. tabacum* were remarkable. Tiny pale spots or bronze scars were observed on the leaves. It is thought that essential oil as well as the photosynthetic capacity of the infected leaves were reduced. It is possible that thrips preferred the other plants for nectars and pollens more than for reproduction.

*Frankliniella* and *Thrips* species are the most harmful genera and present on all continents (Moritz, 2002; Mound, 2012; Pizzol et al., 2014). Western flower thrips, *F. occidentalis* is distributed worldwide and characterized as invasive, polyphagous and occurring on at least 250 plant species from more than 65 families, nearly every species of flowering plants (fruit, vegetable, ornamental, field crops and many weed species). Of the medicinal and aromatic plants *F. occidentalis* was collected on *Capsicum annuum* L. (Solanaceae), *Rosa* spp. (Rosaceae), *Salvia* spp., *O. majorana*, *M. piperita*, *Pelargonium* spp., *N. tabacum*, *C. tinctorius* (Anonymous, 2019). This species is mainly found in flowers but may occur elsewhere on plants. Blumthat et al. (2005) determined that *F. occidentalis* is more common on yellow flowering and nectar-rich plants. Adults of the pest live and feed on flowers and they choose new leaves as food when flowers are absent. They also reproduce more when pollen is present (Hulshof et al., 2003). *Frankliniella occidentalis* became the most dominant and prevalent species within three years after its first introduction in Adana region in 1994 (Atakan et al., 1998). As Tunç & Hastenpflug-Vesmanis (2016) reported, *F. occidentalis* seems to have become the predominant thrips species on many crops and wild plants in Turkey. It is thought that one of the reasons of this population growth is the increasing resistance of the pest to the pesticides used for control (Dağlı, 2018). Onion thrips, *T. tabaci* is known to feed on many vegetable species and field crops as well as on a wide variety of weeds (Doederleini & Sites, 1993). It is a potential pest of cotton and some vegetables (onion, garlic and leek) in Turkey. It is also the vector of TSWV (Tomato spotted wilt virus) (Boonham et al., 2002). Hawaiian flower thrips, *T. hawaiiensis* is a polyphagous, flower-dwelling thrips which was introduced into Turkey in 2015 (Atakan et al., 2015). It was detected on lemon first but recorded also on some vegetables, ornamentals and field crop plants. This indicates that the invasive capacity and its population will increase in the upcoming years. Elekcioğlu (2018) determined 13 thrips species from 35 medicinal and aromatic plants during 2015-2016 at the same localities. *Frankliniella occidentalis*, *T. tabaci* and *T. hawaiiensis* were the prevalent species which the results are in conformity with data collected in this study. Atakan & Pehlivan (2018) determined 11 Thysanoptera species on 9 medicinal and aromatic plant species grown naturally in Balcalı Campus of

Çukurova University. *Thrips tabaci*, *T. major* and *F. occidentalis* were the most prevalent pest thrips species. They found only *A. collaris* as the predator thrips species and at very low numbers.

Table 2. Some plants associated with four common thrips species in Adana Province of Turkey during 2017-2018

| Plant species | Lifespan | Individual numbers of thrips** | | | | |
|---|---|---|---|---|---|---|
| | A, P* | *Fo* | *Tt* | *Th* | *Ac* | Total |
| Lamiaceae | | | | | | |
| *Perovskia atriplicifolia* (Russian sage) | P | 129 | 0 | 0 | 6 | 135 |
| *Dracocephalum moldavica* (Moldavian balm) | P | 90 | 2 | 7 | 10 | 109 |
| *Mentha spicata* (Spearmint) | P | 63 | 0 | 0 | 34 | 97 |
| *Melissa officinalis* (Lemon balm) | P | 41 | 5 | 7 | 13 | 66 |
| *Lavandula angustifolia* (English lavender) | P | 19 | 6 | 0 | 3 | 28 |
| *Salvia officinalis* (Common sage) | P | 19 | 2 | 0 | 0 | 21 |
| *Salvia fruticose* (Anatolian sage) | P | 25 | 0 | 0 | 2 | 27 |
| *Salvia transsylvanica* (Transylvania sage) | P | 11 | 0 | 39 | 0 | 50 |
| *Ocimum basilicum* (Basil) | A | 3 | 3 | 3 | 1 | 10 |
| *Origanum onites* (Turkish oregano) | P | 1 | 2 | 0 | 0 | 3 |
| *Rosmarinus officinalis* (Rosemary) | P | 0 | 37 | 13 | 2 | 52 |
| Asteraceae | | | | | | |
| *Echinacea purpurea* (Purple coneflower) | P | 53 | 0 | 1 | 2 | 56 |
| *Silybum marianum* (Milk thistle) | A | 50 | 3 | 0 | 0 | 53 |
| *Senecio* sp. (Ragwort) | P | 46 | 0 | 0 | 3 | 49 |
| *Achillea millefolium* (Common yarrow) | P | 43 | 6 | 2 | 0 | 51 |
| *Santolina chamaecyparissus* (Cotton lavender) | P | 36 | 45 | 2 | 3 | 86 |
| *Tagetes erecta* (French marigold) | A | 24 | 5 | 8 | 0 | 37 |
| Cappariaceae | | | | | | |
| *Capparis spinosa* (Caper) | P | 77 | 3 | 32 | 0 | 112 |
| Cucurbitaceae | | | | | | |
| *Calendula officinalis* (Pot marigold) | A | 43 | 5 | 1 | 1 | 50 |
| Apiaceae | | | | | | |
| *Pimpinella anisum* (Anise) | A | 2 | 88 | 60 | 2 | 152 |
| *Foeniculum vulgare* (Fennel) | P | 5 | 9 | 0 | 0 | 14 |
| Passifloraceae | | | | | | |
| *Passiflora edulis* (Purple passionflower) | P | 3 | 0 | 40 | 0 | 43 |
| Apocynaceae | | | | | | |
| *Plumeria alba* (White frangipani) | P | 0 | 0 | 69 | 0 | 69 |
| Amaryllidaceae | | | | | | |
| *Tulbaghia violacea* (Society garlic) | P | 15 | 60 | 0 | 0 | 75 |
| Euphorbiaceae | | | | | | |
| *Ricinus communis* (Castor bean) | P | 5 | 0 | 53 | 0 | 58 |
| Linaceae | | | | | | |
| *Linum usitatissimum* (Flax) | A | 2 | 23 | 0 | 0 | 25 |
| Solanaceae | | | | | | |
| *Nicotiana tabacum* (Tobacco) | A | 12 | 0 | 31 | 0 | 43 |
| Ranunculaceae | | | | | | |
| *Nigella sativa* (Black cumin) | A | 6 | 3 | 1 | 5 | 15 |
| Total | | 823 | 307 | 369 | 87 | 1586 |

* A, Annual; P, Perennial;
** *Fo, Frankliniella occidentalis*; *Tt, Thrips tabaci*; *Th, Thrips hawaiiensis; Ac, Aeolothrips collaris.*

In the present study thrips individuals were collected from 80 medicinal and aromatic plant species belonging to 33 families; which 25 of them from Lamiaceae, 15 species from Asteraceae, three species from Ranunculaceae and Apiaceae families each, two species from Solanaceae, Malvaceae, Fabaceae, Geraniaceae, Cucurbitaceae and one each from the other families. Many species found in the present study are known as pests of various crops in different parts of the world. The number of thrips species and total number of specimens collected from medicinal and aromatic plants from the Lamiaceae and Asteraceae families were higher, respectively. The same plant families were reported in a previous study dealing with host plant associations of Thysanoptera (Atakan, 2019). High host-specificities were recorded for *F. occidentalis* and *T. tabaci* more restricted on Asteraceae and Lamiaceae in that study. The author determined that the thrips fauna was dominated by *F. occidentalis*, contributing 81% of the specimens. According to Inoue & Sakurai (2007) some taxonomic groups are known to be preferred by thrips such as Asteraceae, Fabaceae, Rosaceae and Solanaceae in France. In a study in Crotia, thrips species from Aeolothripidae family were mostly found on plants from families Fabaceae, Asteraceae and Brassicaceae. Specimens from Thripidae family were the most abundant on Asteraceae and Fabaceae and family Phlaeothripidae was present, mainly on Poaceae, Asteraceae, and Fabaceae (Raspudić et al., 2009). The essential oils from species within the Lamiaceae family and their volatile constituents have been found to have a broad spectrum of biological activities against *F. occidentalis* and *T. tabaci* (Koschier et al., 2000; De Kogel & Koschier, 2002).

Secondary metabolites are known to work as important feeding stimulants in the selection of host plants by phytophagous insects (Jolivet, 1992). Plants secrete essential oils or protective chemical oils when they are attacked by pests (Rhoades, 1985). The nectar and pollens of flowers and their chemical contents and chemicals secreted by vegetative parts are attractive to pest species in turn of the natural enemies (Riudavets, 1995; Atakan & Pehlivan, 2018). It is thought that this is why flower-inhabiting thrips species are abundant between May and July. In this study, numbers of thrips peaked in May when numbers of flowering plant species were highest. From May, the total number of individuals decreases regularly until July and lowered when most of the annual medicinal and aromatic plant species started to senesce or harvest. However secondary plant metabolites of medicinal and aromatic plants have a wide spectrum of action on arthropod pests, including direct toxicity toward different stages of development, antifeedant, repellent, deterrent and attractive effects, and inhibition of development and oviposition (Chermenskaya et al., 2009; Costa et al., 2013; Zoubiri & Baaliouamer, 2014; Stepanycheva et al., 2018).

Data on thrips host plants in literature is generally lacking (Marullo, 2009). Determination of the host plant of a thrips can be difficult, with many host records being no more than transitory resting places of adults of these highly dispersive insects and a specific host association cannot be assumed just because large numbers of adult thrips are found on a plant (Mound, 2013). A particular plant may be used as a mating or feeding site, but not used for breeding, or a species may breed on various different plants under laboratory conditions, but be host-specific under field conditions (Garms et al., 2013). Comparison of data and that of previous studies revealed that most of the plant species in the present study are reported for the first time as the hosts or the plant associations of the thrips species in Turkey. Further studies are needed to understand the host plant diversity of the thrips species recorded in this study.

The present study shows that the regional faunal studies of thrips are important for reporting new records and to better understand their relationship with host plants. Medical and aromatic plants are becoming increasingly important in Turkey because of their potential to be used in alternative cropping systems, as raw material supply to the food industry and for use in complementary environment in alternative wards, therefore their production has become more widespread in recent years. Thrips are one of the important agents affecting yield and quality in cultivated medical and aromatic plants. Data obtained from this study show that local faunal studies should be conducted in different habitats and host plants to increase the knowledge of thrips species of Turkey. The large number of thrips and their host plants should be a useful guide to other researchers studying detailed taxonomic and faunal studies of thrips in different parts of Turkey.

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*Original article (Orijinal araştırma)*

# Diet-mediated modulation on the development and phenoloxidase activity in the Alder leaf beetle larvae, *Agelastica alni* (L., 1758) (Coleoptera: Chrysomelidae)[1]

Kızılağaç yaprak böceği, *Agelastica alni* (L., 1758) (Coleoptera: Chrysomelidae) larvalarının fenoloksidaz aktivitesi ve gelişiminde diyet etkenli değişiklikler

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## Abstract

Food-induced changes in the phenoloxidase activity and development of *Agelastica alni* (L.,1758) (Coleoptera: Chrysomelidae) larvae reared on unbalanced artificial diets were examined. The study was conducted between 2015-2016. Food quality had an impact on the phenoloxidase activity and growth performance of alder leaf beetle. The maximum pupal mass was recorded in the 0.5:1 P:C (protein:carbohydrate) diet and the minimum pupal mass was recorded from the larvae fed on the 3:1 P:C diet. The amount of carbohydrate consumed affected the pupal mass positively, whereas the amount of protein consumed negatively affected the pupal mass. The highest amount of pupal lipid was found in the 0.5:1 P:C diet and the lowest pupal lipid amount in the 1:3 P:C diet. Also, imbalance diets affected phenoloxidase activity. There was a positive relationship between P ratio of the diet and phenoloxidase activity. Phenoloxidase activity decreases as the amount of carbohydrate consumed by larvae increases. As a result, unbalanced diet affects the immune system of larvae. Carbohydrate also has a significant effect on immune defenses as much as protein. In addition, larvae increase their body size with excessive consumption of carbohydrate.

**Keywords:** *Agelastica alni*, imbalance diet, insect immunity, nutritional ecology, phenoloxidase

## Öz

Bu çalışmada gıda bakımından dengesiz diyetlerle beslenen *Agelastica alni* (L.,1758) (Coleoptera: Chrysomelidae) larvalarının fenoloksidaz aktivitesi ve gelişiminde meydana gelen besin kaynaklı değişiklikler araştırılmıştır. Çalışma 2015-2016 yılları arasında gerçekleştirilmiştir. Besin kalitesi kızılağaç yaprak böceğinin gelişim performansında ve fenoloksidaz aktivitesinde önemli bir etkiye sahiptir. En fazla pupa kütlesi 0.5:1 P:C (protein:karbonhidrat) besininde ve en az pupa kütlesi ise 3:1 P:C besininde beslenen larvalarda kaydedilmiştir. Tüketilen karbonhidrat miktarı pupa kütlesini pozitif olarak etkilerken, tüketilen protein miktarı pupa kütlesini negatif olarak etkilemektedir. Pupa lipid miktarı diyetler arasında farklılık göstermektedir. En fazla pupa lipid miktarı 0.5:1 P:C diyetinde kaydedilirken, en az lipid miktarı 1:3 P:C diyetinde kaydedilmiştir. Dengesiz diyetler fenoloksidaz aktivitesini de etkilemiştir. Besinin protein oranıyla fenoloksidaz aktivitesi arasında pozitif bir ilişki vardır. Fenoloksidaz aktivitesi larvaların tüketmiş olduğu karbonhidrat miktarının artışıyla azalmaktadır. Sonuç olarak, dengesiz diyetler larvaların fenoloksidaz aktivitesini etkilemektedir. Karbonhidrat savunma sistemi üzerinde protein kadar önemli bir etkiye sahiptir. Ek olarak, larvaların vücut büyüklüğü aşırı karbonhidrat tüketimiyle artmaktadır.

**Anahtar Kelimeler:** *Agelastica alni*, dengesiz diet, böcek bağışıklığı, gıda ekolojisi, fenoloksidaz

[1] This study was conducted as a master thesis of first author's at Recep Tayyip Erdoğan University and was presented as a poster presentation at the Ecology Symposium (11-13 May 2017, Kayseri, Turkey).

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## Introduction

As all organisms, insects need energy for growth and reproduction over their life span. Every organism meets the energy from their diets. The nutritional value of diet for animals varies according to the amount of nutrients in the diet and the amount of secondary metabolites of diet (Simpson & Raubenheimer, 2001). Food quality affects the growth (Joern & Behmer, 1997; Prasad & Mukhopadhyay, 2015, Rios et al., 2016), survival, reproductive success and immunity (Yang & Joern, 1994; Ponton et al., 2011) of animals. If the diet does not meet the needs of the organism, it is considered to be an unbalanced diet. Diets with unbalanced macronutrient affect the immune systems and development of larvae (Klemola et al., 2007; Lee et al., 2008; Cotter et al., 2011; Ponton et al., 2013). Immune defenses are important biological features that affect the fitness and development of organisms (Singer et al., 2014; Vogelweith et al., 2015). Invertebrates have a primitive immune system compared to vertebrates (Klemola et al., 2007). The innate system in insects contains specific and nonspecific responses to foreign agents. Phenoloxidase (PO) is one of the most important enzymes in these responses (González-Santoyo & Alex Córdoba-Aguilar, 2012). The PO enzyme is responsible for the activation of melanization in invertebrates. Melanization is responsible for the repair of tissues, defense against other pathogens such as bacteria, fungi and viral agents (Cerenius & Söderhall, 2004). PO is also an enzyme used against various pathogens (Santoyo & Aguilar, 2011). In insects, PO activity in the hemolymph is used to predict resistance to diseases (Adamo, 2004; Vogelweith et al., 2015; Srygley, 2017). That is, it is associated with resistance to some parasites/pathogens between species (Nigam et al., 1997). Dietary quality may also affect PO activity (Cotter et al., 2011; Kangassalo et al., 2018). Studies indicate that the food component that affects immunity is the amount of protein in diets (Lee et al., 2008, Singer et al., 2014). However, there may be differences between species. In addition to the individual effects of nutrients, the rate of macronutrient in the diet is one of the parameters affecting the development and immunity (Cotter et al., 2011). Every organism and growth stage of any organisms require a complex of the nutrient. In the literature, there is no report of a relationship between development parameters of larvae and PO activities.

*Agelastica alni* (L., 1758) (Coleoptera: Chrysomelidae) is an oligophagous leaf insect that usually occurs at a significant population density in alder (*Alnus* sp.) and willow (*Salix* sp.). Almost every year, a significant amount of leaf assimilation surface loss occurs in alder. In some years all the leaves of alder are consumed by *A. alni* (Firidin & Mutlu, 2009). In this study, the effect of unbalanced diets on the PO activity and the development of *A. alni*, which is an important forest pest, was investigated. Therefore, it will make a contribution to the literature and reveal the factors affecting the immunity and development of this species.

## Materials and Methods

### Study organism and diets

*Agelastica alni* larvae were collected from alder leaves in Maçka Çatak Village, Trabzon, Turkey in 2015. The larvae were brought to the laboratory and fed on artificial diets. The artificial diets used in feeding experiments were modified from the study of Yamamoto (1969). Artificial diets were wheat germ-base supplemented with casein. The contents of the base diet developed by Yamamoto (1969) are given in Table 1. In this study, additional total of eight artificial diets were used, with different protein and carbohydrate ratios. The protein and carbohydrate ratios of diets added to the based artificial diet are as follows: 1:1, 2:1, 1:0.5, 1:3, 1:5, 0.5:1, 3:1 and 5:1 P:C (protein:carbohydrate). P:C ratio in the diet was used to create nutrient imbalanced diets.

Table 1. Content of base diet (for 1 kg formula)

| Content | Quantity (g/ml) |
|---|---|
| Wheat germ | 80 g |
| Casein | 30 g |
| Sucrose | 30 g |
| Yeast Brewers | 16 g |
| Vanderzant Vitamine Mixture | 10 g |
| Wesson salt mixture | 8 g |
| Cholesterol | 0.2 g |
| Sorbic acid | 2 g |
| Methyl parapen | 1 g |
| Linseed oil | 1 ml |
| Agar | 20 g |
| Distilled water | 800 ml |

**Larval growth performance**

Trials were performed in two groups. In the first experimental group, the growth performance of larvae and the duration from the larval stage to the pupal stage were recorded. The pupal mass, pupa lipid and protein content of pupa were determined for growth performance. For this purpose, 10 larvae were used for each diet. Individuals were weighed on an electronic balance sensitive to 0.0001 mg; then each one was placed singly into a plastic cup with a cover. Each food block (about 1 g) prepared as described was pre-weighed before being presented to the larva for each treatment. Every second day, any uneaten food by the larvae remaining in the larval chamber was collected and replaced with fresh pre-weighed food block. The uneaten food left by the larva from each feeding chamber was collected separately and dried in an oven (50°C) and weighed after it reached a constant weight. Every other day, each larva was weighed. This procedure was repeated until all of the larvae entered the pupal stage (Lee et al., 2002).

The total lipid amount was calculated using formula from Loveridge (1973) and Simpson & Raubenheimer (2001). The total lipid amount stored in each pupa was determined with three times chloroform extraction (Simpson & Raubenheimer, 2001). The pupae were dried to a constant weight at 50°C. The dry weights of the pupal are noted. Each of the dry pupae was placed in tubes and chloroform was added. The tubes were placed in an automatic shaker for 24 h, then chloroform in tubes evacuated and chloroform re-added to the tubes. This process was repeated three times. At the end of the third chloroform extraction, the pupae were redried and reweighed to calculate their per cent lipid contents.

The lipid free pupae obtained by the chloroform extraction were analyzed for their nitrogen content using Thermo Scientific Flash 2000 series-NCS analyzer instrument and Dumas method (Yi et al., 2013). The ground dry samples weighed approximately 2.5 mg were placed in a thin tin capsule and the capsule was sealed. The capsules were then placed in the autosampler portion of the device. When the sample enters the combustion reactor, it enters into a special furnace heated to 900-1000°C and a small amount of pure oxygen and helium gas is added to the system to allow the samples to burn. In this case, the samples turn into elemental (simple) gases. Element separation is determined without the need for a complex separation system by means of TCD detector and separation in the column. The gas generated by the TCD detector is transferred to the column and the N values are calculated by the peaks formed in the column. At the end of this process, the percentage of nitrogen was multiplied by the constant of 6.25 to convert to the crude protein quantities (Oonincx et al., 2015).

**Phenoloxidase activity**

The second experimental group was established to determine the specific PO activity. In this experimental group, larvae were fed collectively in each food group. Four days after ecdysis to the final larval stage, hemolymph was collected from individuals by piercing the final proleg with a sterile needle.

Hemolymph was collected in Eppendorf tubes and frozen at -20°C until needed. PO activity and the amount of protein were measured according to Lee et al. (2008). For the PO activity assay 100 µL of 10 mM L-Dopa (substrate) was added to 100 µL of buffered hemolymph (400 µL of ice-cold phosphate-buffered saline (PBS, pH 7.4) and the absorbance of the mixture was measured at 492 nm on a Versamax tunable microplate reader after 20 min (no full stop) of incubation at 25°C. PO activity is expressed as PO units, in which one unit represents the amount of enzyme required to increase the absorbance by 0·001/min. Hemolymph protein content was quantified by the method of Bradford (1976). Triplicate samples were used for examining PO activity and protein level.

### Statistical analysis

The amount of food intake by each larva fed on each artificial diet, the pupal mass, the protein contents of the pupae, the lipid contents of the pupae, duration of growth and PO activity were analyzed statistically using SPSS Version 17. A normality test was performed to determine whether the variables were normally distributed. In order to determine the differences between the groups, ANOVA and Tukey test were performed in the data with normal distribution. Correlation testing was performed to determine whether there was a correlation between the amount of food consumed, the amount of pupae protein, the amount of pupae lipid, pupal mass and PO activity. Regression analysis was performed after the relationship was determined.

## Results and Discussion

### Growth performance

Diets affect the amount of food intake and growth performance of larvae. In the feeding experiments, 1:1 P:C group was accepted as the control group. The maximum amount of consumption was found for larvae fed on 2:1 P:C diet and the least amount of consumption was found for larvae fed on 1:5 P:C diet (Figure 1). There was a significant difference in point of food intake between diets (ANOVA, $F=545$, $p<0.00$). However, there was no significant difference between the 1:0.5 P:C diet and the 3:1 P:C diet according to the Tukey test. Dietary carbohydrate ratio ($R=-0.68$, $p<0.01$) has a negative effect on food intake. However, the 1:1 P:C ratio of the diet or protein ratio of the diet had no effect on the amount of food intake ($p>0.05$) (Figure 1).

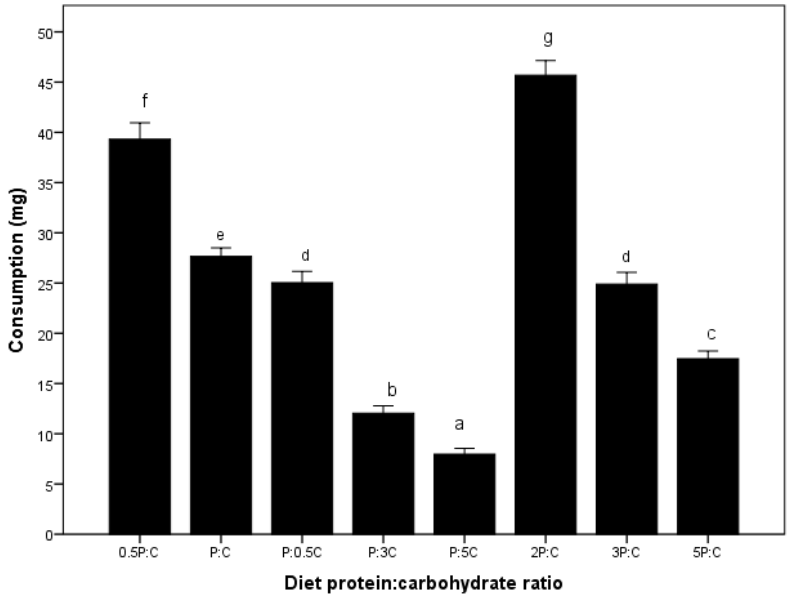


Figure 1. Food intake on different artificial diet. Diets with the same letter are not significantly different (p=0.05).

Diets affect pupal mass (ANOVA, F=11.74, p<0.00). The highest pupal mass was found for individuals fed on 0.5:1 P:C diet and the lowest pupal mass was found for individuals fed on 3:1 P:C diet (Figure 2). The P:C ratio of the diet did not affect pupal mass (p>0.05). However, the amount of carbohydrate consumed and the amount of protein consumed affected pupal mass. While the amount of carbohydrate consumed affects the pupal mass positively (R=0.28, p<0.05), the amount of protein consumed negatively affects pupal mass (R=-0.24, p<0.05), (Figure 2).

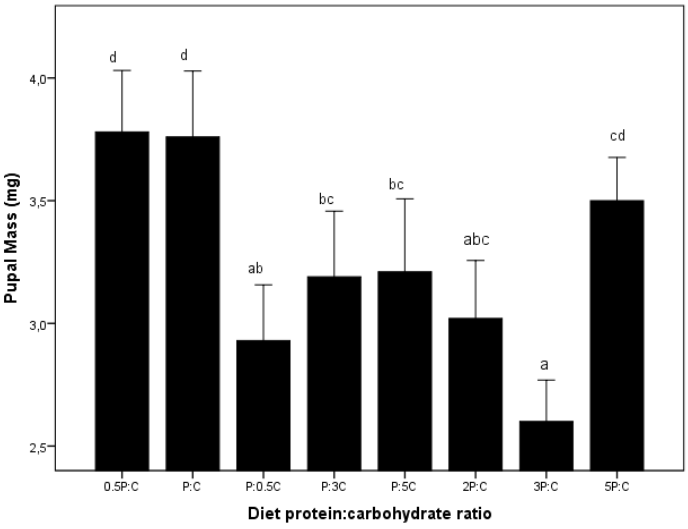


Figure 2. Pupal mass on different artificial diet (mg). Diets with the same letter are not significantly different (p=0.05).

Differences were also found between the pupal lipid for different diets (ANOVA, F=28.7, p<0.01). The highest amount of pupal lipid was determined in the 0.5:1 P:C diet and the lowest pupal lipid amount in the 1:3 P:C diet (Figure 3). The amount of carbohydrate consumed positively affected the amount of pupal lipid (R=0.41, p<0.01). Similarly, the dietary carbohydrate ratio positively affected the amount of pupal lipid (R=0.22, p<0.05). However, there was no effect of the amount of protein consumed to pupal lipid amount (p>0.05). The P:C ratio of the diet negatively affected the amount of pupal lipid (R=-0.26, p<0.05).

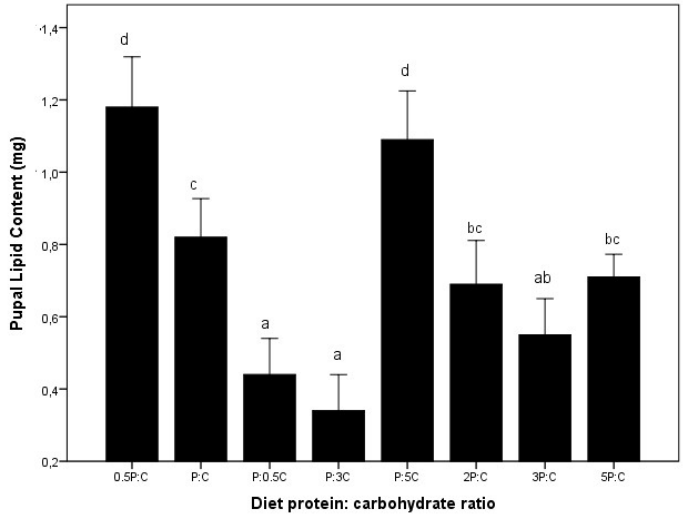


Figure 3. Pupal lipid content on different artificial diet (mg). Diets with the same letter are not significantly different (p=0.05).

The pupal crude protein also varies between diets (F=3.66, p<0.02). The highest pupa crude protein was found from individuals fed on 5:1 P:C diet, while the minimum pupa crude protein was determined from individuals fed on 3:1 P:C diet (Figure 4). However, the P:C ratio of the diet did not affect the pupa crude protein (p>0.05).

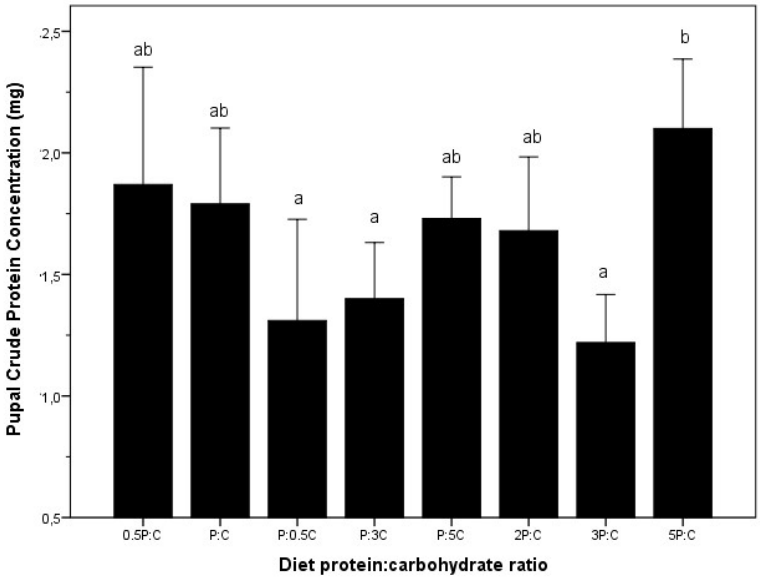


Figure 4. Pupal crude protein concentration on different artificial diet. Diets with the same letter are not significantly different (p=0.05).

**Phenoloxidase Activity**

PO activities of the experimental groups have been affected by diets used (ANOVA, F=21264.6 p<0.01). The highest specific PO activity (U/mg protein) was found for larvae fed on 1:0.5 P:C diet and the lowest PO activity was found for larvae fed on 2:1 P:C diet (Figure 5). The P:C ratio of the diet affected the PO activities of the larvae. There was a positive relationship between P ratio of diet and PO activity (R=0.31, p<0.01). The amount of protein consumed did not affect the PO activity (p>0.05). A negative correlation was found between the C ratio of the diet and PO activity (R=-0.39, p<0.01). Similarly, when the amount of carbohydrate consumed was increased, PO activity decreased (R=-0.68, p<0.01). A positive relationship was found between P:C ratio and PO activity of diet (R=0.52, p<0.01). The amount of pupal lipid also affects the PO activity. PO activity decreased with increasing of pupal lipid amount (R=-0.26, p<0.05).

Our main finding was that nutrient imbalance has an impact on pupal lipid amount and PO activity of alder leaf beetle. Unbalanced diets are known to affect the growth of larvae. According to nutritional ecology, animals develop mechanisms to regulate nutritional imbalances in their diet (Cotter et al., 2011; Prasad & Mukhopadhyay, 2015; Rho & Lee, 2015). Even herbivores can change the amount of consumption to compensate for nutritional imbalance (Waldbauer & Friedman, 1991; Bernays, 1998; Cotter et al., 2011; Ravenscraft & Boggs, 2016). Also, for *A. alni* larvae, food intake varies between artificial diets. However, as the carbohydrate content of the diet increased, it was shown that the amount of food intake decreased. Other factors related to diet had no direct effect on consumption. *A. alni* larvae may have regulated the intake of nutrient by reducing the food intake to ingest amount of carbohydrates required for the development. In addition, carbohydrates are nutritional stimulants for many species (Bernays et al., 2004; Juma et al., 2013). However, for this species sucrose may have a role as a feeding deterrent over a certain concentration.

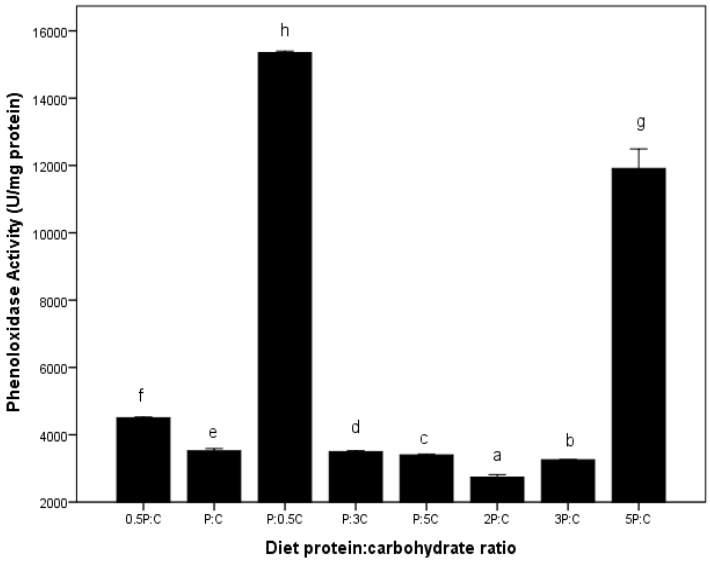


Figure 5. Phenoloxidase activity of larvae on different artificial diet. Diets with the same letter are not significantly different (p=0.05).

As the amount of protein consumed by *A. alni* larvae increases, the pupal mass decreased. This result is consistent with Honek (1993) and Lee et al. (2002). Excess protein ingested along with diet may have increased metabolic activity (Schroeder, 1986). The catabolizing of the excess protein and the removal of the feces from the body can lead to decreased pupal mass. However, insects maintain the balance of pupal crude protein while they fed on diets restricted to protein content. Given the amount of pupal crude protein is an important parameter for the development of insects, larvae may have managed to convert and use nutrition even though the diet is unbalanced (Joern & Behmer, 1997). The amount of pupal lipid increases with the amount of carbohydrates consumed and the carbohydrate rate of the diet. This result is consistent with the literature by Raubenheimer & Jones (2003). Increasing the protein: carbohydrate ratio of the diet resulted in a decrease in the amount of lipid. One way to avoid overly ingesting food is to increase metabolic rate through dietary thermogenesis as seen in some herbivores and omnivores (Trier & Mattson, 2003). This way, lipids can be used as energy reserves in a long-term starvation (Warbrick-Smith et al., 2006).

Lipid has an ecological and evolutionary effect on developmental performance which also relates to immunity of the species (Klemola et al., 2007; Cotter et al., 2011; Ponton et al., 2011; Singer et al., 2014). Studies have shown that diet quality is more effective than the amount of food intake in immune defenses of insects (Siva-Jothy & Thompson, 2002; Klemola et al., 2007; Singer et al., 2014). In our study, there was no significant effect of food intake on PO activity of *A. alni* larvae. However, the P:C ratio of the diet have been found to be important for the PO activities of the larvae. The invertebrate immune system includes the PO-profenoloxidase (PO-PPO) system. PO is a defense component of oxidative and melanism used against eukaryotic parasites (Cerenius & Soderhall, 2004; Lee et al., 2008; Santoyo & Aguilar, 2011; Vogelweith et al., 2015). This activity is an important component of the innate immune system (Lee et al., 2008). PO activity in hemolymph can be used to measure disease resistance (Adamo, 2004). Therefore, high PO activity can be interpreted as high resistance to pathogens. The increase in P:C ratio of the diet also causes an increase in PO activity. Therefore, nutritional imbalance may be perceived as a stress condition by larvae and may cause an increase in PO activity. PO may also increase due to population density or response to parasites (Klemola et al., 2007). Low-quality diets cause to undergo stress on autumn moths, *Epirrita autumnata* (Borkhausen, 1794) (Lepidoptera: Geometridae) and thus show high PO activity (Klemola et al., 2007). For *A. alni* larvae, the increase in the P:C ratio of the diet increases the PO

activity. Protein deficiency in the diet can affect the immune system of insects (Lee et al., 2008; Srygley et al., 2009). Therefore, an increase in the rate of PO activity with an increase in protein ratio is expected. However, one of the interesting results in study is that the increase in the C ratio of the diet and the amount of carbohydrates consumed causes the PO activity of the larvae to decrease. Srygley et al. (2009) suggested that protein is more important than carbohydrate in immune defenses. According to our results, carbohydrate might play n important roles in immune defense as well as protein. The amount of carbohydrates consumed and the carbohydrate rate of the diet has a positive effect on the level of lipids in the pupae. Pupal lipid content also suppresses the PO activity. Adamo et al. (2008) reported that the high lipid level in the hemolymph caused a decrease in the concentration of apolipophorin III protein. Apolipophorin protein also has a role in the activation of pro-PO cascade (Adamo et al., 2008; Zdybicka-Barabas & Cytrynska, 2010). Therefore, consumption of large amounts of carbohydrates increases the amount of pupal lipids, and the increase in the amount of pupal lipids reduces the concentration of apolipophorin III protein and suppresses the PO activity. However, the effect of the amount of lipid in hemolymph is reported (Adamo et al., 2008; Zdybicka-Barabas & Cytrynska, 2010). According to the results of our study, it must have concluded that the increase in the amount of storage lipid causes the concentration of apolipophorin III protein to decrease. This has another advantage for larvae. With the increase in the amount of carbohydrates consumed, the larvae used nutrition for growth rather than immunity. Syrgley (2017) stated that protein consumption has a more important role in the determinant of fitness than carbohydrate. On the contrary, the amount of protein consumed decreases the pupal mass. The amount of protein consumed has no effect on the amount of pupal lipid. The excess C ratio in the diet may have encouraged the use of nutrient for the development of larvae. In the evolutionary process, larvae must have developed a strategy, that by increasing its body size, they have developed fecundity in order to increase fitness rather than strengthen immune defense to increase fitness, because for many insects, body size is associated with fecundity (Garrad et al., 2016; Srygley, 2017; Togashi & Yamashita, 2017).

As a result, unbalanced diets may impair immune function and affect defenses against pathogens (Amar et al., 2007; Vogelweith et al., 2015). In addition, limiting nutrition can stress insects. This situation leads to the suppression or increase of immune defenses. When considered the results obtained from different experimental diets in the present study, carbohydrates also might be an important compound in insect immunity. For example, the diet 3:1 and 2:1 P:C did not show high PO activity despite the increased protein content. All of these data suggest that the insect immunity might be a result of complex feeding containing enough of the essential macronutrients.

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*Original article (Orijinal araştırma)*

# Resilience of breeding *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae: Scolytinae) to ingestion by vertebrates[1]

## Omurgalılar tarafından yutulan *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae: Scolytinae)'yı yetiştirmenin esnekliği

**Dirk H. R. SPENNEMANN[2*]**

## Abstract

Volant and terrestrial predators consume a wide range of palm drupes, some of which may be infested by spermatophagus beetles. Field observations suggest that the larvae of some beetle species survive the passage through the gastrointestinal tract. To assess the resilience of the date stone beetle, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae: Scolytinae) to ingestion by vertebrates, specimens reared from infested *Phoenix canariensis* (Chabaud, 1882) (Arecales: Arecaceae) seeds were exposed *in vitro* to simulated gastric and intestinal fluids in a laboratory setting at Charles Sturt University (Albury, Australia) in 2018. The observed mortality among beetles protected in their galleries inside the seeds was low (11-24%). The continued breeding success was affected by numerous beetles abandoning the seeds after immersion. Total mortality occurred among unprotected beetles exposed for 12 h or longer. This study demonstrates that as mortality of adult beetles inside ingested seeds is very low, vertebrate vectors may aid in the medium- to long-distance dispersal of the species.

**Keywords:** Biogeography, digestion-resistant insects, endochory, insect pests, physiology

## Öz

Kanatlı ve karada yaşayan predatörler, bazıları spermatofag böcekler ile bulaşık olabilen çok sayıda palmiye meyvesini tüketmektedirler. Arazi gözlemleri, bazı böcek türlerinin larvalarının mide-bağırsak kanalından geçişte hayatta kaldıklarını göstermektedir. Bu makale, Hurma böceği, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae: Scolytinae)'nın omurgalılar tarafından yutulmaya karşı direncini değerlendirmek amacıyla, enfekte olmuş *Phoenix canariensis* (Chabaud, 1882) (Arecales: Arecaceae) tohumlarından yetiştirilen böcek örnekleri 2018 yılında Charles Sturt Üniversitesi (Albury, Avustralya)'ndeki bir laboratuvar ortamında *in vitro* da simüle edilmiş mide ve bağırsak sıvılarına maruz bırakılmıştır. Galerilerinde tohumların içinde korunan böcekler arasında gözlenen ölüm oranı düşük (%11-24) saptanmıştır. Sürekli yetiştirme başarısı, daldırmadan sonra tohumları terk eden çok sayıda böcekten dolayı etkilenmiştir. Toplam ölüm oranı, 12 saat veya daha uzun süre maruz kalan korunmasız böcekler arasında meydana gelmiştir. Bu çalışma, yutulan tohumlar içindeki ergin böceklerin ölüm oranı çok düşük olduğundan, omurgalı vektörleri sayesinde bu türlerin orta ila uzun mesafe dağılımına yardımcı olabileceğini göstermektedir.

**Anahtar sözcükler:** Biyocoğrafya, sindirime dirençli böcekler, endokori, zararlı böcekler, fizyoloji

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## Introduction

The dispersal success of palms is enhanced by volant and terrestrial vectors which not only effect medium- to long-range transport of the seed away from the host tree (Zona & Henderson, 1989; Spennemann, 2018e), but also causes scarification of the seed coat during the passage through the gastrointestinal system which increases the likelihood of germination (Traveset, 1998; Rodriguez-Perez et al., 2005; Silverstein, 2005). The dispersal success of some palms, such as *Phoenix* sp., is affected by the presence and extent of infestation with date stone beetles, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae: Scolytinae). Experiences during a seed germination experiment showed that *Phoenix canariensis* (Chabaud, 1882) (Arecales: Arecaceae) seeds infested by these scolytids germinated (as evidenced by an emergent radicle), but that much of the albumen of the pericarp was consumed, thus dramatically reducing the viability of the developing hypocotyl to develop into a viable seedling (Spennemann et al., 2018).

*Coccotrypes dactyliperda*, is a spermatophagus beetle which is closely associated with the date palm, *Phoenix dactylifera* L., 1783 (Arecales: Arecaceae) complex and can be considered endemic to the Middle East and North Africa. Due to the trade in dates as fruit for consumption, and during the nineteenth century also due to the distribution of palm seeds for horticultural endeavors and as vegetable ivory for button manufacture, *C. dactyliperda* has become a true cosmopolitan species that can be found in most subtropical and temperate zones (Spennemann, 2018b).

As with other crypto-parasites, the entire life cycle of *C. dactyliperda* occurs inside the seed. Mated females start to lay eggs 3 to 5 d after inhabiting a new seed. Unmated *C. dactyliperda* females are able to lay eggs that produce male offspring and then proceed to mate with these to produce offspring of mixed sex. Depending on the size of the seed, multiple broods and even generations of beetles can hatch and reproduce inside a seed before emerging. A single date seed can concurrently house large numbers of eggs, larvae, pupae and imagines, with in excess of 80 individuals (females, males, pupae, larvae and ova). On record is the development period of a full life cycle from egg to adult for females which is temperature- and humidity-dependent, and has been reported as ranging from 22 d (at 28°C) to 49 d (at 20°C). During the winter period imagines of female *C. dactyliperda* enter a hibernation or dormancy period inside the seed in which they hatched. After emergence from hibernation and subsequent oviposition, the first generation of female beetles to leave the brood chamber emerge during late-June to early-July (in the northern hemisphere) (Spennemann, 2019a).

*Coccotrypes dactyliperda* tend to either colonize within fallen seeds, or bore into fresh drupes on the infructescence causing their abscission. A study in Israel found that *C. dactyliperda* were responsible for about 20% of the annual seed bank found underneath palms due to feeding-induced abscission (Bar-Shalom & Mendel, 2001). At the end of the breeding season in October, some 10% of the seeds on the ground were colonized, but after hibernation and the first generation in March, 95% of the seeds that had remained on the ground showed evidence of infestation (Bar-Shalom & Mendel, 2001).

Setting aside the random event that an infested drupe might be consumed by a bird during the short period prior to abscission, any dispersal of *Coccotrypes*-infested drupes will be due to birds (Spennemann, 2018d, 2019b) as well as terrestrial vertebrates, primarily canids and ursids (Spennemann, 2018e) feeding on fallen drupes.

The infested seed may either be exposed to mastication and subsequent ejection from the oral cavity (e.g., fruit bats; Spennemann, 2018c); to partial digestion and subsequent regurgitation from the crop/gizzard (e.g., currawong; Spennemann, 2018d); or to partial digestion and subsequent defecation (e.g., canids). The survival of a *Coccotrypes* female and its brood will depend on the duration of exposure to gastrointestinal liquids (e.g., saliva, gastric acid and intestinal fluids), during gastrointestinal transit as well as the nature of ingestion and duration of mastication (if any).

The broader literature shows that while insect-infested fruit were rejected by birds (Manzur & Courtney, 1984) as well as primates (Benítez-Malvido et al., 2016), infestations of the seed, without significantly spoiling the pericarp (flesh) had a lesser adverse effect. Hernández (2011) reviewed instances where seeds infested by insects had passed partially (regurgitated) or fully (defecated) through the digestive system of birds (Nalepa & Piper, 1994; Guix & Ruiz, 1995, 1997; Hernández & Falcó, 2008), ungulates such as tapirs (Olmos et al., 1999; Giombini et al., 2009), marsupials (Rouco & Norbury, 2013), as well as primates (Bravo & Zunino, 1998; Bravo, 2008). Seed-inhabiting adult beetles in particular, had a high survival chance. However, in other examples, such as bruchid beetles, the ingestion and immersion in gastric fluids kills off large proportions of larvae inside the seeds (Lamprey et al., 1974; Coe & Coe, 1987; Or & Ward, 2003).

The extant literature is silent on the extent that mastication and ingestion of affected palm drupes might kill the beetles, pupae or larvae in the seed itself. The latter, in particular, have a permeable skin and therefore are susceptible to chemicals even in low concentrations, compared with the chitinous exoskeleton of adult beetles. In favor of survival are the comparatively short gastrointestinal transit times (see below), and the small diameter of the access hole (≤1 mm) which can easily be clogged by masticated matter. Moreover, female *C. dactyliperda* exhibit the behavior to block the entrance/exit of the brood chamber with their body to prevent larvae from falling out and stray matter to ingress (Spennemann, 2019a). As the bore hole is only marginally bigger than the diameter of the female beetle itself, that blocking may be very effective, as long as the beetle is resistant to the fluids it is exposed to.

While no experimental data exist that shed light on the ability of *C. dactyliperda* to survive ingestion, one anecdotal example, dating to the first half of the nineteenth century, has been documented in the literature. Hippolyte Lucas reports from Algeria in 1846 that the ingestion of infested *Chamerops humilis* L. seeds by Golden Jackals, *Canis aureus* L., 1758, (Carnivora: Canidae), which in North Africa are widely noted as feeding on fallen dates of *P. dactylifera* and other palms (Spennemann, 2018e), was reputedly not detrimental to the survival of *C. dactyliperda* beetles and their larvae (Lucas in Anonymous, 1846; Lucas, 1849).

*Coccotrypes dactyliperda* have shown a great resilience to adverse external environmental conditions. In previous work, the author exposed cohorts of *C. dactyliperda* to insecticides (Spennemann et al., 2018) as well as subzero temperatures (Spennemann, 2019c). While the galleries could not provide sufficient protection to prolonged exposure to freezing (80% mortality after 7 h at -8°C), the beetles were surprisingly resilient to some but not all insecticides. While mortality was only 14.3% following a 20 min immersion in 10% bleach, the morality was 33.3% among seeds that had been immersed in the synthetic pyrethroid Cyhella®. An exposure to the organophosphate Fenitrothion®, however, caused 87.5% mortality (Spennemann et al., 2018).

This paper will report on the findings of experiments designed to simulate the ingestion of *C. dactyliperda* by vertebrates and the exposure of breeding females to gastric and intestinal fluids.

## Materials and Methods

To simulate ingestion by predators, beetles in their seeds were exposed to an immersion in simulated gastric and intestinal fluids (for composition see below). The experiment did not simulate the mechanical mixing and potential grinding action that occurs in the crop or stomach, nor the movement the seeds would experience in the intestinal tract. Two sets of samples were prepared, one which was immersed in 50 ml clear fluids and one that was immersed in a simulated masticated pulp comprised of 50 g fruit pulp mixed with 50 ml simulated gastric fluid (SGF) or simulated intestinal fluid (SIF). Samples using plain tap water served as a control.

The experiment was conducted in October 2018 in the PC2 laboratory of the Peter Till Laboratories, Faculty of Science, Charles Sturt University (Albury, Australia).

**Source of the beetles**

The beetles used here were drawn from a population bred a for multifactorial experiment, assessing food choices and emergence times (Spennemann, 2018a). The original beetle population originated from *P. canariensis* seeds collected at Alma Park, NSW, Australia (Spennemann et al., 2018).

**Preparing seed used in the experiment**

For the exposure experiments, beetles were either used unprotected (i.e., by themselves in a vial) or protected in their galleries (to simulate real conditions). In order to avoid previous experiences with collective exposures, where single seeds were penetrated by multiple beetles (Spennemann, 2018a), single beetles were placed with a single seed each into 7.5 ml plastic vials. These beetles were allowed to tunnel for 6 to 9 d. As mated females start to lay eggs between 1 and 3 d after inhabiting a new seed (Herfs, 1950; Zchori-Fein et al., 2006), the eight-day period was deemed sufficient for each beetle to establish a breeding gallery. As some beetles refused to take to a seed and had to be replaced after 3 d, the seeds were then extracted and, as cohorts of 10, were subjected to various treatments (see below). Common to all seeds were single penetration holes of 1 mm diameter (Figure 1a).


Figure 1. Examples of penetrated seeds used for the experiment: a) single penetration hole (at bottom of image); b) single penetration hole (bottom) and two exit holes (top of image).

The actual depth of the penetration was not ascertained at the start of the immersion. Some had commenced tunneling but had abandoned these tunnels. This was not noticed at the commencement of the experiment and was only recognized when the seeds were cut open at the end of the experiment. This accounts for the smaller sample numbers in Table 3.

The beetle-infested seeds used for the long-term immersion in simulated intestinal fluid mixed with pulp (12 and 24 h) were drawn from a breeding population where many of the beetles were already in a more advanced stage of development and the seeds already exhibited multiple exit holes (Figure 1b). These seeds had, therefore, a greater probability of moisture intrusion into the gallery.

**Simulated gastric and intestinal fluids**

Simulated gastric and intestinal fluids are commonly used to study questions such as the digestibility and performance of foods (Fu et al., 2002; Stappaerts et al., 2014), the solubility and dissolution behavior of drugs (Khadra et al., 2015; Wang et al., 2015) and drug delivery methods (Chen et al., 2018). The composition of the simulated gastric and intestinal fluids used in this experiment follows the formulation described by Wang et al. (2015):

*Simulated gastric fluid* - 0.8 g of sodium chloride was dissolved in 100 ml of reverse osmosis (RO) water. 2.8 ml of 10M HCl was then added to the NaCl solution to adjust the pH 1.2. To this, 1.28 g of Pepsin was added and gently stirred. The volume was then made up to 400 ml with RO water. The solution had a final pH of 1.55.

*Simulated intestinal fluid* - 2.72 g of monobasic potassium phosphate was dissolved in 100 ml of RO water, then 44.7 ml of 0.2 N NaOH was added to adjust the pH to 6.8. To this, 4 g of Pancreatin was added and gently stirred. The volume was then made up to 400 ml with RO water. The solution had a final pH of 6.84.

To create simulated masticated fruit pulp, the flesh was removed, from 300 fresh *P. canariensis* drupes (peeled *in toto* or flesh cut off in strips), which yielded 319 g of flesh. The sample of flesh pieces was mixed thoroughly. 25% were retained as peeled and the remaining 75% were chopped in a domestic kitchen blender. 50% of this mass was removed, with the remainder pureed. All three fractions (Figure 2) were then combined.


Figure 2. Fractions of palm flesh pulp. From left: as peeled; chopped; pureed.

The minimum time of exposure of the beetles to the clean seeds was 7 d and the maximum time was 10 d. To ensure that these time differences did not affect the outcomes in a systematic fashion, the 80 vials were randomly allocated to one of eight cohorts of 10 [numbers 1-80 allocated at random, then groups in sets of 10 (A-H)]. These eight cohorts were then randomly allocated to the experiments.

At the time of exposure to the experimental fluids or pulp mixtures, a cohort of ten live beetles was directly added to the container to simulate the incidental ingestion of crawling specimens. Once exposed to the sample solution, the samples were vigorously shaken for 10 s at 5-min intervals, but were otherwise left to sit on the lab bench.

**Duration**

Assessed was the survival of *C. dactyliperda* at intervals from 0.3 to 24 h. The duration of exposure to the fluids was informed by data on gastrointestinal transit times in the primary vectors. Among birds there appears to be not only a correlation between body size (and thus gape size) and the size of fruit consumed, but also between body size and retention time in the crop until regurgitation, with larger birds retaining larger seeds for longer periods (Levey, 1987). Among Pied Currawongs, *Strepera graculina* (Shaw, 1790) (Passeriformes: Artamidae) for example, it takes between 5 to 15 min after ingestion to regurgitate the rough and indigestible matter (e.g., seeds, epicarp, fibrous parts of pericarps, bones and feathers) in the form of pellets (Bass, 1995). Among canids, gastrointestinal transit time appears to be related to body size. It is significant that not all stomach content is necessarily defecated at the same time, with some content delayed up to 30 h. Observed transit times range from 3 h [Red Foxes, *Vulpes vulpes* L., 1758 (Carnivora: Canidae)] to 55 h [large domestic dogs, *Canis familiaris* L., 1758 (Carnivora: Canidae)] (Neseni et al., 1955; Szuman & Skrzydlewski, 1962; Weber, 2006; Boillat et al., 2010).

Exposure time for each liquid was set at 20 min, with the exception of a sequential exposure to both simulated gastric and intestinal fluid (20+20 min). To assess the effects of longer gastrointestinal transit times, isolated beetles (in cohorts of 10) were placed into 7.5-ml vials and exposed to SGF and SIF for up to 24 h.

**Assessment and disposal of survivors**

At the end the respective set time intervals the samples were drained on filter paper, and the seeds extracted. The residue was searched to assess the survival of the isolated beetles. Any specimens found alive were killed in 90% alcohol. The entire pulp residue was then placed into a freezer at -12°C for 5 d to kill any beetles that had not been located. A similar procedure was followed for the beetles emerging from the seeds (see below) and the final disposal of the infested seeds. At the termination of the experiment 27 d after immersion (34-37 d after commencement), all seeds that had not yielded two emergences were cut open and inspected. Given the life cycle of *C. dactyliperda* comprised of an egg incubation period (6-10 d), larval stage (12-15 d) and pupal stage (3-4 d) (Spennemann, 2019a), the presence of pupae was interpreted as evidence of successful breeding.

The extracted seeds were placed in numbered vials to observe the emergence success and timing. At the time of exposure, the brood chamber would have comprised of the adult female as well as eggs and some larvae (for life cycle times see Spennemann, 2019a). The emergence of more than one adult beetle was taken as evidence of successful continued breeding as the second individual must have developed from eggs (or larvae) present at the time of immersion, or from subsequent oviposition events.

**Statistical analysis**

Statistical analysis of the small sample was limited to exploratory analysis (Tukey, 1980), primarily using the statistical functions in MS Excel and the two-proportion z-test (Stangroom, 2019).

## Results

The results of the simulated ingestion by drupe predators are set out in Table 1. Note that despite diligent searching, one beetle each could not be located in the pulp samples $H_2O$ and SGF (indicated by the + sign). At least in the instance of the control sample ($H_2O$) it must be assumed that the missing beetle was alive. As anticipated, all isolated beetles survived the control experiment despite the vigorous shaking.

Intriguingly, the survival rate of the beetles exposed to SGF was likewise high, where exposure to liquid SIF resulted in 50% mortality. The presence of pulp buffered the effects of SIF, with nil mortality in the sample of SIF alone, and 30% mortality in the sequential SGF-SIF experiment. The higher mortality in the latter experiment may have been caused by the greater volume of liquid in that sample (50 g of pulp immersed in 50 ml of SGF and SIF each) as exposed to the stand-alone SIF experiment (50 g of pulp immersed in 50 ml of SIF).

Table 1. Resistance of *Coccotrypes dactyliperda* to ingestion (in % of seeds) using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (Statistically significant ($p < 0.05$) difference from the control shown in bold italics)

| | | liquid | | | | pulp | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 20 min | | 40 min | | 20 min | | 40 min | | 12 h | | 24 h | |
| | | single | in seed | single | in seed | single | in seed | single | in seed | single | in seed | single | in seed |
| Treatment | $H_2O$ | 100 | 71.4 | — | — | 90+ | 62.5 | — | — | — | — | — | — |
| | SGF | 90 | 66.7 | — | — | 90+ | 85.7 | — | — | — | — | — | — |
| | SIF | ***50*** | 62.5 | — | — | 100 | 44.4 | — | — | 0 | 88.9 | 0 | 87.5 |
| | SGF/SIF | — | — | ***50*** | 66.7 | — | — | 70 | 44.4 | — | — | — | — |

A subsequent experiment examined the effects of longer exposure of single beetles to both simulated fluids. Notably, the mortality among the beetles exposed to SIF was much less in this experiment, at least in the early stages (Table 2). Although it is possible that, despite repeated vigorous mixing, the increased surface tension in the smaller vial contributed to the beetles remaining partially afloat, the beetles were fully exposed to the fluids. All beetles immersed in tap water were alive after a 24 -h period. In a different experiment with an identical methodology, *C. dactyliperda*, were alive even after a 48-h immersion in tap water (Spennemann et al., 2018). Beetles immersed in SGF survived to a large degree until more than 6 h had passed and then mortality increased (Table 2; Figure 3). The observed differences between the control and SGF became statistically significant (two proportion z-test; $p < 0.05$) only after 12 h of immersion (24 h, z=2.58, $p$=0.01). Immersion in SIF had a much greater impact, mortality remained low until 1 h after immersion, when it dropped dramatically. At 1.5 h only 30% were still alive, which was a statistically significant decrease (z=3.28, $p$=0.001). Mortality remained at the 30% level until after 9 h of immersion, when it rose to 100%.

Table 2. Resistance of single *Coccotrypes dactyliperda* to ingestion (immersion in liquid) using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (Statistically significant ($p < 0.05$) difference from the control shown in bold italics)

| Treatment | Exposure time (h) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | 0.75 | 1 | 1.5 | 2 | 3 | 4 | 6 | 9 | 12 | 24 |
| $H_2O$ | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | 10 |
| SGF | 10 | 9 | 10 | 8 | 8 | 8 | 8 | 9 | 6 | 7 | ***5*** |
| SIF | 10 | 10 | 8 | ***3*** | ***2*** | ***3*** | ***3*** | ***3*** | ***3*** | ***0*** | ***0*** |

The beetles that resided in the seeds when exposed to fluids exhibited a low mortality (Table 3). Only in the case of an immersion in SGF and SIF mixed with pulp did some beetles die (22.2%). That rate of mortality was not statistically significantly different from immersion in $H_2O$ ($\chi^2$=1.66; p=0.197), or SIF ($\chi^2$=2.12; p=0.145) or SGF ($\chi^2$=1.67; p=0.197) alone. The continued breeding success, however, was affected by numerous beetles that abandoned the seeds after immersion. As this also occurred in the $H_2O$ controls (Table 3), it was caused by environmental factors in the vials rather than the exposure per se. Indeed, the observed differences in the rate of abandonment (Table 3) are not statistically significant (Table 4).

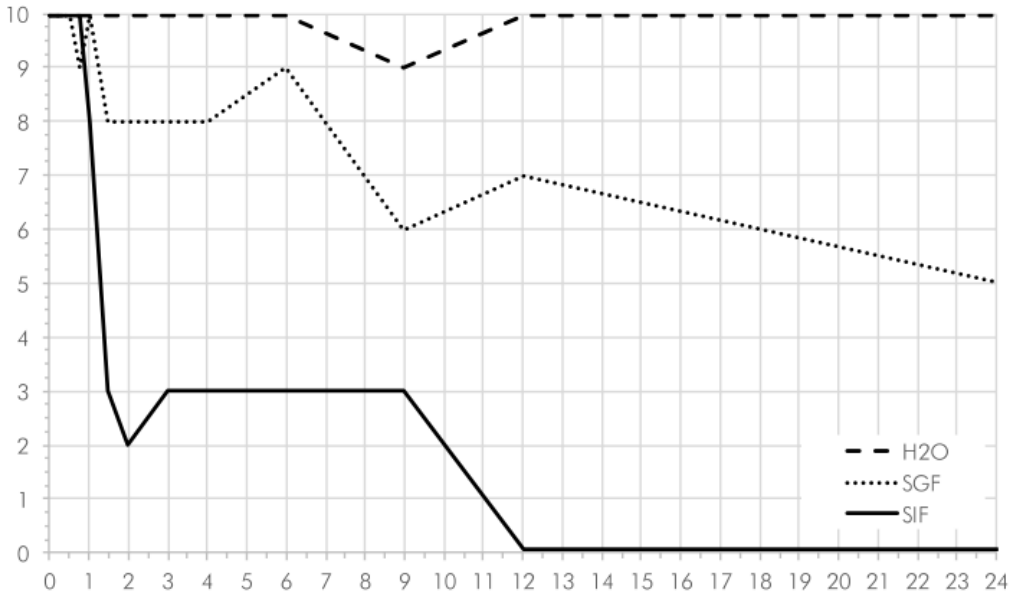


Figure 3. Resistance of single *Coccotrypes dactyliperda* to ingestion using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) over time (24 h).

Table 3. Resistance of *Coccotrypes dactyliperda* breeding in seeds to ingestion using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (in % of seeds)

| | | Success | Abandoned | Mortality | n |
|---|---|---|---|---|---|
| $H_2O$ | clear | 71.4 | 28.6 | 0.0 | 7 |
| | pulp | 62.5 | 37.5 | 0.0 | 8 |
| SGF | clear | 66.7 | 33.3 | 0.0 | 6 |
| | pulp | 85.7 | 14.3 | 0.0 | 7 |
| SGF/SIF | clear | 66.7 | 33.3 | 0.0 | 9 |
| | pulp | 44.4 | 33.3 | 22.2 | 9 |
| SIF | clear | 62.5 | 37.5 | 0.0 | 8 |
| | pulp | 44.4 | 55.6 | 0.0 | 9 |
| | pulp 12 | 88.9 | 11.1 | 0.0 | 9 |
| | pulp 24 | 87.5 | 12.5 | 0.0 | 8 |

Table 4. Differences in percentages of abandoned seeds between the control and simulated gastric fluid (SGF) or simulated intestinal fluid (SIF)

| | $H_2O$ | SGF | SIF | SGF/SIF | |
|---|---|---|---|---|---|
| $H_2O$ | — | $\chi 2$=0.031; p=0.8604 | $\chi 2$=0.124; p=0.7245 | $\chi 2$=0.038; p=0.038 | clear fluid |
| SGF | $\chi 2$=0.959; p=0.3275 | — | $\chi 2$=0.027; p=0.8699 | $\chi 2$=0.001; p=0.9805 | |
| SIF | $\chi 2$=0.524; p=0.4691 | $\chi 2$=2.686; p=0.1013 | — | $\chi 2$=0.023; p=0.8807 | |
| SGF/SIF | $\chi 2$=0.023; p=0.8807 | $\chi 2$=0.750; p=0.3865 | $\chi 2$=0.809; p=0.3683 | — | |
| | | | pulp | | |

## Discussion

The literature reports a number of instances where insects successfully hatched from ingested infested seed. The base assumption is that the seeds were infested when the drupe still had a substantial amount of pericarp adhering, which acted as an attractant to the fruit disperser. Some species, such as *Revena rubiginosa* (Boheman, 1836), (Coleoptera: Curculionidae) will perforate a developing drupe for oviposition in the seed, but will not inhabit the seed itself (Martín et al., 2009). Larvae emerged successfully from *R. rubiginosa*-infested *Syagrus romanzoffiana* (Cham.) Glassman (Arecales: Arecaceae) drupes regardless of whether the seeds were regurgitated (Guix & Ruiz, 1995) or defecated by birds (Guix & Ruiz, 1997) or defecated by ungulates (tapirs) (Olmos et al., 1999).

While *R. rubiginosa in S. romanzoffiana* seeds seem to survive the passage through a tapir's digestive tract (Olmos et al., 1999), *Pachymeris cardo* (Fåhraeus, 1839) (Coleoptera: Chrysomelidae) (a bruchid beetle) in *Maximiliana maripa* (Aubl.) Drude (Arecales: Arecaceae) seed exhibited a high mortality in tapir guts (Fragoso, 1997). This is supported by other examples of bruchid beetles, where the ingestion and immersion in gastric fluids kills a large proportion of larvae inside seeds (Lamprey et al., 1974; Coe & Coe, 1987; Or & Ward, 2003). The high mortality among bruchids can be explained by the fact that oviposition, and subsequent early larval development, occurs on the surface of the drupe/seed rather than inside (Ernst et al., 1990) and that the damage to the epicarp is more extensive (due to numerous penetration holes by multiple larvae).

*Coccotrypes dactyliperda*, as described, will penetrate a drupe (or seed) and will complete is entire life cycle inside the seed. This results in a single penetration hole, limiting the ingress of gastric acids. Moreover, it can be speculated that adult beetles, with their chitinous exoskeleton, are more resilient to the impact of low-concentration acidity than larvae with their soft and moisture-dependent skin. Even if larvae were to die due to exposure, but if the adult beetle survives, the female may either lay fresh eggs or abandon the seed and recommence breeding elsewhere. The data presented here support this conceptual model. It could be shown that *C. dactyliperda* inside seeds will survive ingestion and subsequent regurgitation or defecation by vertebrates. Some will continue breeding, while others will abandon their seed to search for a new seed. The experiments also indicate that *C. dactyliperda* will survive exposure to gastric and intestinal fluids for a short time even if they are not protected by the seed. This suggests that while they may be killed prior to defecation by terrestrial mammals, they might survive the comparatively short period between ingestion and regurgitation among birds.

As these were *in vitro* rather than *in vivo* experiments, the experiments could neither take into account the mechanical action during mastication, nor that which takes place in the crop and gizzard of the birds or the stomach on terrestrial vertebrates. It is speculated that such action might crush or otherwise affect beetles crawling among the food mass and that the action may push some of the food mass into the tunnel. Among mammals, seed damage caused by mastication is usually low (Koike et al., 2008), unless the seeds have been hollowed out so far that they can be crushed easily (Or & Ward, 2003).

## Conclusions

Based on the experiments, it is clear that *C. dactyliperda* breeding in *P. canariensis* seeds, and presumably seeds of other palm species, will survive ingestion and passage through the gastrointestinal tract and may be transported and dispersed by vertebrates with long gastrointestinal transit times, such as canids. Thus, vertebrate vectors may aid in the medium- to long-distance dispersal of the beetle. While the beetles may survive the gastrointestinal passage and may successfully reproduce after defecation, the ultimate success of the population will depend on whether the emerging beetles will be able to find suitable host seeds at the new locality.

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*Original article (Orijinal araştırma)*

# A new species and a new record of *Tephritis* Latreille, 1804 (Diptera: Tephritidae) from Turkey

Türkiye'den *Tephritis* Latreille, 1804 (Diptera: Tephritidae)'in yeni bir türü ve yeni bir kaydı

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## Abstract

*Tephritis* Latreille, 1804 is one of the largest genera of Tephritini (Diptera: Tephritidae), which includes about 190 described species. Forty species of this genus have been recorded up to date from Turkey. In the summer 2018, a previously undescribed species of *Tephritis* was collected by sweeping from *Scorzonorea tomentosa* L. (Asteraceae: Cichorieae), in Erzurum Province of Turkey, described herein as *Tephritis turkeri* sp. nov. Also, other Tephritid species *Tephritis zernyi* Hendel, 1927 is recorded for the first time from Turkey. Material is kept in the collection of Gaziantep University Entomology Laboratory.

**Keywords:** Erzurum, new record, new species, Tephritidae, *Tephritis*, Turkey

## Öz

*Tephritis* Latreille, 1804 tanımlanmış 190 türü ile Tephritini (Diptera: Tephritidae)'nin en büyük cinslerinden bir tanesidir. Bugüne kadar, Türkiye'den bu cinsin kırk türü kaydedilmiştir. 2018 yazında, daha önce tanımlanmamış *Tephritis* türü Türkiye'nin Erzurum ilinden *Scorzonorea tomentosa* L. (Asteraceae: Cichorieae) bitkisinden süpürülerek toplanmış ve bu makalede *Tephritis turkeri* sp. nov olarak tanımlanmıştır. Ayrıca, diğer Tephritid türü *Tephritis zernyi* Hendel, 1927 Türkiye'den ilk kez kaydedilmiştir. Örnekler Gaziantep Üniversitesi Entomoloji Laboratuvarı koleksiyonunda muhafaza altına alınmıştır.

**Anahtar sözcükler:** Erzurum, yeni kayıt, yeni tür, Tephritidae, *Tephritis*, Türkiye

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## Introduction

Fruit flies are high profile insects among commercial fruit and vegetable growers, marketers, exporters, government regulatory agencies, and the scientific community. Locally, producers face huge losses without some management scheme to control fruit fly populations. At the national and international levels, plant protection agencies strictly regulate the movement of potentially infested products (McPheron & Steck, 1996). Some species of the Tephritidae infest the flower heads of the Asteraceae host plants, collectively belonging to several tribes, with or without the induction of galls (Freidberg, 1984).

*Tephritis* Latreille, 1804 is one of the largest genera of the tribe Tephritini (Diptera: Tephritidae) which includes about 168 described species (Norrbom et al., 1999). More than 20 species of *Tephritis* have been described from the Middle Eastern and Central Asian areas of Palearctic Region in the last 20 years (Korneyev & Korneyev, 2019). Larvae of *Tephritis* flies feed on Asteraceae (Merz, 1999). For most comprehensive list of host plants see Korneyev, 2016. Most species live in flower heads, but a few species have been reared from stem or even stem-base galls (Merz, 1999).

Species of *Tephritis* have wide range distribution in Turkey and 40 species have been recorded or described from Turkey (Koçak & Kemal, 2013; Korneyev, 2016; Korneyev & Korneyev, 2019).

Main purpose of this study was to contribute to Turkish fruit fly fauna with new species and new record. In the paper, *Tephritis turkeri* sp. nov. is described and illustrated, and *Tephritis zernyi* Hendel, 1927 is recorded for the first time from Turkey.

## Materials and methods

### Collection of specimens

Adult specimens were collected in the summer of 2018 in Erzurum Province of Turkey (Figure 1). Specimens of *T. turkeri* sp. nov. were swept with insect net over possible host plant *Scorzonera tomentosa* L. (Asteraceae) which is an endemic plant for Turkey. *Tephritis zernyi* was swept from *Arctium minus* Bernh. (Asteraceae). Collected samples are pinned and deposited in the collection of the Entomology Laboratory, Department of Biology, Faculty of Arts and Sciences, Gaziantep University, Gaziantep, Turkey (GUGT).

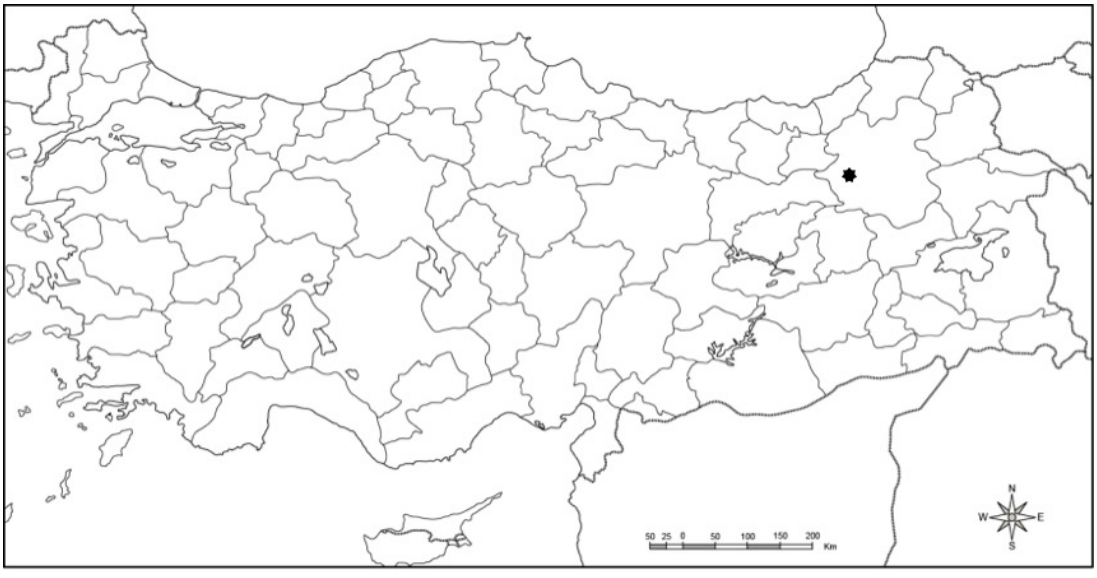


Figure 1. Map of the sampling site (*) in Turkey.

**Preparation of specimens**

Both male and female genitalia were prepared using following procedure: the abdomen was excised from specimen, boiled in NaOH solution (10%) for 45 min, washed with distilled water and genitalia was removed from abdomen. For deporting water, genitalia were held in 96% alcohol during the following 15 min. For deporting alcohol, genitalia were held in xylene 3-5 min. Then, genitalia were placed between microscope slide glass cover slip and were pasted with Entellan.

Terminology and abbreviations generally follow White et al. (1999); additional abbreviations used are: AL, aculeus length; BL, body length; WL, wing length.

## Results

***Tephritis* Latreille, 1804**

Type species: *Musca arnicae* Linnaeus, 1758

See Hendel (1927), Merz (1994), and Korneyev & Korneyev (2019) for diagnosis and description.

***Tephritis turkeri* sp. nov.**

Type material. Holotype ♀: Turkey, Erzurum, Aşkale, Kop Mountain, 39°54' N, 41°14' E, 2400 m, 28.06.2018. leg. M. Kütük and M. Yaran. Paratypes 4♀♀, 36♂♂: same locality and date as the holotype leg. M. Kütük and M. Yaran. Materials deposited at Gaziantep University, Gaziantep, Turkey (GUGT).

**Diagnosis**

*Tephritis turkeri* sp. nov. can be distinguished by the following combination of characters: wing with moderately developed pattern not extending into anal cell and anal lobe, brown spots at the end of vein $R_{4+5}$ and M joined to each other and widened as typical apical fork but separated from main dark area (as in *Tephritis bimaculata* Freidberg, 1981); cell $r_{4+5}$ at the level of dm-cu with one large hyaline spot and with four round small hyaline spots; cell m with three large hyaline area separated two narrow dark rays; dark rays of cell m reach hind margin of wing; abdomen with white setae; aculeus incised on tip.

The new species is very similar to *Tephritis crepidis* Hendel, 1927 in having wing with wide, subrectangular spots at apices of $R_{4+5}$ and M either separated or narrowly fused to each other or remaining dark pattern, pterostigma with yellowish or hyaline spot, anal cell and anal lobe mostly or entirely hyaline, crossvein r-m with four small dots surrounding it, oviscape entirely dark setulose without white setulae even in basiventral part, aculeus incised at apex, but very shallowly, spermathecae moderately long, and male phallus without spinulae on the preglans. It differs from *T. crepidis* by having cell $r_{2+3}$ with two hyaline spots, without the third subapical spot (in *T. crepidis*, third spot usually present), four small dots surrounding crossvein r-m separated (in *T. crepidis*, as a rule, fused forming a pair of hyaline bars), anal cell without dark spots (in *T. crepidis*, usually with two conspicuous gray spots at vein $A_2$ and cell apical half) and somewhat smaller size (mean wing 3.7 in *T. turkeri* vs. 4.5 in *T. crepidis*).

The new species is also similar to *T. bimaculata*, *Tephritis jabeliae* Freidberg, 1981, *Tephritis spreta* (Loew, 1862), and some specimens of *Tephritis dioscurea* (Loew, 1856) in having two hyaline spots in $R_{2+3}$ combined with the spots on the apices of veins $R_{4+5}$ and M separated from the remaining wing pattern, clearly differing from them by having the subbasal hyaline spot in the cell $r_{4+5}$ 8-shaped or split into two smaller spots (subrectangular in *T. bimaculata*, *T. jabeliae*, *T. spreta* and *T. dioscurea*) and oviscape much shorter, usually as long as or slightly longer than tergites 5 and 6 combined.

**Description**

Head. Yellow to brown, except ocellar triangle black, occiput black and wide v-shaped; frons as wide as long; eye 1.25 times as high as long; ocellar, frontal and orbital setae dark brown, other setae and setulae yellowish white; first flagellomere of antenna 1.25 times as long as wide; antenna yellow to brown; arista dark brown to black; gena yellow with black setulae; palpus yellow with black setulae (Figure 2c-d).

Thorax. Ground color black, densely gray microtrichose; setulae white and acuminate; all setae brown and acuminate, except posterior notopleural setae whitish; basal scutellar setae two times as long as apical scutellar setae; halter yellow (Figure 2e).

Wing. Pattern brown, apical fork developed and separated from main dark area; three of examined specimens have apical spots isolated from each other (as shown Figure 3a). Basal cells bc, bm and bcu hyaline, cell c hyaline with narrow brown spot at middle; pterostigma brown with yellowish hyaline spot; cell $r_1$ brown from posterior to pterostigma with two trapeziform hyaline spots; hyaline spots of cell $r_1$ separated by narrow dark ray; apex of $r_1$ brown, without hyaline spot; cell $r_{2+3}$ hyaline at base, with dark area posterior to pterostigma; in cell $r_{2+3}$ three hyaline spots posterior to spots of $r_1$ separated by narrow dark rays or partly merged one small hyaline spot under the distal spot; apical half of cell br dark, usually with two small hyaline spots and tiny dot; crossvein r-m dark and two side of vein with two small hyaline spots at the level of vein $R_{2+3}$; vein $R_{4+5}$ bare; cell $r_{4+5}$ at the level of dm-cu with one large hyaline spot and with four round small hyaline spots; brown spots at the end of vein $R_{4+5}$ and M joined each other and widened as typical apical fork but separated from main dark area; basal half of cell dm hyaline, rest part of cell is brown, with one large hyaline spot and two smaller hyaline spots in near the crossvein dm-cu; cell m with three large hyaline area separated two narrow dark rays; dark rays of cell m reach hind margin of wing; cell cu with pale dark irregular pattern; rest of cell almost hyaline; vein A darkened at base, anal cell otherwise hyaline; anal lobe hyaline.

Legs. Brown; femora with dark bands and brown and whitish mixed setulae.

Abdomen. Ground color black; densely gray microtrichose and white setulose; male tergite 5 with and female tergites 5 and 6 black marginal setae. Male terminalia: Epandrium oval and wide as in other *Tephritis* (Figure 3b). Phallus glans moderately short and membranous (Figure 3c); preglans bare. Female terminalia: Oviscape shining dark brown to black, widely white setulose on basal part and, brown setulose and setose posteriorly, as long as tergites 4-6 combined (Figure 2f); aculeus 5.5 times as long as wide, moderately narrowed towards apex(Figure 3d); apex of aculeus abruptly narrowed at apex, almost truncated, with two apical lobes, separated by a very shallow (half as deep as lobe width) incision on tip (Figure 3f); spermathecae 6-7 times as long as wide, papillose (Figure 3e). Measurements*:* Male: BL = 4.7-4.9 mm; WL = 3.5-3.7 m (n = 10). Female: BL = 5.2-5.4 mm; WL = 3.7-4.0 mm (n = 5); AL = 1.6 mm.

Host plant. Unknown. Adults of *T. turkeri* sp. nov. were swept from *S. tomentosa*, an endemic plant in Turkey, which is its possible host plant.

**Etymology**

This species is named after Mustafa Türker Kütük, the son of first author.

**Zoobank number**

This work and the nomenclatural acts it contains have been registered in the ZooBank. The ZooBank Life Science Identifier (LSID) for this publication is urn:lsid:zoobank.org:pub:79B3937C-8581-4E0A-9AE2-019DE7E38A84.

***Tephritis zernyi* Hendel, 1927**

Specimens examined. 3♀♀, Turkey, Erzurum, Kop Mountain, 40°01' N, 40°31' E, 2378 m, 28.06.2018. leg. M. Kütük and M. Yaran.

Host plant. *A. minus*.

Comment. Detailed description and diagnosis of *T. zernyi* were provided by Korneyev & Korneyev, 2019. In this study, *T. zernyi* was recorded for the first time from Turkey (Figure 4a-c).


Figure 2. Adult figures of *Tephritis turkeri* sp. nov. a) holotype ♀; b) paratype ♂; c) head (lateral); d) head; e) thorax; f) female abdomen (Scale bar=1 mm).

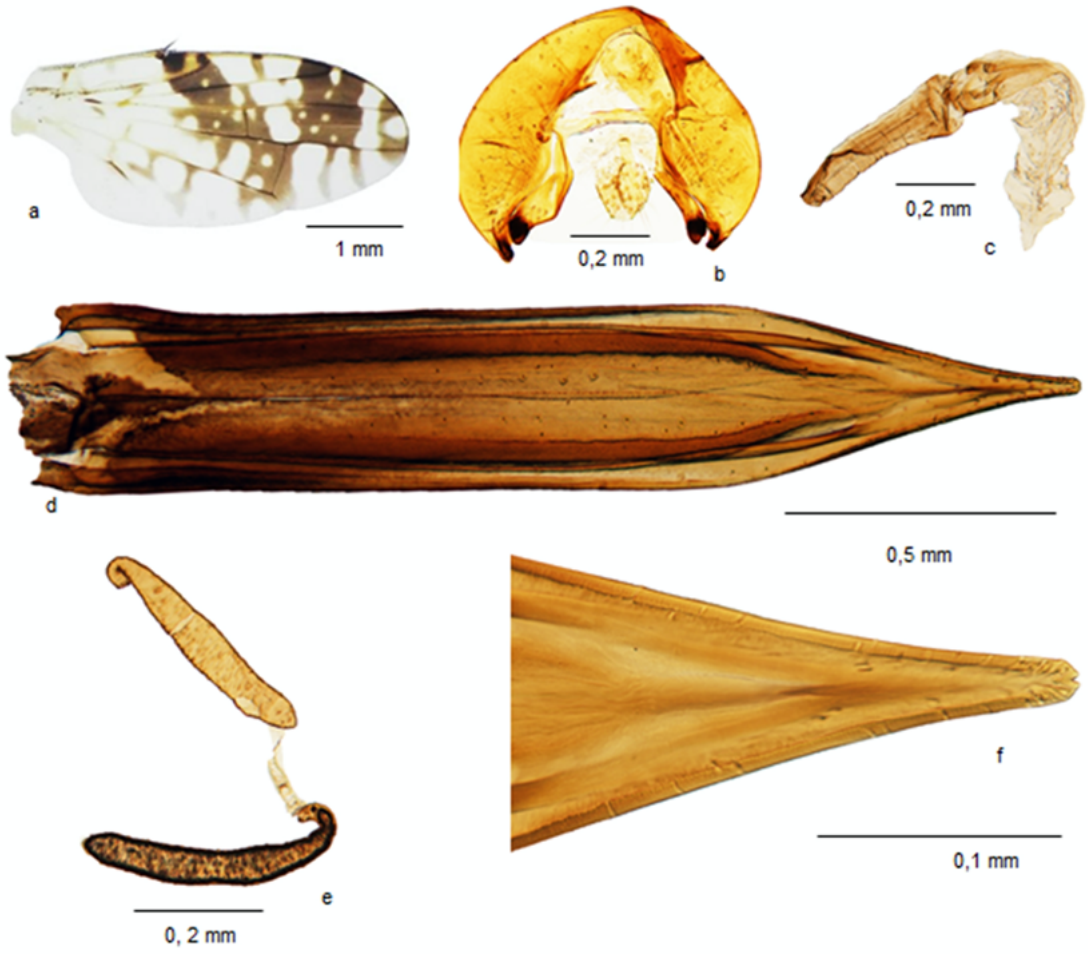


Figure 3. Wing and genitalia of *Tephritis turkeri* sp. nov. a) wing; ♂ b) epandrium; c) glans; d) aculeus; e) spermatheca; f) apex of aculeus.

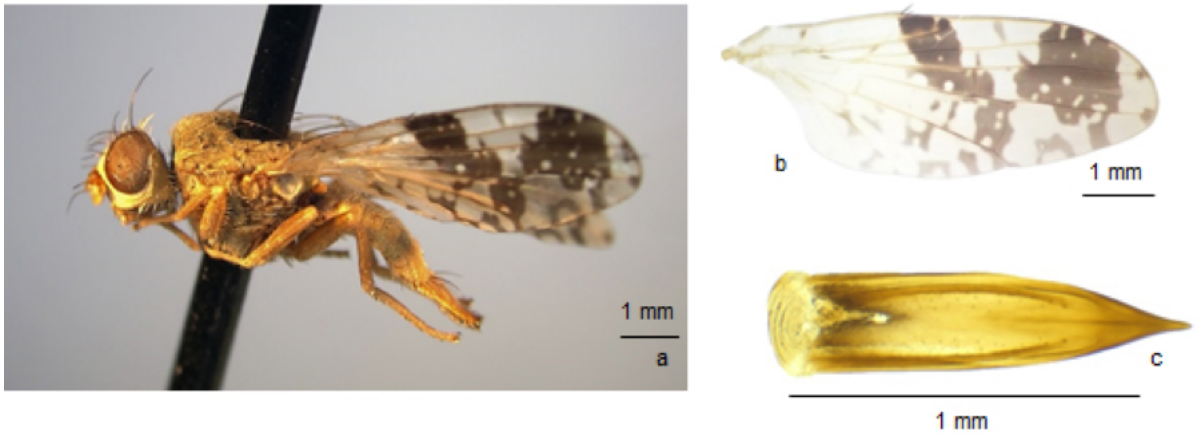


Figure 4. *Tephritis zernyi* a) adult; b) wing; c) aculeus (scale bar=1 mm).

## Discussion

*Tephritis turkeri* new species is very similar to *T. bimaculata*, *T. jabeliae* and some populations of *Tephritis conyzifloliae* Merz, 1992 and with apical fork separated from main dark area. It can be easily distinguished from *T. bimaculata* with dark wing pattern, small hyaline spots in cell $r_{4+5}$ and cell dm and three large hyaline area in cell m (in *T. bimaculata* posterior hyaline area of cell m separated with dark ray) and can be easily distinguished from *T. jabeliae* with larger hyaline spot on apex of wing, unique hyaline spots in cell $r_1$, $r_{2+3}$ and cell $r_{4+5}$ and apex of aculeus (in *T. turkeri* sp. nov. aculeus incised on tip). Also, it is similar to *T. conyzifloliae* and *T. crepidis* with incised aculeus on tip and separated apical fork (some populations see Korneyev & Korneyev, 2019). In addition to that, it can be easily distinguished from *T. conyzifloliae* and *T. crepidis* with different size and type hyaline areas of wing cells and completely hyaline anal cell and anal lobe.

Freidberg & Kütük (2002) revised *Tephritis pulchra* (Loew, 1844) group and only the species of *T. pulchra* group were associated with genus *Scorzonera*. *Tephritis turkeri* sp. nov. were swept from *S. tomentosa* which is an endemic plant from Turkey. Although any biological has not been conducted for this paper, we suppose that this plant can be a host for *T. turkeri* sp. nov.

The new species, however, shows strong similarity with *T. crepidis*, which develops in flower heads of *Crepis* spp., the genus of the tribe Cichorieae of the family Asteraceae, to which *Scorzonera* also belongs.

*Tephritis zernyi* was recorded in this study for the first time from Turkey. One hundred and seventy species of fruit flies had previously been described or recorded from Turkey (Korneyev, 2016; Görmez & Kütük, 2020). Together with those reported here, the number of the fruit flies determined in Turkey is 172.

It is clear that Turkey has many different biotopes and ecological conditions. Many species, genera and families will be waiting to be discovered in Turkey, and some species might become extinct before they can be collected and described (Yaran, 2019).

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*Original article (Orijinal araştırma)*

# Occurrence and abundance of cereal nematodes in Konya and Karaman Provinces in Turkey[1]

Konya ve Karaman (Türkiye) illerinde tahıl nematodlarının dağılımı ve popülasyon yoğunluğu

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## Abstract

Distribution and populations of cereal nematodes in Konya and Karaman Provinces were investigated in 2016 and 2017. Root lesion nematodes, *Pratylenchus thornei* Sher & Allen, 1953, *Pratylenchus neglectus* Rensch, 1924 and *Pratylenchus vulnus* Allen & Jensen, 1951 (Tylenchida, Pratylenchidae), were found at 69, 7 and 17 locations, respectively. Cereal cyst nematode, *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 (Tylenchida, Heteroderidae), was found at 33 locations. Stem and bulb nematode, *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936 (Tylenchida, Anguinidae), was not found in any samples. *Pratylenchus* spp. were found in 40.5% (6±1 nematodes 100 g dry soil$^{-1}$) and *Heterodera* spp. in 29% (3±1cysts 250 g dry soil$^{-1}$) of the soil samples. *Ditylenchus* spp. (32.6%, 18±3 nematodes 100 g dry soil$^{-1}$) and *Tylenchus* spp. (21.4%; 12±3 nematodes 100 g dry soil$^{-1}$) were widely distributed. *Paratylenchus* spp. (0.9%; 1±1 nematodes 100 g dry soil$^{-1}$) and *Pratylenchoides* spp. (0.5%; 1±1 nematodes 100 g dry soil$^{-1}$) were found in few locations and in low abundance. *Aphelenchus* spp. (39.1%; 33±5 nematodes 100 g dry soil$^{-1}$) and *Aphelenchoides* spp. (51.2%; 52±7 nematodes 100 g dry soil$^{-1}$) were identified as the fungal-feeding nematodes. *Acrobeloides* (65.1%; 109±13 nematodes 100 g dry soil$^{-1}$) and *Cephalobus* (19.1%; 7±1 nematodes 100 g dry soil$^{-1}$) were the most abundant bacterial-feeding nematode genera.

**Keywords:**Free living nematodes, *Heterodera* spp., morphometrics, *Pratylenchus* spp., species-specific PCR

## Öz

Konya ve Karaman illerinde tahıl alanlarında nematodlarının dağılımı ve popülasyonları 2016 ve 2017 yıllarında araştırılmıştır. Kök yara nematodları; *Pratylenchus thornei* Sher & Allen, 1953, *Pratylenchus neglectus* Rensch, 1924 ve *Pratylenchus vulnus* Allen & Jensen, 1951 (Tylenchida, Pratylenchidae) sırasıyla 69, 7 ve 17 lokasyonda tespit edildi. Tahıl kist nematodu *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 (Tylenchida, Heteroderidae) 33 lokasyonda bulunmuştur. Soğan sak nematodu, *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936 (Tylenchida, Anguinidae) incelenen örneklerde bulunmamıştır. *Pratylenchus* spp. toprak örneklerinde %40.5 (6±1 nematod 100 g kuru toprak$^{-1}$) ve *Heterodera* spp. %29 (3±1 kist 250 g kuru toprak$^{-1}$) oranında tespit edilmiştir. *Ditylenchus* spp. (%32.6; 18±3 nematod 100 g kuru toprak$^{-1}$) ve*Tylenchus* spp. (%21.4, 12±3 nematod 100 g kuru toprak$^{-1}$)'nin yaygın olarak dağıldığı belirlenmiştir. *Paratylenchus* spp. (%0.9, 1±1 nematod 100 g kuru toprak$^{-1}$) ve *Pratylenchoides* spp. (%0.5; 1±1 nematod 100 g kuru toprak$^{-1}$) birkaç lokasyonda ve düşük yoğunlukta bulunmuştur. *Aphelenchus* spp. (%39.1; 33±5 nematod 100 g kuru toprak$^{-1}$) ve *Aphelenchoides* spp. (%51.2; 52±7 nematodes 100 g kuru toprak$^{-1}$) fungal beslenen türler olarak belirlenmiştir. *Acrobeloides* (%65.1; 109±13 nematod 100 g kuru toprak$^{-1}$) ve *Cephalobus* (%19.1; 7±1 nematod 100 g kuru toprak$^{-1}$) en yoğun bulunan bakteriyel beslenen nematod cinsleridir.

**Anahtar sözcükler:**Serbest yaşayan nematodlar, *Heterodera* spp., morfometrik ölçümler, *Pratylenchus* spp., türe özgü PCR

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## Introduction

Cereals have an important place in the human diet and in animal feed throughout the world. Konya Province has the highest cereal production on the Central Anatolian Plateau of Turkey. Excluding maize, cereals are produced over 1.2 Mha, which is the 11.7% of Turkey production area (about 10 Mha) in Konya and Karaman Provinces. Cereal production in the provinces is about 3.5 Mt, 12.5% of the total production in Turkey (28 Mt) (TÜİK, 2019).

Nematodes are round worms that include parasitic and free living species found in the rhizosphere region of the soil. Nematodes are grouped according to their feeding characteristics (Yeates et al., 1993). Around the world, the most damaging nematode species in cereals are root lesion and cereal cyst nematodes, and likewise in Turkey (Nicol et al., 2003; Imren et al., 2012; Abd-Elgawad & Askary, 2015; Toktay et al., 2015). The yield losses in wheat caused by cereal cyst and root lesion nematodes were reported up to 50% on the Central Anatolian Plateau of Turkey (Nicol & Ortiz-Monastterio, 2004). Cereal cyst nematodes of *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984, *Heterodera latipons* Franklin, 1969 and *Heterodera mani* Mathews, 1971 (Tylenchida, Heteroderidae) were found in cereals on the Central Anatolian Plateau (Enneli et al., 1994; Rumpenhorst et al.,1996; Ozturk et al., 1998; Abidou et al., 2005; Yavuzaslanoglu et al., 2012). *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* Rensch, 1924 (Tylenchida, Pratylenchidae) were prevalent species of root lesion nematodes on the Central Anatolian Plateau (Yavuzaslanoglu et al., 2012).

Morphology and morphometrics are the basic methods in nematode diagnosis. However, generally there is considerable variation between the specimens investigated witin a species; the studies require experience and are time consuming. In particular, *Pratylenchus* spp. has considerable intraspecific variation. Additionally, the low number of diagnostic features, depending on the reproductive strategy of the species necessitates molecular identification (Castillo &Vovlas, 2007). Molecular characterization is a practical and reliable method, and supports nematode diagnosis studies. To date, data on root lesion nematodes on the Central Anatolian Plateau of Turkey were based on morphological and molecular identification. The study aimed to investigate the root lesion nematodes using molecular methods in addition to morphology and morphometrics.

The long-term assessment of nematode communities in cereal growing areas provides important information on the damaging potential of parasitic nematodes and also effect of free living nematodes on soil fertility and sustainability. Hence, monitoring nematode communities in regulated terms is required; especially nematode communities on the production areas of strategic plant species. For this purpose, the occurrence and abundance of nematodes in cereal production areas in Konya and Karaman Provinces which represent the largest cereal production area of Turkey were investigated in 2016-2017. The most damaging plant-feeding nematode species were identified using the species-specific PCR technique.

## Materials and Methods

### Sampling locations

Wheat and barley planted fields in Konya and Karaman Provinces on the Central Anatolian Plateau were sampled for the prevalence and population estimation of the nematodes in the years of 2016 and 2017. A total of 215 plant and soil samples were collected representing the districts systematically stopping every 3-4 km. Sixty-one samples were collected from Karaman Province and 154 samples were collected from Konya Province. The sampling was performed in April 2016 and 2017 for migratory nematodes. Cereal cyst nematode (CCN) cysts were sampled from the same locations with migratory nematodes in July-August 2017. The samples were taken every 15-20 paces in a zigzag pattern from 15-20 points constituting two kg of a bulk sample in a field. A 2.5 cm diameter soil corer was used for soil sampling to a depth of 30 cm. The plant samples were collected from the same points as the soil cores. The sampled cereal fields were mapped using a GPS.

**Nematode extraction and population estimation**

The nematodes were extracted from the plant and soil samples using a modified Baermann funnel technique (Hooper, 1986a). Three plants were used for the nematode extraction which was performed separately for each plant. The nematode counts from the plant samples were presented as average number per plant. These plant samples were incubated for 1 d and the soil samples for 2 d for the nematode extraction. The percentage of the moisture of each soil samples was calculated drying 10 g of fresh soil in an oven at 90ºC for 2 d. The nematode counts from the soil samples were converted to the number of nematodes 100 g of dry soil$^{-1}$. The nematodes obtained from the soil samples were divided into trophic groups according to Yeates et al. (1993).

The cysts of the CCN were extracted using a flotation technique (Kort, 1960) from a 250 g soil sample. Number of cysts in each soil sample was counted under a stereo microscope. The plant roots were washed gently with water and evaluated for the cyst formation under the stereo microscope.

**Species identification of key plant-feeding nematodes**

The nematodes obtained from the plant and soil samples were counted at genus level and divided into trophic groups according to Yeates et al. (1993). The permanent slides of the plant-feeding nematodes for the morphological species identification were prepared using all the available specimens (Hooper; 1986b). Measurements and morphometric calculations were compared with the literature.

Five species of *Pratylenchus* spp. [*P. thornei, P. neglectus, Pratylenchus vulnus* Allen & Jensen, 1951*, Pratylenchus scribneri* Steiner, 1943 and *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941(Tylenchida, Pratylenchidae)], three species of *Heterodera* spp. [*H. filipjevi, H. latipons* and *Heterodera avenae* Wollenweber, 1924 (Tylenchida, Heteroderidae)] and *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936 (Tylenchida, Anguinidae) were investigated using the species-specific PCR in addition to morphological diagnosis. This molecular method was applied as described by Karaca (2018) and Yavuzaslanoglu et al. (2018). Species-specific primers used for identification of root lesion nematodes multiplying D3 expansion regions of 26S-rDNA were presented on Table 1. Nematodes were identified obtaining the specific bands at 290, 278, 286, 288 and 287 bp for *P. neglectus*, *P. penetrans*, *P. scribneri*, *P. thornei* and *P. vulnus,* respectively (Al-Banna et al., 2004).

Table 1. Species of root lesion nematodes (*Pratylenchus* spp.) identified using 26S-rDNA D3 elongation regions, short name of used primers, 5'-3' forward sequences, annealing temperature, PCR product size and references obtained

| Nematode species | Short name of primer | 5'-3' Primer sequence | Annealing temperature (°C) | PCR product size (bp) | Reference |
|---|---|---|---|---|---|
| *P. neglectus* | PNEG | ATGAAAGTGAACATGTCCTC | 63 | 290 | Al-Banna et al., 2004 |
| *P. penetrans* | PPEN | TAAAGAATCCGCAAGGATAC | 62 | 278 | Al-Banna et al., 2004 |
| *P scribneri* | PSCR | AAAGTGAACGTTTCCATTTC | 63 | 286 | Al-Banna et al., 2004 |
| *P. thornei* | PTHO | GAAAGTGAAGGTATCCCTCG | 68 | 288 | Al-Banna et al., 2004 |
| *P. vulnus* | PVUL | GAAAGTGAACGCATCCGCAA | 68 | 287 | Al-Banna et al., 2004 |

Primers specific to rDNA-ITS regions of cereal cyst nematodes are shown in Table 2. *Heterodera filipjevi*, *H. avenae* and *H. latipons* were identified with obtained specific bands at 170, 242 and 204 bp, respectively (Toumi et al., 2013; Yan et al., 2013).

Molecular identification of *D. dipsaci* were performed using nine species-specific primers suggested by Vrain et al. (1992); Marek et al. (2005); Zouhar et al. (2007); Marek et al. (2010) and Vovlas et al. (2011) (Table 3). Specific bands for identification of *D. dipsaci* were investigated at 327, 396, 517, 263, 333, 967, 256, 325, 245 bp, for PF1-PR1, PF2-PR2, DdpS1-rDNA2, DitNF1-rDNA2, DipU F-DipU R, 18S-26S, DipU F-Dip1 R, DIT2 F-DIT2 R, DIT5 F-DIT5 R primers, respectively.

Table 2. Species of identified cereal cyst nematodes (*Heterodera* spp.) using rDNA-ITS regions, short name of used primers, 5'-3'sequences, annealing temperature, PCR product size and references obtained

| Nematode species | Short name of primer | 5'-3' Primer sequence | Annealing temperature (°C) | PCR product size (bp) | Reference |
|---|---|---|---|---|---|
| *H. filipjevi* | HfITS-F1 | F: CCCGTCTGCTGTTGAGA | 58 | 170 | Yan et al., 2013 |
| | HfITS-R1 | R: ACCTCAGGCTTTTATTATCAC | | | |
| *H. avenae* | HaITS-F | F: ATGCCCCCGTCTGCTGA | 64 | 242 | Yan et al., 2013 |
| | HaITS-R | R: GAGCGTGCTCGTCCAAC | | | |
| *H. latipons* | Hlat-actF | F: ATGCCATCATTATTCCTT | 50 | 204 | Toumi et al., 2013 |
| | Hlat-actR | R: ACAGAGAGTCAAATTGTG | | | |

Table 3. The primers and sequences, PCR product size, target regions on nematode genome and references used for species-specific detection of *Ditylenchus dipsaci*

| Primer | 5'-3' Primer sequence | PCR product size (bp) | Target region | Reference |
|---|---|---|---|---|
| PF1 | 5′-AACGGCTCTGTTGGCTTCTAT-3 | 327 | Flanking ITS regions | Marek et al., 2005 |
| PR1 | 5′-ATTTACGACCCTGAGCCAGAT-3′ | | | |
| PF2 | 5′-TCGCGAGAATCAATGAGTACC-3′ | 396 | Flanking ITS regions | Marek et al., 2005 |
| PR2 | 5′-AATAGCCAGTCGATTCCGTCT-3′ | | | |
| DdpS1 | 5′-TGGCTGCGTTGAAGAGAACT-3′ | 517 | 5.8S rDNA | Vrain et al., 1992 |
| rDNA2 | 5′-TTTCACTCGCCGTTACTAAGG-3′ | | | |
| DitNF1 | 5′-TTATGACAAATTCATGGCGG-3′ | 263 | 18S rDNA - ITS1 | Vrain et al., 1992 |
| rDNA2 | 5′-TTTCACTCGCCGTTCTAAGG-3′ | | | |
| DipU F | 5' -CCCATTTTTGAACTTTTTTACAAG-3' | 333 | Flanking ITS regions | Vovlas et al., 2011 |
| DipU R | 5' -CTAGATTAGCAAAGACGTATATC-3' | | | |
| 18S | 5' -TTGATTAGGTCCCTGCCCTTT-3' | 967 | ITS1-5.8S-ITS2 | Marek et al., 2010 |
| 26S | 5' -TTTCACTCGCCGTTACTAAGG-3' | | | |
| DipU F | 5' -CCCATTTTTGAACTTTTTTACAAG-3' | 256 | ITS1-ITS2 | Marek et al., 2010 |
| Dip1 R | 5' -GAAAAGCACCCAACCAGTACC-3' | | | |
| DIT2 F | 5′-GCAATGCACAGGTGGATAAAG-3′ | 325 | Flanking ITS regions | Zouhar et al., 2007 |
| DIT2 R | 5′-CTGTCTGTGATTTCACGGTAGAC-3′ | | | |
| DIT5 F | 5′-GAAAACCAAAGAGGCCGTAAC-3′ | 245 | Flanking ITS regions | Zouhar et al., 2007 |
| DIT5 R | 5′-ACCTGATTCTGTACGGTGCAA-3′ | | | |

### Statistical analysis

Statistically significant differences of the population densities of the nematode genera among districts were investigated using analysis of variance and Student's t-test. Statistical analyses were performed using the JMP5.01.a program (JMP, 2009).

## Results and Discussion

The occurrence and abundance of the nematodes on wheat and barley fields in Konya and Karaman Provinces, which is the largest cereal production area of Turkey was investigated in detail in all the districts represented with 215 samples. Seventy-six percent of the samples contained plant-feeding nematodes.

The occurrence of the economically important nematode species of *Pratylenchus* spp. was 40.5%, supporting previous reports (Yavuzaslanoglu et al., 2012) (Table 4). The maximum *Pratylenchus* spp. population was recorded in Karapınar District of Konya Province with a mean of 14±14 nematodes 100 g dry soil$^{-1}$. The plant samples contained a higher number of nematodes in Kadınhanı, Selçuklu and Cihanbeyli Districts of Konya Province; a mean of 13±8, 16±16 and 9±4 nematodes 100 g dry soil$^{-1}$, respectively (Table 5). However, the population density was relatively lower in comparison to previous reports. It was a maximum 111 nematodes 100 g dry soil$^{-1}$ in the current study, while it had been reported to be up to 274 nematodes in 100 g dry soil in 2003 (Yavuzaslanoglu et al., 2012).

Table 4. Prevalence of individual nematode genera and feeding groups identified in samples

| Province | District | Number of samples collected | *Pratylenchus* spp. | *Heterodera* spp. | *Pratylenchus* spp. + *Heterodera* spp. | *Ditylenchus* spp. | *Tylenchus* spp. | *Paratylenchus* spp. | *Pratylenchoides* spp. | Plant Feeding Nematodes | *Aphelenchus* spp. | *Aphelenchoides* spp. | Fungal Feeding Nematodes | *Cephalobus* spp. | *Eucephalobus* spp. | *Acrobeles* spp. | *Acrobeloides* spp. | *Rhabditis* spp. | Bacterial Feeding Nematodes | *Dorylaimid*aorder |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Karaman | Ayrancı | 9 | 2 | 2 | 0 | 4 | 1 | 0 | 0 | 5 | 5 | 3 | 7 | 1 | 1 | 0 | 6 | 0 | 8 | 1 |
| | Başyayla | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 2 | 0 |
| | Ermenek | 4 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 3 | 2 | 4 | 0 | 1 | 0 | 3 | 0 | 3 | 0 |
| | Central | 44 | 17 | 8 | 3 | 18 | 8 | 0 | 0 | 32 | 24 | 28 | 33 | 13 | 0 | 1 | 34 | 1 | 37 | 4 |
| | Sarıveliler | 2 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 2 | 2 | 2 | 1 | 0 | 0 | 2 | 0 | 2 | 0 |
| Konya | Ahırlı | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Akşehir | 4 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 1 | 3 | 4 | 2 | 0 | 1 | 1 | 0 | 3 | 0 |
| | Altınekin | 3 | 3 | 2 | 2 | 2 | 1 | 0 | 0 | 3 | 0 | 2 | 2 | 0 | 0 | 0 | 3 | 0 | 3 | 0 |
| | Beyşehir | 5 | 2 | 1 | 1 | 4 | 2 | 0 | 0 | 5 | 3 | 4 | 4 | 1 | 0 | 0 | 5 | 0 | 5 | 2 |
| | Bozkır | 4 | 2 | 1 | 1 | 2 | 0 | 1 | 0 | 4 | 1 | 1 | 1 | 1 | 0 | 0 | 3 | 0 | 3 | 1 |
| | Central | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| | Çeltik | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 1 |
| | Cihanbeyli | 26 | 12 | 11 | 6 | 2 | 5 | 0 | 1 | 18 | 5 | 3 | 7 | 7 | 0 | 2 | 14 | 0 | 17 | 11 |
| | Çumra | 11 | 4 | 4 | 1 | 5 | 1 | 0 | 0 | 9 | 9 | 8 | 10 | 1 | 0 | 2 | 10 | 0 | 10 | 0 |
| | Doğanhisar | 3 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 3 | 3 | 2 | 3 | 1 | 1 | 0 | 3 | 0 | 3 | 0 |
| | Ereğli | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| | Güneysınır | 5 | 1 | 1 | 1 | 3 | 0 | 0 | 0 | 4 | 3 | 3 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 0 |
| | Hüyük | 2 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 |
| | Ilgın | 7 | 1 | 4 | 0 | 2 | 2 | 0 | 0 | 6 | 2 | 6 | 6 | 0 | 0 | 0 | 6 | 0 | 6 | 1 |
| | Kadınhanı | 8 | 4 | 4 | 3 | 6 | 4 | 0 | 0 | 8 | 1 | 5 | 5 | 0 | 0 | 0 | 8 | 0 | 8 | 0 |
| | Karapınar | 8 | 3 | 1 | 0 | 1 | 1 | 0 | 0 | 5 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| | Karatay | 15 | 8 | 5 | 2 | 4 | 5 | 0 | 0 | 13 | 5 | 9 | 9 | 1 | 0 | 0 | 9 | 0 | 10 | 1 |
| | Kulu | 12 | 6 | 4 | 2 | 0 | 2 | 0 | 0 | 9 | 0 | 4 | 4 | 4 | 1 | 2 | 3 | 0 | 7 | 7 |
| | Sarayönü | 6 | 5 | 4 | 3 | 4 | 3 | 0 | 0 | 6 | 2 | 6 | 6 | 0 | 0 | 0 | 6 | 0 | 6 | 0 |
| | Selçuklu | 5 | 1 | 2 | 0 | 2 | 2 | 0 | 0 | 4 | 1 | 5 | 5 | 0 | 0 | 0 | 4 | 0 | 4 | 0 |
| | Seydişehir | 6 | 1 | 2 | 1 | 0 | 2 | 1 | 0 | 4 | 3 | 3 | 4 | 1 | 0 | 0 | 4 | 0 | 5 | 1 |
| | Tuzlukçu | 6 | 3 | 1 | 1 | 0 | 1 | 0 | 0 | 3 | 1 | 3 | 3 | 2 | 0 | 2 | 1 | 0 | 3 | 4 |
| | Yalıhöyük | 4 | 2 | 1 | 1 | 3 | 1 | 0 | 0 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 |
| | Yunak | 7 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 1 | 3 | 3 | 0 | 1 | 3 | 0 | 4 | 0 |
| Number of total samples | | 215 | 87 | 62 | 28 | 70 | 46 | 2 | 1 | 163 | 84 | 110 | 134 | 41 | 4 | 11 | 140 | 1 | 161 | 34 |
| Frequency % | | 100 | 40 | 29 | 13 | 32 | 21 | 0,9 | 0,5 | 76 | 39 | 51 | 62 | 19 | 1 | 5 | 65 | 0,5 | 75 | 15 |

Table 5. Mean±standard error of mean and (range) population densities of nematode genera and order Dorylaimida in the samples collected from Konya and Karaman Provinces in Turkey. All values are (100 g dry soil)$^{-1}$, except where indicated

| Province | District | *Pratylenchus* spp. | *Pratylenchus* spp. plant$^{-1}$ | *Heterodera* spp. (250 g dry soil)$^{-1}$ | *Heterodera* spp. plant$^{-1}$ | *Ditylenchus* spp. | *Tylenchus* spp. | *Paratylenchus* spp. | *Pratylenchoides* spp. | *Aphelenchus* spp. | *Aphelenchoides* spp. | *Cephalobus* spp. | *Eucephalobus* spp. | *Acrobeles* spp. | *Acrobeloides* spp. | *Rhabditis* spp. | Dorylaimida order |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Karaman | Ayrancı | 5±3 (0-25) | 0 | 1±1 (0-8) | 0 | 10±4 (0-25) | 3±3 (0-24) | 0 | 0 | 43±24 (0-204) | 31±16 (0-127) | 2±2 (0-21) | 5±5 (0-41) | 0 | 129±60 (0-527) | 0 | 3±3 (0-25) |
| | Başyayla | 11±11 (0-22) | 10±10 (0-20) | 0 | 0 | 22±22 (0-44) | 0 | 0 | 0 | 54±10 (44-63) | 88±67 (21-154) | 0 | 0 | 0 | 625±456 (169-1081)* | 0 | 0 |
| | Ermenek | 11±6 (0-22) | 10±6 (0-20) | 0 | 0 | 11±6 (0-22) | 0 | 0 | 0 | 95±67 (0-293) | 22±13 (0-45) | 0 | 6±6 (0-23) | 0 | 84±63 (0-270) | 0 | 0 |
| | Central | 4±2 (0-44) | 5±1 (0-40) | 2±1 (0-36) | 1±1 (0-2) | 28±10 (0-410) | 8±3 (0-91) | 0 | 0 | 44±11 (0-290) | 53±12 (0-342) | 8±2 (0-46) | 0 | 1±1 (0-22) | 93±22 (0-758) | 1±1 (0-21) | 2±1 (0-22) |
| | Sarıveliler | 0 | 0 | 0 | 0 | 57±12 (45-69) | 11±11 (0-23) | 0 | 0 | 215±146 (69-360) | 171±14 (158-185) | 12±12 (0-23) | 0 | 0 | 567±244 (323-811) | 0 | 0 |
| Konya | Ahırlı | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Akşehir | 11±6 (0-22) | 0 | 0 | 0 | 0 | 5±5 (0-21) | 0 | 0 | 5±5 (0-21) | 16±5 (0-22) | 11±6 (0-22) | 0 | 5±5 (0-21) | 28±28 (0-111) | 0 | 0 |
| | Altınekin | 7±7 (0-21) | 13±6 (0-20) | 15±11 (0-36) | 0 | 83±48 (0-166) | 28±28 (0-83) | 0 | 0 | 0 | 97±50 (0-166) | 0 | 0 | 0 | 166±78 (22-291) | 0 | 0 |
| | Beyşehir | 9±9 (0-46) | 4±4 (0-20) | 1±1 (0-7) | 0 | 35±11 (0-60) | 10±6 (0-26) | 0 | 0 | 119±65 (0-348) | 172±77 (0-371) | 5±5 (0-24) | 0 | 0 | 181±57 (20-348) | 0 | 19±14 (0-70) |
| | Bozkır | 17±17 (0-68) | 5±5 (0-20) | 1±1 (0-1) | 0 | 17±11 (0-44) | 0 | 6±6 (0-23) | 0 | 11±11 (0-44) | 44±44 (0-176) | 6±6 (0-22) | 0 | 0 | 23±10 (0-47) | 0 | 6±6 (0-23) |
| | Çeltik | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7±7 (0-22) | 15±8 (0-23) | 30±20 (0-69) | 0 | 0 | 0 | 0 | 15±15 (0-44) |
| | Central | 0 | 10±1 (0-20) | 2±1 (0-3) | 0 | 0 | 0 | 0 | 0 | 11±11 (0-22) | 0 | 0 | 0 | 0 | 22±22 (0-43) | 0 | 0 |
| | Cihanbeyli | 5±2 (0-22) | 9±4 (0-100) | 5±2 (0-37) | 1±1 (0-4) | 3±2 (0-45) | 6±3 (0-66) | 0 | 5±5 (0-133) | 12±8 (0-194) | 8±5 (0-112) | 14±7 (0-154) | 0 | 3±3 (0-66) | 76±40 (0-939) | 0 | 16±5 (0-89) |
| | Çumra | 6±3 (0-22) | 2±2 (0-20) | 2±1 (0-14) | 0 | 20±9 (0-88) | 2±2 (0-22) | 0 | 0 | 88±28 (0-263) | 49±12 (0-108) | 2±2 (0-22) | 0 | 4±3 (0-23) | 149±35 (0-389) | 0 | 0 |
| | Doğanhisar | 14±14 (0-42) | 0 | 0 | 0 | 7±7 (0-21) | 50±39 (0-127) | 0 | 0 | 43±1 (42-44) | 78±58 (0-190) | 15±15 (0-44) | 30±30 (0-89)* | 0 | 299±203 (0-698) | 0 | 0 |
| | Ereğli | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 23 | 0 | 0 |
| | Guneysınır | 0 | 4±4 (0-20) | 1±1 (0-1) | 0 | 45±24 (0-136) | 0 | 0 | 0 | 106±45 (0-217) | 121±95 (0-497) | 0 | 0 | 0 | 111±65 (0-339) | 0 | 0 |
| | Hüyük | 11±11 (0-21) | 0 | 0 | 0 | 44±20 (24-63) | 180±180 (0-359)* | 0 | 0 | 32±32 (0-63) | 127±127 (0-254) | 0 | 0 | 0 | 604±369 (236-973) | 0 | 0 |
| | Ilgın | 0 | 3±3 (0-20) | 3±1 (0-7) | 0 | 11±7 (0-47) | 7±5 (0-27) | 0 | 0 | 21±14 (0-93) | 135±63 (0-434) | 0 | 0 | 0 | 129±65 (0-488) | 0 | 3±3 (0-21) |
| | Kadınhanı | 13±6 | 13±8 | 2±1 | 1±1 | 40±15 | 59±50 | 0 | 0 | 3±3 | 67±23 | 0 | 0 | 0 | 190±58 | 0 | 0 |
| | Karapınar | 14±14 | 8±3 | 0 | 0 | 3±3 | 3±3 | 0 | 0 | 3±4 | 48±48 | 0 | 0 | 0 | 4±4 | 0 | 0 |
| | Karatay | 6±3 | 5±2 | 6±3 | 0 | 10±6 | 17±9 | 0 | 0 | 34±21 | 93±56 | 1±1 | 0 | 0 | 187±73 | 0 | 2±2 |
| | Kulu | 4±3 | 10±3 | 3±2 | 0 | 0 | 5±4 | 0 | 0 | 0 | 7±3 | 22±10 | 2±2 | 4±3 | 10±5 | 0 | 17±6 |
| | Sarayönü | 7±4 | 13±4 | 14±9 | 1±1 | 46±20 | 22±11 | 0 | 0 | 10±7 | 85±20 | 0 | 0 | 0 | 123±23 | 0 | 0 |
| | Selçuklu | 4±4 | 16±16 (0- | 1±1 | 0 | 33±28 | 25±17 | 0 | 0 | 4±4 | 58±23 | 0 | 0 | 0 | 151±51 | 0 | 0 |
| | Seydişehir | 3±3 | 0 | 2±2 | 1±1 | 0 | 8±5 | 7±7 | 0 | 29±21 | 36±19 | 4±4 | 0 | 0 | 59±33 | 0 | 4±4 |
| | Tuzlukçu | 7±5 | 7±4 | 1±1 | 0 | 0 | 11±11 | 0 | 0 | 7±7 | 33±18 | 19±15 | 0 | 7±4 | 4±4 | 0 | 26±14 |
| | Yalıhöyük | 11±6 | 5±5 | 2±2 | 0 | 52±32 | 6±6 | 0 | 0 | 6±6 | 22±22 | 0 | 0 | 0 | 28±21 | 0 | 0 |
| | Yunak | 3±3 | 6±6 | 7±7 | 1±1 | 0 | 0 | 0 | 0 | 21±15 | 13±13 | 12±6 | 0 | 3±3 | 19±12 | 0 | 0 |

*highest population density.

Three species of root lesion nematodes were found in the cereal fields surveyed. *Pratylenchus thornei* was the most widely distributed root lesion nematode, identified from 69 sampling locations in Çumra, Karatay, Karapınar, Central, Guneysınır, Bozkır, Yalıhöyük, Seydişehir, Beyşehir, Hüyük, Doğanhisar, Ilgın, Akşehir, Tuzlukçu, Yunak, Kulu, Cihanbeyli, Altınekin, Selçuklu, Sarayönü and Kadınhanı Districts in Konya and Central, Ayrancı, Başyayla, Ermenek Districts in Karaman Province using D3b-R/Ptho-F primer set (Figure 1). The species-specific bands were obtained at 288 bp as suggested by

Al-Banna et al. (2004) (Figures 2 and 3). The morphology and morphometricss for *P. thornei* agreed with the previous literature (Sher & Allen, 1953; Elekcioglu, 1992; Kepenekçi, 1999; Osmanoglu, 2006; Imren & Elekcioglu, 2008) (Table 6).

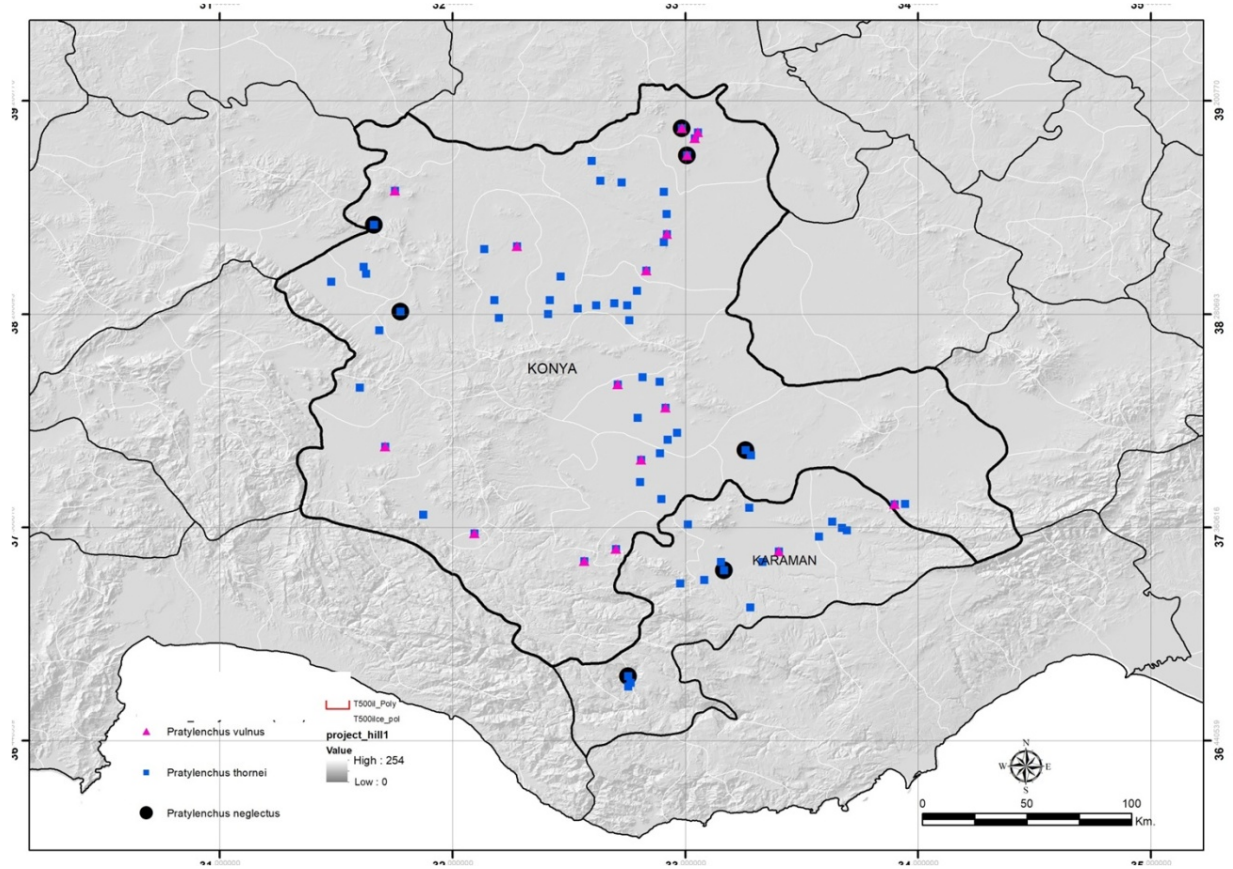


Figure 1. Root lesion nematodes identified in the sampling locations.

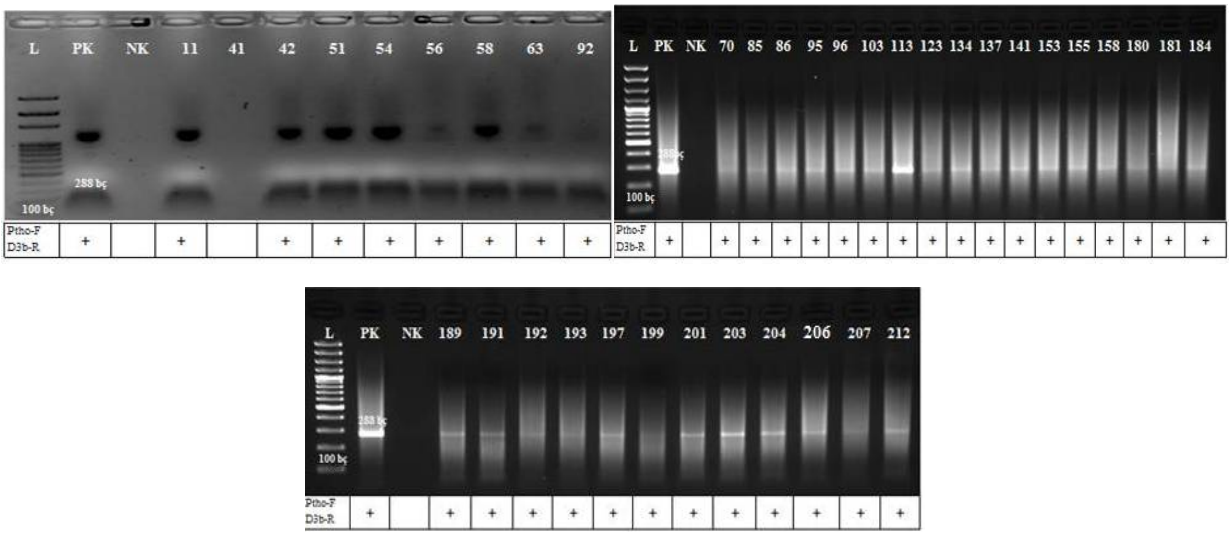


Figure 2. Identification of *P. thornei* in plant samples using PTHO primer in Konya and Karaman provinces. L, 100-bp ladder; PK: positive control; NK, negative control; numbers are survey samples numbers.

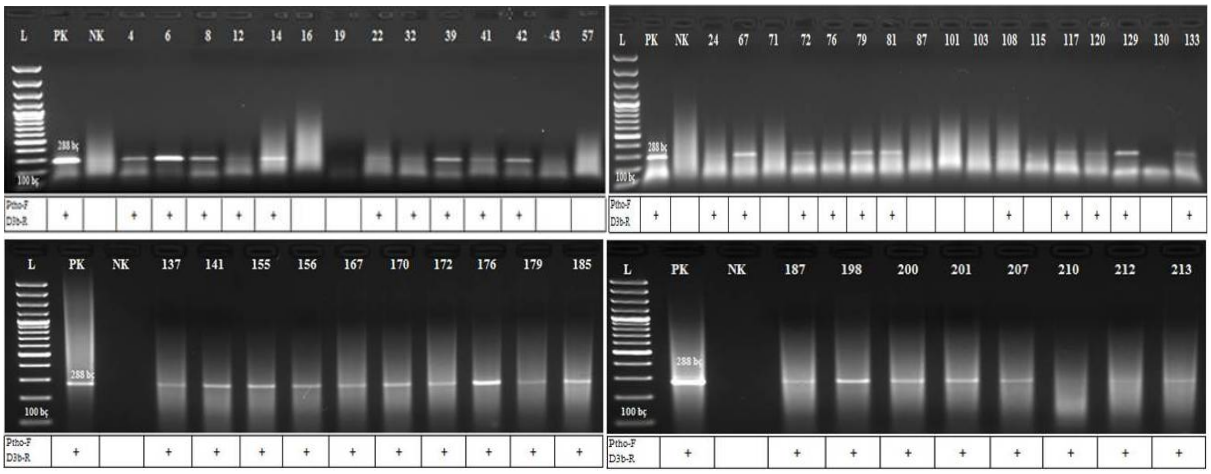


Figure 3. Identification of *P. thornei* in soil samples using PTHO primer in Konya and Karaman provinces. L, 100-bp ladder; PK, positive control; NK, negative control; numbers are survey samples numbers.

Table 6. Morphometrics of *Pratylenchus thornei* identified in this study and in references

| Measurements* | This study | Sher & Allen, 1953 | Kepenekci, 1999 | Osmanoglu, 2006 | Imren & Elekcioglu, 2008 |
|---|---|---|---|---|---|
| n | 5 | | 20 | 8 | 14 |
| L (mm) | 0,54 | 0,45-0,77 | 0,48-0,63 | 0,50-0,68 | 0,48-0,60 |
| a | 29,64 | 26-39,6 | 26,9-34,4 | 33,28-38,83 | 29,9-36,6 |
| B | 6,13 | 5,5-8,0 | 4,7-5,9 | 4,26-6,18 | 4,8-6,2 |
| b' | 5,36 | | 4,2-5,1 | 6,16-8,26 | |
| c | 18,8 | 18-22 | 16,6-21,0 | 16,64-25,5 | 16,0-26,6 |
| c' | 2,5 | | 2,3-2,9 | 2,07-3,36 | 2,01-2,72 |
| MB (%) | 49,37 | | 51,7-57,6 | 40,51-51,30 | |
| Stylet (µm) | 14,75 | 17-19 | 16-18 | 15,68-16,90 | 16,2-18,5 |
| Tail (µm) | 28,78 | | 25-36 | 21,56-36,26 | 22,5-30,0 |
| V (%) | 77,54 | 73-80 | 73,8-79,2 | 73-77 | 71,6-79,0 |

* L (mm), total body length; a, body length/the largest width part of body; b, body length/distance from esophagus intestine overlapping part to anterior end of body; c, body length/tail length; c', tail length/body width at anus; MB (%), distance from anterior to median bulb/esophagus length × 100; V (%), distance from anterior end of body to vulva/body length × 100.

*Pratylenchus neglectus* species was found in seven locations in Karapınar, Ilgın, Tuzlukçu and Kulu Districts in Konya and Başyayla and Central Districts in Karaman Province using D3b-R/Pneg-F primer set at 290 bp as suggested by Al-Banna et al. (2004) (Figures 1, 4 and 5). One specimen of *P. neglectus*investigated by morphology and morphometricss agreed with the literature (Sher & Allen, 1953; Akgül & Okten, 1997; Kepenekci, 1999; Imren & Elekcioglu, 2008) (Table 7).

*Pratylenchus vulnus* was found in 17 locations in Çumra, Güneysınır, Bozkır, Yalıhöyük, Beyşehir, Yunak, Kulu, Cihanbeyli, Karatay and Kadınhanı Districts in Konya and Central and Ayrancı Districts in Karaman Province using D3b-R/Pvul-F primer set at 287 bp (Figures 1, 6 and 7).

*Pratylenchus vulnus* was recorded for the first time in cereals in Turkey in addition to the reported root lesion nematode species of *P. thornei* and *P. neglectus* (Yavuzaslanoglu et al., 2012). It was second most common species of root lesion nematodes in survey area after *P. thornei*. The prevalence of *P. neglectus* was lower than expected on Central Anatolian Plateau based on earlier reports (Yavuzaslanoglu et al., 2012).

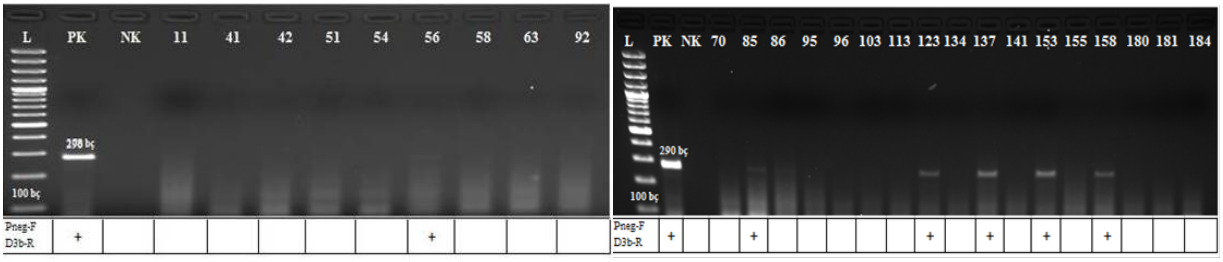


Figure 4. Identification of *P. neglectus*in plant samples using PNEG primer in Konya and Karaman provinces. L, 100-bp ladder; PK, positive control; NK, negative Control; numbers are survey samples numbers.

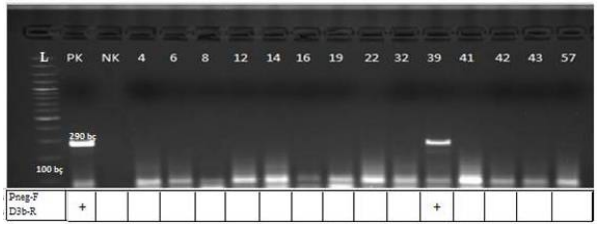


Figure 5. Identification of *P. neglectus*in soil samples using PNEG primer in Konya and Karaman provinces. L, 100-bp ladder; PK, positive control; NK, negative control; numbers are survey samples numbers.

Table 7. Morphometrics of *Pratylenchus neglectus* identified in this study and in references

| Measurements* | This study | Sher & Allen (1953) | Akgül & Okten (1997) | Kepenekçi (1999) | Imren & Elekcioglu (2008) |
|---|---|---|---|---|---|
| n | 1 | | 8 | 20 | 6 |
| L (mm) | 0,51 | 0,31-0,55 | 0,34-0,46 | 0,38-0,51 | 0,398-0,460 |
| a | 25,75 | 18-25 | 18,9-32,2 | 21,7-27,8 | 18,2-26,1 |
| B | 5,02 | 4,0-6,3 | 3,75-6,52 | 4,1-6,0 | 4,1-5,6 |
| b' | 4,58 | | 3,53-5,92 | 3,9-4,8 | |
| C | 17,68 | 16-22 | 16,7-31,7 | 14,9-23,3 | 14,01-23,00 |
| c' | 2,26 | | 1,8-2,3 | 1,5-3,1 | 1,9-2,6 |
| MB (%) | 46,86 | | 41,0-59,4 | 43,0-63,1 | |
| Stylet (µm) | 15,38 | 16-18 | 11,7-16,2 | 16-20 | 14,2-19 |
| Tail (µm) | 29,04 | | 13,5-20,7 | 20-31 | 20,0-28,4 |
| V (%) | 79,45 | 80-88 | 74,8-81,3 | 79,7-84,8 | 77,0-86,4 |

* L (mm), total body length; a, body length/the largest width part of body; b, body length/distance from esophagus intestine overlapping part to anterior end of body; c, body length/tail length; c', tail length/body width at anus; MB (%), distance from anterior to median bulb/esophagus length × 100; V (%), distance from anterior end of body to vulva/body length × 100.

The study showed the value of molecular studies which are useful for differentiation of morphologically similar and difficult to distinguish species. Morphologically *P. vulnus* is quite similar to *P. thornei* and is difficult to differentiate. Also, *P. vulnus* has high intraspecific morphometric variation and low number of diagnostic features so requires molecular identification (Castillo & Vovlas, 2007).

*Pratylenchus penetrans* and *Pratylenchus scribneri* were not found in any of the samples using the species-specific primers (D3b-R/PPEN-F and D3b-R/PSCR-F).

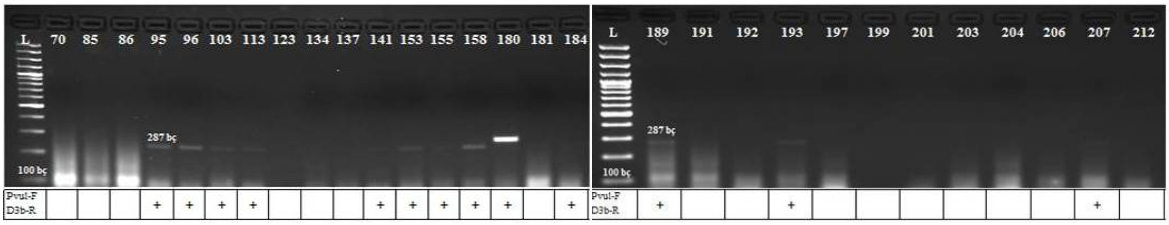


Figure 6. Identification of *P. vulnus* in plant samples using PVUL primer in Konya and Karaman provinces. L, 100-bp ladder; numbers are survey samples numbers.

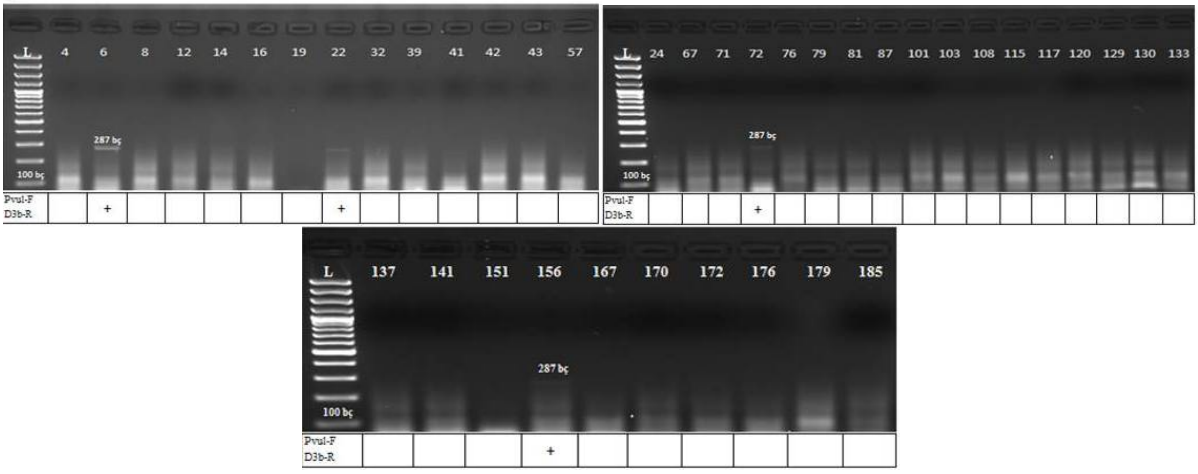


Figure 7. Identification of *P. vulnus* in soil samples using PVUL primer in Konya and Karaman provinces. L, 100-bp ladder; numbers are survey samples numbers.

The prevalence of cereal cyst nematodes was about 29%.Cereal cyst nematode cyst numbers were the highest in Altınekin and Sarayönü Districts of Konya Province; a mean of 15±11 and 14±9 cysts 250 g dry soil$^{-1}$, respectively. The number of cysts from the plant samples was between 0-4 cysts (Table 5). Population density of *Heterodera* spp. was found higher in comparison to the previous report in 2003 (Yavuzaslanoglu et al., 2012).

*Heterodera filipjevi* was found in 33 locations in Çumra, Karatay, Central, Beyşehir, Hüyük, Ilgın, Tuzlukçu, Yunak, Cihanbeyli, Altınekin, Sarayönü and Kadınhanı Districts in Konya Province and in Central and Ayrancı Districts in Karaman Province using the HfITS-R/HfITS-F primer set at 170 bp as suggested by Yan et al. (2013) (Figures 8 and 9). *Heterodera filipjevi* was identified by morphology and morphometrics of the vulval region and these measurements agreed with previous reports (Subbotin, 1999; Abidou, 2005; Handoo, 2002; Imren et al., 2012) (Table 8).The study showed that *H. filipjevi* was the main cereal cyst nematode in this part of the Central Anatolian Plateau (Enneli et al., 1994; Rumpenhorst et al.,1996; Ozturk et al*.,* 1998; Abidou et al*.,* 2005; Yavuzaslanoglu et al., 2012).

*Heterodera latipons* and *H. avenae* were not found in the samples using the species-specific primers (HaITS-R, HaITS-F, Hlat-actF and Hlat-actR). In previous research, it was established that the distribution of the cereal cyst nematodes in Turkey was closely related to climatic conditions prevalent throughout Anatolia, with *H. filipjevi* is found on the Central Anatolian Plateau and *H. avenae* in temperate zone of East Anatolia (Toktay et al., 2015). *Heterodera latipons* was reported to be predominant in Southeast Anatolia with a mediterranean climate (Imren et al., 2012). Molecular and morphological identification studies supported the previous observations on the distribution of cereal cyst nematodes in Turkey.

The prevalence of root lesion and cereal cyst nematodes together was about 13% in survey area which was lower than in earlier studies (Rumpenhorst, 1996; Yavuzaslanoglu et al., 2012).

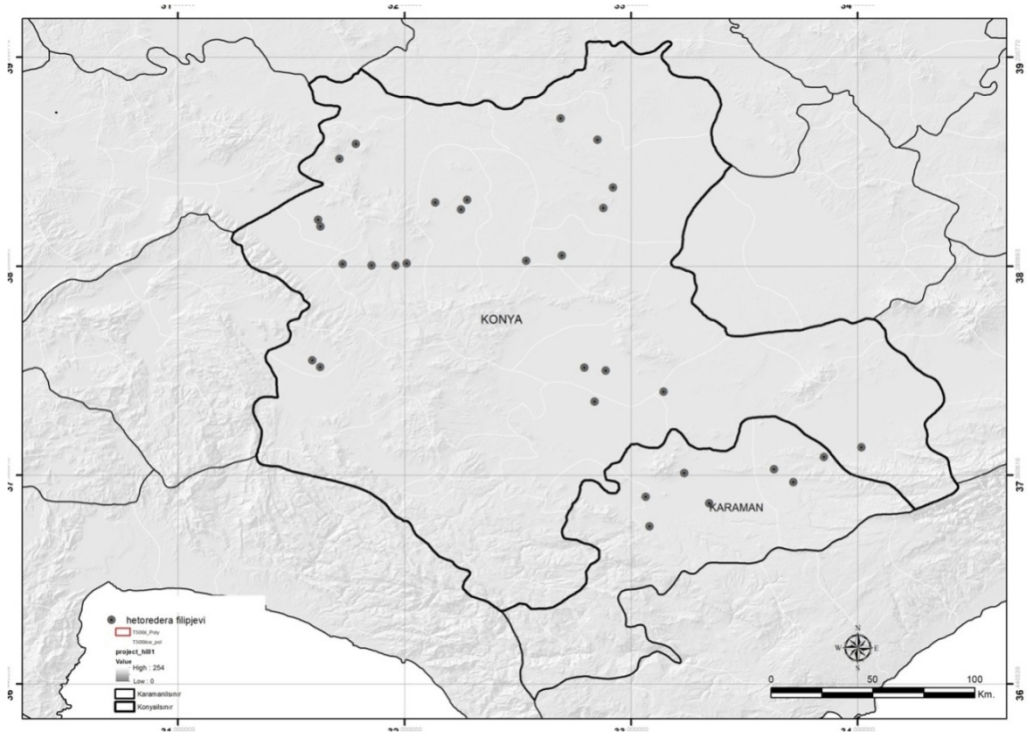


Figure 8. *Heterodera filipjevi* identified in the sampled locations.

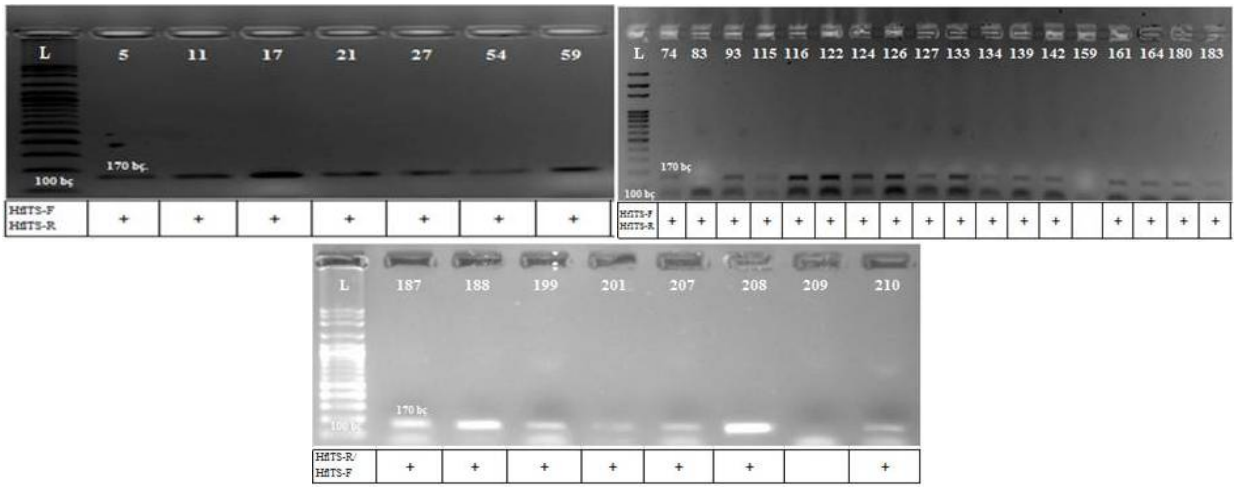


Figure 9. Identification of *H. filipjevi* in soil samples using HfITS-F1 HfITS-R1 primer in Konya and Karaman Provinces. L, 100-bp ladder; numbers are survey samples numbers.

Table 8. Morphometrics of *Heteroderafilipjevi* identified in this study and in references

| Measurements (µm) | This study | Subbotin (1999) | Abidou (2005) | Handoo (2002) | Imren et al. (2012) |
|---|---|---|---|---|---|
| Fenestra length | 64,13 | 50,00 | 50.41 | 52,00 | 66,24 |
| Semifenestra length | | 27,00 | 25.30 | | 23,04 |
| Vulval bridge width | 15,60 | 11.80 | 7.27 | 8 (6-9) | 19,52 |
| Vulval slit length | 29,60 | 11.00 | 6.95 | 7(6-8) | 24,64 |
| Fenestra width | 13,00 | | | 28 (21-33) | |

*Ditylenchus* (32.6%)*, Tylenchus* (21.4%)*, Paratylenchus* (0.9%) and *Pratylenchoides* (0.5%) were the other plant-feeding nematodes found in the wheat and barley fields surveyed.

The maximum population density of the *Ditylenchus* spp. was 410 nematodes 100 g dry soil$^{-1}$ in Karaman Province Central District (mean: 28±10 nematodes 100 g dry soil$^{-1}$). The *Ditylenchus* spp. populations were higher in Altınekin (mean: 83±48 nematodes 100 g dry soil$^{-1}$), Sarıveliler (mean: 57±12 nematodes 100 g dry soil$^{-}$), Yalıhüyük (mean: 52±32 nematodes 100 g dry soil$^{-1}$), Sarayönü (mean: 46±20 nematodes 100 g dry soil$^{-1}$) and Guneysınır (mean: 45±24 nematodes 100 g dry soil$^{-1}$) Districts (Table 5). No species-specific bands for *D. dipsaci* in any of the samples tested were with the nine species-specific primer sets used.

*Tylenchus* spp. populations were significantly different between districts (P<0.05). The highest population was recorded in Hüyük District in Konya Province, with a mean of 180±180 nematodes 100 g dry soil$^{-1}$ (Table 5).

*Paratylenchus* spp. was recorded in Bozkır (mean: 6±6 nematodes 100 g dry soil$^{-1}$) and Seydişehir (mean: 7±7 nematodes 100 g dry soil$^{-1}$) Districts in Konya Province (Table 5).

*Pratylenchoides* spp. was only found in Cihanbeyli District in Konya Province, with a mean of 5±5 nematodes 100 g dry soil$^{-1}$ (Table 5).

Fungal-feeding nematodes were in 62% of the soil samples. *Aphelenchoides* spp. (51.2%) and *Aphelenchus* spp. (39.1%) were observed (Table 4). The population density of the fungal-feeding nematodes was generally high in all districts. The maximum number of *Aphelenchus* spp. was recorded in Sarıveliler District in Karaman Province (mean: 215±146 nematodes 100 g dry soil$^{-1}$). *Aphelenchoides* spp. was the highest in Beyşehir District in Konya Province (mean: 172±77 nematodes 100 g dry soil$^{-1}$) (Table 5).

Bacterial-feeding nematodes were found in 75% of samples. *Acrobeloides* was the most prevalent bacterial-feeding nematode genera in 65.1% of samples, followed by *Cephalobus*in 19.1% of samples. *Eucephalobus* (1.9%), *Acrobeles* (5.1%) and *Rhabditis* (0.5%) prevalence was relatively low. The most abundant bacterial-feeding nematode genus was *Acrobeloides.* The maximum population density was in Başyayla District in Karaman Province at 625±456 nematodes 100 g dry soil$^{-1}$ (P<0.05) (Table 5). *Eucephalobus* spp. populations were significantly different between districts (P<0.05). The maximum population density was 30±30 nematodes 100 g dry soil$^{-1}$ in Doğanhisar District in Konya Province. *Eucephalobus* spp. was recorded in Ayrancı (mean: 5±5 nematodes 100 g dry soil$^{-1}$) and Ermenek Districts (mean: 6±6 nematodes 100 g dry soil$^{-1}$) in Karaman Province and in Kulu District (mean: 2±2 nematodes 100 g dry soil$^{-1}$) in Konya Province. It was not found in the other districts (Table 5). The population densities of *Cephalobus* spp. were the highest in Çeltik District in Konya Province (30±20 nematodes 100 g dry soil$^{-1}$) (Table 5). *Acrobeles* spp. was recorded in seven districts. The maximum population density was in Tuzlukçu District in Konya Province (mean: 7±4 nematodes 100 g dry soil$^{-1}$ ) (Table 5). *Rhabditis* spp. was only found in the Central District in Karaman Province (mean: 1±1 nematodes 100 g dry soil$^{-1}$) (Table 5).

Nematodes in the Dorylaimida were found in 15.8% of the samples (Table 4). The population densities of the nematodes in the Dorylaimida were significantly different between districts (P<0.05). The maximum population density was 26±14 nematodes 100 g dry soil$^{-1}$ in Tuzlukçu District in Konya Province (Table 5).

The soil environment and host plant are the main factors affecting nematode survival and population growth (Wallace et al., 1993). In addition, climate change and global warming affect spatial distribution and damage potential of pathogens and pests (Iglesias et al., 2001; Ghini et al., 2008; Morgan & Wall, 2009). Changes in prevalence and population density of the main damaging plant parasitic nematode species are probably due to the changes in soil conditions such as temperature and moisture with application of different crop rotation over years.

The study provides useful information on the prevalence and population densities of *Heterodera* spp. and *Pratylenchus* spp. which are the major pests of the economically important cereal crops for making risk analysis effectively and taking appropriate control actions.

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*Original article (Orijinal araştırma)*

# Fumigant toxicity of essential oil of *Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae) to *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)

*Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae) esansiyel yağının *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)'a karşı fumigant toksisitesi

**Hatice BAŞ[1*]** **Doğan Erhan ERSOY[2]**

## Abstract

In this study, vapor of essential oil obtained by the hydrodistillation of *Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae) was tested on the different stages of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae). The larvae, pupae and adult stages of *T. molitor* were exposed to different doses of *H. perforatum* essential oil for 24 h. After exposure, mortality rate, $LC_{50}$, $LC_{90}$ and $LC_{99}$ values, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx)], acetylcholinesterase (AChE) activity and malondialdehyde (MDA) levels were measured in the insects. *Tenebrio molitor* was cultured at Gazi University Department of Biology and all analyses were done in Yozgat Bozok University in 2017 and 2018. The results indicated that the pupae of *T. molitor* were the most tolerant and adults were the most sensitive. Mortality increased with the increasing concentration of essential oil. Also, increasing doses of essential oil caused decreasing in SOD, CAT, GST GPx and AChE activities and increasing in MDA level. These results indicate that essential oil of *H. perforatum* can be used against *T. molitor* in a pest control program.

**Keywords:** Antioxidant enzymes, GC-MS, *Hypericum*, insecticidal activity, meal worm, pesticide

## Öz

Bu çalışmada, *Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae) distilasyonundan elde edilen uçucu yağın, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)'ün farklı gelişme dönemleri üzerindeki etkisi test edilmiştir. Larva, pupa ve ergin dönemdeki *T. molitor* bireyleri, 24 saat boyunca farklı dozlarda *H. perforatum* uçucu yağına maruz bırakılmıştır. Uygulama sonrası, böceklerin ölüm oranları, $LC_{50,}$ $LC_{90}$ ve $LC_{99}$ değerleri, antioksidan enzim aktiviteleri [süperoksit dismutaz (SOD), katalaz (CAT), glutatyon-S-transferaz (GST) ve glutatyon peroksidaz (GPx)], asetilkolinesteraz (AChE) aktivitesi ve malondialdehit (MDA) seviyeleri ölçülmüştür. *Tenebrio molitor* Gazi Üniversitesi Biyoloji Bölümü'nde kültüre alınmış ve analizler 2017 ve 2018 yıllarında Yozgat Bozok Üniversitesi'nde yapılmıştır. Sonuçlar, *T. molitor*'un pupa döneminin en toleranslı; ergin döneminin ise en hassas dönem olduğunu belirtmektedir. Ölüm oranları, maruz kalınan uçucu yağ konsantrasyonunun artmasıyla artmıştır. Ayrıca, artan uçucu yağ dozları, SOD, CAT, GST GPx ve AChE aktivitelerinde azalmaya, MDA düzeyinde ise artışa neden olmuştur. Bu sonuçlar, *H. perforatum* uçucu yağının, *T. molitor* ile mücadelede önemli bir potansiyele sahip olduğunu ortaya koymuştur.

**Anahtar sözcükler:** Antioksidan enzimler, GC-MS, *Hypericum*, insektisidal aktivite, un kurdu, pestisit

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## Introduction

During food storage, especially corn, oat and wheat, major problems can occur because of the presence of insects like yellow mealworm *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) (Garcia et al., 2003). Insects in stored bran and grains can contaminate foods via their feces and fragments of old cuticle parts, and also indirectly by saprophytic microorganisms. All of these factors can cause quality loss (Garcia et al., 2003; Pinto, 2008). *Tenebrio molitor* adults lay elongated eggs on substances. When larvae hatch, they produce chitinous exoskeleton in a few days and their white color changes to yellow. Generally pupal stage can take up 5-6 days. After pupal stage, insects become adult and mate. Adults lay eggs in 4-17 days following mating (Siemianowska et al., 2013).

Insects can be controlled with chemical treatments such as pesticides; however, using of these chemicals may have adverse effects on environment and non-target organisms (Ghini et al., 2002; Lee et al., 2004). Thus, safer control methods, like using natural extracts and secondary compounds of plants as biopesticides, are becoming important for pest control (Lima et al., 2011). Botanical pesticides are studied because they have low toxicity for humans, decreased toxic effects on environment and rapid degradation. These properties make them suitable pesticides for insects in organic agriculture. Essential oils which have botanical origin, are effective insecticides (Regnault-Roger, 1997; Cosimi et al., 2009). Essential oils can be obtained from seeds, flowers, leaves, buds, twigs, wood, bark and roots of plants, and can have anti-protozoan, antihelminthic and insecticidal activities (Upadhyay, 2010). There are many studies that report the use of essential oils against insects. Essential oils from *Conyza newii* Oliv. & Hiern, *Plectranthus montanus* Benth., *Lippia javanica* Spreng., *Lippia ukambensis* (Vatke) Verdc., *Tetradenia riparia* (Hochst.) Codd and *Tarchonanthus camphoratus* L. (Omolo et al., 2005), seven *Citrus* spp., two *Origanum* spp., three *Cymbopogon* spp., two *Pimenta* spp., two *Eucalyptus* spp., three *Mentha* spp. and two *Juniperus* spp. (Choi et al., 2003) have been reported to have insecticidal activity.

*Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae), St. John's wort contains essential oils that are potentially insecticidal. Recently, the consumption of compounds derived from *H. perforatum* has increased strongly, and it has become one of the most consumed medicinal plants worldwide (Wills et al., 2000). It has an extensive variety of medicinal applications, such as diseases of the alimentary tract, eczema, skin wounds and burns (Butterweck, 2003; Saddiqe et al., 2010). There are many studies that have focused on the effects of *H. perforatum* extracts against bacteria and viruses, and various diseases (Serkedjieva et al., 1990; Çakir et al., 1997). Also, *H. perforatum* can be used as antihelmintic and antiseptic (Çakir et al., 1997). In addition to medical use, *Hypericum* species have insecticidal activity. The the insecticidal effects of *Hypericum* spp. have been assessed against *Culex pipiens* (L., 1758) (Diptera: Culicidae) (Rouis et al., 2013), *Tribolium castaneum* (Herbst, 1797) (Coleptera: Tenebrionidae) (Parchin & Ebadollahi, 2016), *Manduca sexta* L., 1763 (Lepidoptera: Sphingidae) (Samuels & Knox, 1989) and *Sitophilus granarius* (L., 1758) (Coleptera: Dryophthoridae) (Kordali et al., 2012). Considering previous studies, plant materials of *Hypericum* spp. may be candidates for future works about management of pests. Therefore we aimed to evaluate the chemical composition and insecticidal effects of the essential oil extracted from *H. perforatum* against *T. molitor.*

Oxidative stress, the imbalance between free radical production and antioxidant defenses, is associated with chemical exposition. Malondialdehyde (MDA) levels and antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx)] are important indicators of oxidative stress. The antioxidant enzymes such as SOD, CAT, GPx and GST may neutralize the oxidative stress, so, increases and decreases in their levels give us important information about the cell damage caused by oxidative stress (Baş & Kalender, 2011).

Given that synthetic pesticides have many adverse effects on non-target animals and cause environmental pollution, it is important to identify alternative methods to control pests. So, we investigated the efficacy of *H. perforatum* essential oil, against *T. molitor* in this study. Data on the insecticidal activity of *H. perforatum* are limited in the literature. This study will contribute to the literature because it is the first study to investigate the effects of *H. perforatum* against *T. molitor*.

## Materials and Methods

### Plant material, isolation and analysis of the essential oil

*Hypericum perforatum* was collected in June 2017 in Uşak Province of Turkey. The plant samples were cleaned and dried via herbarium techniques of Davis (1972). Two hundred g of dried aerial parts of plants were distilled for 4 h in water (3L) using a Clevenger type apparatus. The essential oil obtained was kept at 4°C until beginning of the study. Essential oil of *H. perforatum* was determined via GC-MS using Agilent Technologies (Santa Clara, CA, USA) 6890N Network GC System 5973 MSD, ionization energy: 70 eV; 19091 N-136 HP-Innowax column 60 m x 0.25 mm i.d.; helium 1 mL $min^{-1}$. One µl of sample was injected into the GC-MS analysis system. Injection temperature was 250°C. Column temperature was initially held at 60°C for 3 min, then raised to 280°C at a rate of 5°C/min. Then, the temperature was ramped by 5°C/min to 300°C. The amounts of essential oil components were estimated from the area under GC peaks. Identification of compounds were done by mass spectral comparison by electronic libraries (Wiley, NIST, Adams). Retention indices were calculated according to the equation given by Kovats (1958).

### *Tenebrio molitor* culture

A culture of *T. molitor* was established at the Metin Aktaş Zoology Museum (Department of Biology, Gazi University) and all analyses were done in the General Biology and Seed Science Technology Laboratories (Yozgat Bozok University) in 2017 and 2018.

Insects were cultivated in plastic containers (30 x 25 x 15 cm). Corn flour (30%) and wheat flour (70%) were added as a food source and pasteboards were added for egg deposition. Thinly sliced potato or apple was added each week for water source and regulating for humidity of containers. Twenty individuals of different stages of the insect (larvae, pupae and adult) were selected randomly from one population.

### Bioassays

*Hypericum perforatum* essential oil was tested for its fumigant effect against different stages of the *T. molitor*. Adult insects were placed into 1 L glass jars. Six replicates were tested for each concentration of *H. perforatum* essential oil and each replicate consisted of 20 adults. We used 2.5 × 2.5 cm filter paper strips for application of essential oil then the filter paper was attached to the bottom of the cover of glass jars. Adults of the mealworms were exposed to different concentrations of essential oil (0, 1, 2, 3, 4, 5 and 6 µl/L air) for 24 h. We determined the mortalities and values of $LC_{50,}$ $LC_{90}$ and $LC_{99}$. The larval and pupal stages of *T. molitor* were handled in the same method as the adult stage of insect. Different concentrations of essential oil were applied to larvae (0, 2.5, 5, 7.5, 10, 12.5 and 15 µl/L air) for 24 h. The pupal stage of *T. molitor* was exposed to essential oil (0, 5, 10, 15, 20, 25 and 30 µl/L air) for 24 h. The control groups of larval, pupal and adult stages of *T. molitor,* involved the same conditions without essential oil. We have treated different doses to different stages of the insect because all of the adults died when we treated 6 µl/L air, but not all individuals died in the larval and pupal stages. So, we treated different doses until all died.

### Toxicological assays

All of the chemicals (ethanol, thiobarbituric acid, trichloroacetic acid, pyrogallol, triton-X-100, $H_2O_2$, 1-chloro 2, 4-dinitrobenzene and nicotinamide adenine dinucleotide phosphate) used in this study to determine the antioxidant enzyme activities, MDA level, and AChE activity were obtained from Sigma-Aldrich (Germany).

### Tissue collection and preparation

Larvae, pupae and adults were cooled on ice (5 min) then sterilized with ethanol. After this step, they were cut into small pieces and put into Eppendorf tubes which were filled with homogenization buffer at pH 7.4. Samples were kept at -80°C until examined. Before starting work, Eppendorf tubes were kept at 25°C until the samples thawed.

The extracts of larvae, pupae and adults were prepared with a homogenizer at 4°C and centrifuged (NUVE NF800) for 15 min. After centrifugation, the supernatants were taken for examination of CAT, GST, SOD, GPx, MDA and acetylcholinesterase (AChE). MDA levels and the activities of antioxidant enzymes and AChE were measured by measuring the absorbance via a UV-VIS spectrophotometer (Biotech Engineering, Spectroscan 60 DV). Protein concentrations were estimated according to the method of Lowry et al. (1951).

### Measurement of malondialdehyde levels

The levels of MDA were measured by thiobarbituric acid (TBA) test as described by Ohkawa et al. (1979). First we add to 10% trichloroacetic acid to a sample then centrifuged for 10 min. The supernatant was collected and added to TBA. After incubation of tissue homogenates with TBA, MDA reacts with it to form a pink colored complex at 95°C. Next, the samples were cooled and we measured the absorbance at 532 nm. We defined the MDA level as nM/mg protein.

### Measurement of antioxidant enzyme activities

We measured the enzyme activity of CAT based on procedure of Aebi (1984). This procedure is based on determining the hydrolysis of $H_2O_2$. The absorbance was determined at 240 nm using spectrophotometer. The activity was given as mM/mg protein. The enzymatic activity of GPx was measured by the procedure which was identified by Paglia & Valentine (1987). The reaction was searched at 340 nm and the activity was indicated as nM/mg protein. The method of Marklund & Marklund (1974) was used for assessment of SOD activity at 440 nm and the enzymatic activity was indicated as U/mg protein. GST activity was measured at 340 nm (Habig et al., 1974). This procedure is based on assaying the generation of 1-chloro-2,4-dinitrobenzene and glutathione conjugate. The GST activity was estimated as μM/mg protein.

### AChE enzyme activity

The effects of the *H. perforatum* essential oil on activity of AChE was measured by the procedure of Ellman et al. (1961). The assay solution contained 0.015 M acetylthiocholine iodide, 0.01 M 5,5'-dithiobis(2-nitrobenzoic acid), 0.1 M Na-K phosphate buffer at pH 8.0 and ethopropazine. The reaction was monitored at 412 nm wavelength using a spectrophotometer. The activity was estimated as U/mg protein.

### Statistical analysis

The data were analyzed by one-way analysis of the variance in SPSS program 20.0 for Windows and Tukey test for multiple comparisons at a significance level of 0.05. $LC_{50}$, $LC_{90}$ and $LC_{99}$ values were estimated by probit analysis with SPSS (Abbott, 1925).

## Results

### Chemical compounds of *Hypericum perforatum*

The chemical composition of *H. perforatum* essential oil (25 compounds were determined by GC-MS) is shown in Table 1. α-Pinene (51.2%), 3-carene (7.3%), α-caryophyllene (5.2%) are the main compounds of essential oil.

Table 1. Percentage composition of the essential oil of *Hypericum perforatum*

| Compounds | RI (Retention Index) | Percantage (%) |
|---|---|---|
| )α-pinene | 939 | 51.2 |
| Sabinene | 976 | 2.4 |
| β-pinene | 979 | 3.2 |
| β-Myrcene | 990 | 3.6 |
| 2-carene | 1001 | 1.0 |
| Eugenol | 1356 | 0.6 |
| α-phellandrene | 1004 | 0.4 |
| 3-carene | 1010 | 7.3 |
| p-cymene | 1024 | 0.7 |
| Limonene | 1029 | 2.0 |
| ɣ-terpinene | 1058 | 2.2 |
| Myrtenol | 1194 | 0.6 |
| α-longipinene | 1351 | 0.3 |
| α-copaene | 1376 | 0.5 |
| β-caryophyllene | 1419 | 0.8 |
| Aromadendrene | 1439 | 0.3 |
| α-caryophyllene | 1454 | 5.2 |
| Allo- | 1461 | 0.5 |
| ɣ-muurolene | 1477 | 0.3 |
| Germacrene-D | 1482 | 0.6 |
| ɣ-cadinene | 1513 | 0.3 |
| Calamenene | 1518 | 0.5 |
| Longifolene | 1556 | 0.4 |
| Cedrol | 1601 | 0.3 |
| Cadalene | 1674 | 3.2 |
| Terpenes | 87.8 | |
| Monoterpenes | 74.6 | |
| Sesquiterpenes | 13.2 | |
| Non-terpenes | 0.6 | |
| Unidentified | 11.6 | |
| Total | 88.4 | |

**Fumigant activity results**

The mortality percentages of life stages increased with increment of the concentrations of essential oil (adults $F = 30.5$, $df = 5,114$, $P < 0.05$; pupae $F= 87.2$, $df = 5,114$, $P < 0.05$; larvae $F = 51.5$, $df = 5,114$, $P < 0.05$).

Considering the results of probit analysis, $LC_{50,}$ $LC_{90}$ and $LC_{99}$ values of the essential oil were 3.06, 4.99 and 6.56; 13.3, 23.6 and 32.0; and 8.24, 13.9 and 18.5 µl/L air for the adult, pupal and larval stages of *T. molitor* respectively (Table 2).

The fumigant effects of essential oil were more significant on the adults than the larvae and pupae of *T. molitor*. The most resistant developmental stage of the insect was the pupae. The data of this research determined that the fumigant toxicity of *H. perforatum* essential oil differed between the life stages of the insect.

Table 2. $LC_{50}$, $LC_{90}$ and $LC_{99}$ values (µl/L air) of *Hypericum perforatum* essential oil against different life stages of *Tenebrio molitor*

| Time (24 h) | N | $LC_{50}$ | $LC_{90}$ | $LC_{99}$ | df | Chi-Square | Slope | Sig. | |
|---|---|---|---|---|---|---|---|---|---|
| Pupae | 20 | 13.3 | 23.6 | 32.0 | 5 | 2,61 | 0,12±0,02 | 0,760 | a |
| 95% confidence limits | | 11.0-15.4 | 20.7-28.0 | 27.6-39.3 | | | | | |
| Larvae | 20 | 8.24 | 13.9 | 18.5 | 5 | 4,37 | 0,23±0,03 | 0,497 | a |
| 95% confidence limits | | 7.10-9.44 | 12.3-16.6 | 16.0-22.9 | | | | | |
| Adult | 20 | 3.06 | 4.99 | 6.56 | 5 | 2,08 | 0,67±0,09 | 0,838 | a |
| 95% confidence limits | | 2.64-3.48 | 4.44-5.84 | 5.73-7.94 | | | | | |

N, number of the tested stages; a, since the significance level is greater than 0.15, no heterogeneity factor is used in the calculation of confidence limits.

**AChE enzyme activity, MDA levels and antioxidant enzyme activity results**

The MDA levels of insects increased with the increasing exposure doses of essential oil of *H. perforatum* against different stages of *T. molitor,* significantly (Figure 1).

The antioxidant enzyme activities (CAT, GST, SOD and GPx) decreased by increasing application doses of essential oil of *H. perforatum* against different stages of *T. molitor,* significantly (Tables 3 to 5).

The AChE enzyme activity decreased by increasing exposure doses of essential oil of *H. perforatum* against different stages of *T. molitor,* significantly (Tables 3 to 5).

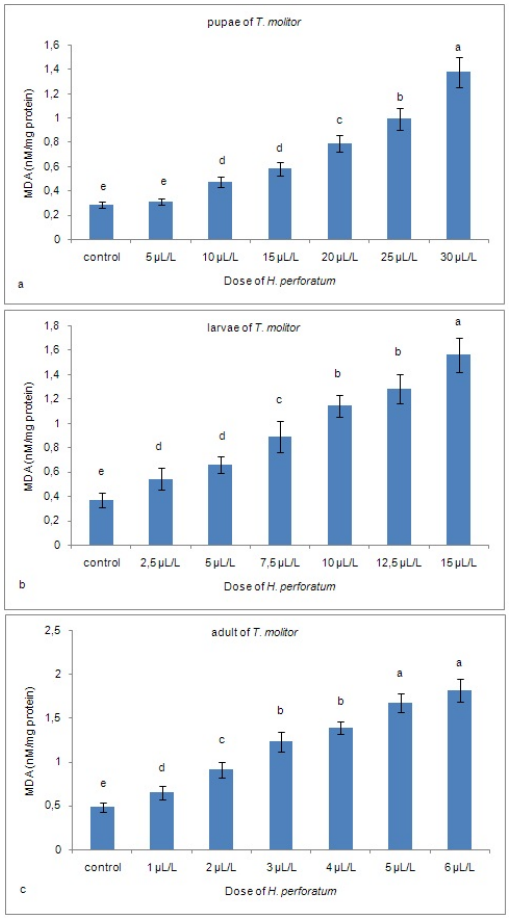


Figure 1. Effect of *Hypericum perforatum* essential oil on MDA levels of different life stages of *Tenebrio molitor*: a) pupae, b) larvae and c) adults. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate standard deviation (SD) of means.

Table 3. Effect of *Hypericum perforatum* essential oil doses on enzyme activities of pupae of *Tenebrio molitor*

| Enzyme | Control | | 5 µl/L | | 10 µl/L | | 15 µl/L | | 20 µl/L | | 25 µl/L | | 30 µl/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CAT (mM/mg protein) | 47.0±3.21 | a | 41.4±2.42 | b | 36.3±2.58 | c | 31.8±1.96 | d | 26.5±3.15 | e | 20.8±2.51 | f | 18.5±2.87 | f |
| GPx (nM/mg protein) | 0.221±0.011 | a | 0.218±0.02 | a | 0.183±0.013 | b | 0.156±0.011 | c | 0.13±0.021 | c | 0.094±0.012 | d | 0.068±0.012 | e |
| SOD (U/mg protein) | 0.03±0.0018 | a | 0.0268±0.0011 | b | 0.0241±0.0014 | c | 0.0212±0.0011 | d | 0.0179±0.002 | e | 0.014±0.0016 | f | 0.0127±0.0023 | f |
| GST (µM/mg protein) | 3.12±0.171 | a | 2.72±0.21 | b | 2.32±0.165 | c | 1.95±0.187 | d | 1.6±0.132 | e | 1.29±0.141 | f | 0.27±0.223 | f |
| AChE (U/mg protein) | 0.025±0.0022 | a | 0.0237±0.0019 | a | 0.0194±0.0012 | b | 0.0182±0.0024 | b | 0.0143±0.0011 | c | 0.0108±0.0017 | d | 0.0072±0.0015 | e |

Values are mean±standard deviation. Significance at P < 0.05. Within each row, means followed by the same letter are not significantly different.

Table 4. Effect of *Hypericum perforatum* essential oil doses on enzyme activities of larvae of *Tenebrio molitor*

| Enzyme | Control | | 2.5 µl/L | | 5 µl/L | | 7.5 µl/L | | 10 µl/L | | 12.5 µl/L | | 15 µl/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CAT (mM/mg protein) | 58.2±4.1 | a | 55.8±3.72 | a | 48.3±3.53 | b | 46.0±2.78 | b | 39.4±2.84 | c | 32.1±3.07 | d | 24.0±4.21 | e |
| GPx (nM/mg protein) | 0.27±0.012 | a | 0.243±0.013 | b | 0.235±0.011 | b | 0.203±0.013 | c | 0.18±0.016 | c | 0.145±0.014 | d | 0.101±0.021 | e |
| SOD (U/mg protein) | 0.036±0.0021 | a | 0.034±0.0015 | a | 0.0311±0.0012 | b | 0.0297±0.0023 | b | 0.0257±0.0014 | c | 0.0214±0.0023 | d | 0.019±0.0026 | d |
| GST (µM/mg protein) | 4.76±0.224 | a | 4.68±0.18 | a | 4.29±0.174 | b | 4.07±0.235 | b | 3.62±0.164 | c | 3.23±0.188 | d | 2.81±0.197 | e |
| AChE (U/mg protein) | 0.037±0.0026 | a | 0.035±0.0014 | a | 0.029±0.0017 | b | 0.024±0.0015 | c | 0.02±0.0018 | d | 0.017±0.0026 | d | 0.011±0.0021 | e |

Values are mean±standard deviation. Significance at P < 0.05. Within each row, means followed by the same letter are not significantly different.

Table 5. Effect of *Hypericum perforatum* essential oil doses on enzyme activities of adults of *Tenebrio molitor*

| Enzymes | Control | | 1 µl/L | | 2 µl/L | | 3 µl/L | | 4 µl/L | | 5 µl/L | | 6 µl/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CAT(mM/mg protein) | 53.8±4.01 | a | 46.1±2.91 | b | 39.0±3.52 | c | 32.2±3.27 | d | 29.8±2.98 | d | 23.5±3.04 | e | 21.7±2.74 | e |
| GPx (nM/mg protein) | 0.24±0.013 | a | 0.231±0.011 | a | 0.196±0.022 | b | 0.157±0.014 | c | 0.14±0.023 | c | 0.105±0.011 | d | 0.097±0.013 | d |
| SOD (U/mg protein) | 0.032±0.0011 | a | 0.0292±0.0013 | b | 0.0288±0.0022 | b | 0.0251±0.0011 | c | 0.022±0.0015 | d | 0.0185±0.0018 | e | 0.00178±0.0021 | e |
| GST (µM/mg protein) | 3.95±0.227 | a | 3.51±0.192 | b | 3.01±0.23 | c | 2.58±0.166 | d | 2.56±0.236 | d | 2.1±0.191 | e | 1.97±0.248 | e |
| AChE (U/mg protein) | 0.031±0.0015 | a | 0.028±0.0019 | a | 0.023±0.0016 | b | 0.0022±0.0021 | b | 0.018±0.0014 | c | 0.016±0.0023 | c | 0.011±0.0013 | d |

Values are mean±standard deviation. Significance at P < 0.05. Within each row, means followed by the same letter are not significantly different.

## Discussion

Chemical control is the mostly used method against insect pests (Jembere et al.,1995). However, these harmful insects have developed resistance against many chemicals (Upadhyay, 2010). Also, other problems have been observed such as adverse effects on non-target organisms, especially natural enemies and toxicity to users and mammals, residue problems and environmental pollution (Cosimi, 2009). So, we evaluated an alternative method in this study. Potential insecticidal compounds have been extracted from plants which have shown growth inhibition of and toxicity to many harmful insects which damage field crops (Koul et al., 2000; Upadhyay, 2010). Previous studies have shown that essential oils of these plants have insecticidal activity against insects in stored cereals (Tripathi et al., 2000; Verma et al., 2000), field crops (Isman et al., 2001) and households (Singh et al., 2000). These studies on essential oils motivated us to focus on essential oil experiments for finding alternative methods.

There are a wide variety of chemical compounds such as terpenes, sesquiterpenes, and aromatic compounds in essential oils (Ogendo et al., 2008). These natural compounds may have volatile properties so, they may have fumigant activity and this character can be used against pests. These volatile compounds have harmful effects against insects and effect reproduction and longevity of different life stages of them. Table 1 shows the percentage composition of the volatile oil of *H. perforatum*.

Essential oils from pennyroyal, eucalyptus, rosemary and marjoram have shown insecticidal activity against *Pediculus humanus* subsp. *capitis* De Geer, 1778 (Psocodea: Pediculidae). In this study, $LT_{50}$ values were 14.7, 12.6, 22.4 and 19.6, respectively (Yang et al., 2004). Essential oils from some plants grown in Africa have shown fumigant effects against *Anopheles gambiae* Giles, 1902 (Diptera: Culicidae). $LD_{50}$ values were $3.8 \times 10^{-3}$ for *T. camphoratus*; $4.3 \times 10^{-3}$ for *L. javanica*; $2.8 \times 10^{-3}$ for *P. montanus;* $4.4 \times 10^{-3}$ for *T. riparia* and $4.7 \times 10^{-3}$ for *L. ukambensis* (Omolo et al., 2005). Essential oil from *Ipomoea cairica* (L.) Sweet have shown insecticidal effect against larvae of *Aedes aegypti* (L., 1762)*, Culex tritaeniorhynchus*

Giles, 1901*, Culex quinquefasciatus* Say, 1823 and *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae). The $LC_{50}$ and $LC_{90}$ values estimated for *C. tritaeniorhynchus, A. aegypti, A. stephensi* and *C. quinquefasciatus* were 14.8 and 78.3; 22.3 and 92.7; 14.9 and 110; and 5.9 and 162 ppm, respectively (Thomas et al., 2004). Essential oils, carvacryl, mentha, eucalyptus and citronella, have shown high repellency to *Aedes albopictus* Skuse, 1894 (Diptera: Culicidae) (Yang & Ma, 2005). In another study, *Cinnamomum camphora* (L.) J.Presl. and *Artemisia princeps* Pamp. have shown toxicity and repellency to *Bruchus rufimanus* Boheman, 1833 (Coleptera: Chrysomelidae) and *Sitophilus oryzae* Schoenherr, 1838 (Coleptera: Dryophthoridae) (Liu et al., 2006). Also, the toxicity of essential oils from the bark and leaves of *Drimys winteri* Forst. and *Laurelia sempervirens* Tul. against *T. castaneum* were studied (Zapata & Smagghe, 2010). In another study, 43 essential oils were studied against *Lycoriella ingenua* (Dufour, 1839) adults (Choi et al., 2006). Fumigant toxicity of essential oil of *Lippia origanoides Kunth* against *T. molitor* was investigated in a work (Lima et al., 2011). Also, the fumigant effect of essential oils of nine plant species from Clusiaceae and Asteraceae against *S. granarius* were assessed in another study (Kordali et al., 2012). In our study, it was revealed that *H. perforatum* essential oil has toxicity to *T. molitor*, like these studies. $LC_{50,}$ $LC_{90}$ and $LC_{99}$ values of the essential oil of *H. perforatum* were 3.06, 4.99 and 6.56; 13.3, 23.6 and 32.0; and 8.24, 13.9 and 18.5 µl/L air against adults, pupae and larvae of *T. molitor,* respectively. These values indicate that *H. perforatum* may be an alternative biopesticide against *T. molitor.* Similar results to our study were obtained in previous studies on different target insects. The insecticidal effects of *Hypericum* spp. have been assessed against *T. castaneum* (Parchin & Ebadollahi, 2016), *C. pipiens* (Rouis et al., 2013), *S. granarius* (Kordali et al., 2012) and *M. sexta* (Samuels & Knox, 1989). Despite these results Dastagir et al. (2016) reported that *H. perforatum* did not show cytotoxic, insecticidal and antibacterial activity in vitro at different doses. However, it is possible that the doses applied in that study were too low.

The major component of *H. perforatum* was found to be as α-pinene (51.2%). Also, Çakir et al. (1997) was found that α-pinene is main compound (61.7%) which found in *H. perforatum* essential oil. α-pinene is an organic compound of the terpene class and monoterpenoids were known to have insecticidal activity. In a previous study, monoterpenes were described to have an insecticidal effect on some substantial pests (Lee et al., 1997). So, α-pinene may have caused the mortalities seen in this study. The results show that essential oils of both plants were toxic to all stages of *T. molitor*. Data of this study showed that the adults of *T. molitor* was more sensitive to essential oil than other stages ($LC_{99}$ 6.56 µl/L air). Pupae of *T. molitor* was found to be more tolerant than other stages ($LC_{99}$ 32.0 µl/L air). We obtained complete mortality by vapor of *H. perforatum* essential oil in 6, 15 and 30 µl/L air for 24 h against adults, larvae and pupae of *T. molitor,* respectively. Gözek (2007) demonstrated that adult and larval stages of *Tribolium confusum* Jaquelin Du Val, 1868 (Coleptera: Tenebrionidae) were the most tolerant stages to treatments of garlic essential oil. On the other hand, Sümer Ercan et al. (2013) found that adult of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) was the most sensitive stage to exposure of *Prangos ferulaceae* Lindl. essential oil. The larval stage of *E. kuehniella* was more tolerant than the egg stage after exposure to essential oils of *Thymus argaeus* Boiss. & Balansa and *Thymus sipyleus* Boiss. (Ercan et al., 2018). So, the effectiveness of different essential oils may differ between different stored product pests.

MDA is the major product of polyunsaturated fatty acid peroxidation process. It indicates the level of lipid peroxidation (LPO) and is used as a marker of oxidative stress (Büyükgüzel et al., 2010; Baş & Kalender, 2016). In this research, oxidative stress evidenced by increased MDA level might be related to antioxidant enzyme activity reduction. Antioxidant enzymes such as CAT, GPx, GST and SOD are substantial cell protectors against oxidative stress caused damage (Baş & Kalender, 2011; Messarah et al., 2012). SOD enzyme is responsible for superoxide radical dismutation into $H_2O_2$ and $O_2$. CAT is responsible for catalyzing $H_2O_2$ conversion into $H_2O$ and $O_2$ (Büyükgüzel & Kalender, 2009). The role of GPx enzyme is preventing oxidative damage of cell membranes by catalyzing $H_2O_2$ conversion to $H_2O$ (Baş & Kalender, 2011; Messarah et al., 2012). GST is one of the major antioxidant enzymes, it has important

roles in oxidative damage inhibition via detoxifying LPO products (Büyükgüzel & Kalender, 2009). There are many studies that investigated antioxidant enzyme activities and MDA levels for examining degree of oxidative stress in insect tissues (Büyükgüzel & Kalender, 2007; Büyükgüzel et al., 2010; Aslanturk et al., 2011). Our research demonstrated a significant decrease in SOD, CAT, GST and GPx activities in all of the examined developmental stages of *T. molitor*. Changes reported in this study on antioxidant enzyme activities may be due to the reactive oxygen species generation. Moreover, some components of essential oils can show neurotoxic effects on pests and monoterpenes can be act as competitive inhibitors of AChE in insect tissues (Kostyukovsky et al., 2002). In this research *H. perforatum* essential oil caused decreasing of AChE activity. A previous study suggested that AChE enzyme activity may be decreased by essential oil exposing (Polatoğlu et al., 2016). Mortalities of *T. molitor* may be observed by these changes on activities of antioxidant enzymes and AChE and MDA values.

## Conclusions

This is the first report on the insecticidal fumigant activity of *H. perforatum* essential oil against different developmental stages of *T. molitor.* This study showed that mortality rates and MDA levels were increased, and CAT, GST, SOD and GPx activities were decreased by the essential oil. The tested doses of essential oil gave complete mortality of pupae, larvae and adults of *T. molitor*. The results support the hypothesis that *H. perforatum* essential oil causes oxidative stress and induces LPO process. So, *H. perforatum* essential oil has been demonstrated to have toxicity to *T. molitor* and could be used as an alternative to synthetic chemical control of this insect. The use of *H. perforatum* essential oil may be a more healthy and reliable method for controlling insects.

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*Original article (Orijinal araştırma)*

# The status of the red mason bee in the orchards of Ankara and Çankırı Provinces, Turkey[1]

Kırmızı duvarcı arısının Ankara ve Çankırı (Türkiye) illerinin meyve bahçelerindeki durumu

**Yasemin GÜLER[2*]**

## Abstract

Research on the red mason bee, *Osmia bicornis* (L., 1758) (Hymenoptera: Megachilidae), which is an important pollinator particularly for stone fruits, in Turkey is limited to the last decade. After the first report in sweet cherry orchards of Afyonkarahisar, this study aimed to determine the presence and the density of the red mason bee and to collect data on its nesting biology between 2014 and 2016 in the mixed orchards of Ankara and Çankırı Provinces. Although the red mason bee was detected in almost all orchards sampled using the Malaise trap, the nesting activity was recorded in only four orchards in 2014 and 2015. Reeds with an inner diameter of 6-9 mm and a length of 15-25 cm were used as the artificial trap-nests. It was determined that the percentage of the nesting success varies between 6 and 48%. The cocoons that originated from the nests were placed in the incubator to stimulate diapause. Then, the temperature of the incubator was gradually increased to complete the life cycle of the species with starting the bud stage of stone fruits in early spring. Consequently, the emergence rates of the adults from the 135 cocoons collected in 2014 were 36 to 95%. It was not recorded the adult emergence from any of the 143 cocoons obtained from the dissections of the nests in 2015. The sex ratios, both between orchards and from 2014 to 2015 in the same orchard ranged between 1:1.5-1:4 (♀:♂). The results are discussed based on stress conditions such as weather, limited pollen and nectar sources and insecticide application.

**Keywords:** Malaise trap, Megachilidae, *Osmia bicornis*, sex ratio, trap-nest

## Öz

Özellikle sert çekirdekli meyvelerin önemli bir polinatörü olan Kırmızı duvarcı arısı, *Osmia bicornis* (L., 1758) (Hymenoptera: Megachilidae) ile ilgili araştırmalar, Türkiye'de son on yıl ile sınırlıdır. Afyonkarahisar'daki kiraz bahçelerinde ilk çalişmanın ardından 2014 ile 2016 yılları arasında Ankara ve Çankırı illerindeki karışık meyve bahçelerinde yürütülen bu çalışma, Kırmızı duvarcı arısının varlığını ve yoğunluğunu belirlemek ile yuvalanma biyolojisi hakkında veri elde etmeyi amaçlamıştır. Kırmızı duvarcı arısı, Malaise tuzak kullanılarak örneklenen hemen tüm bahçelerde belirlenmiş olmasına rağmen, yapay yuvalanma 2014 ve 2015 yıllarında sadece dört bahçede kaydedilmiştir. İç çapı 6-9 mm, uzunluğu 15-25 cm olan kamışlar yapay tuzak yuva olarak kullanılmışlardır. Yuvalanma başarısının %6 ile 48 arasında değiştiği belirlenmiştir. Yuvalardan elde edilen kokonlar diapozu başlatması için inkübatöre yerleştirilmiştir. Daha sonra inkübatörün sıcaklığı, erken ilkbaharda sert çekirdekli meyvelerin tomurcuk kabarması döneminin başlaması ile türün yaşam döngüsünü tamamlaması için kademeli olarak arttırılmıştır. Sonuç olarak, 2014 yılında toplanan 135 kokondan ergin çıkışı oranı %36 ile 95 olmuştur. 2015 yılına ait yuvaların diseksiyonundan elde edilen 143 kokonun hiç birinde ergin çıkışı kaydedilmemiştir. Eşey oranları hem tüm bahçeler arasında hem de aynı bahçenin 2014 ve 2105 yıllarında 1:1.5 ile 1:4 (♀:♂) arasında çeşitlilik göstermiştir. Sonuçlar, iklim, sınırlı polen ve nektar kaynakları ile insektisit uygulaması gibi stres koşullarına dayandırılarak tartışılmıştır.

**Anahtar sözcükler:** Malaise tuzak, Megachilidae, *Osmia bicornis*, eşey oranı, tuzak yuva

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## Introduction

Mason bees, *Osmia* spp. Panzer, 1806 (Hymenoptera: Megachilidae) are important in the pollination of early flowering fruits, such as almond, plum and cherry (Bosch & Blas, 1994; Eeraerts et al., 2019b), because they are able to be activity even in cool and rainy weather conditions below 12ºC, which limit the pollinator activity of the honey bee (Vicens & Bosch, 2000b). In addition, only a few female individuals of *Osmia* spp. are sufficient for pollination of a single flowering fruit tree, in contrast with hundreds of the honey bee workers (Krunić & Stanisavljevic, 2006a). Another advantage is that the nesting areas are aboveground in contrast to many other species of solitary bees, so that their populations can be managed using low cost artificial nests (Krunić & Stanisavljević, 2006b; Benedek, 2008). Research on the use of mason bees in orchards started in the second half of the twentieth century. Today, several species are used commercially for pollination of flowers in orchards in the USA, Japan, England, Denmark, Spain, Serbia and Italy (Sekita, 2001; Krunić & Stanisavljević, 2006a; Benedek, 2008; Matsumoto & Maejima, 2010). One of these species is *Osmia bicornis* (L., 1758) (syn. *Osmia rufa* L., 1758) (Hymenoptera: Megachilidae), the red mason bee.

In addition to being one of the few solitary bee species active in early spring, the red mason bee has a wide distribution area in Europe, Caucasus and Central Asia (Banaszak & Romasenko, 1998) as well in Turkey (Özbek, 2013; Güler et al., 2014). It is univoltine and spends the winter as adult in the cocoon (Raw, 1974; Strohm et al., 2002). It uses nests abandoned by other bees, dry stalks, cavities opened to the ground by other insects, wall crevices and empty snail shells (Müller, 2018). The nests consist of cells, which contain septa between them, are arranged linearly and plastered with a mixture of saliva-clay (Güler, 2012). This species collects pollen and nectar from tens of plant species belonging to 19 families (Müller, 2018). One of these families is Rosaceae, which contains both economically and commercially important fruit species (Bertrand et al., 2019). Güler & Özkök (2016) reported that Rosaceae pollen is one of the most encountered pollen within the artificial nests of *O. bicornis* in sweet cherry orchards.

Data on to presence of the mason bees in orchards of Turkey are limited to the last decade. Currently, five mason bee species [*O. bicornis*, *Osmia brevicornis* (Fabricius, 1798), *Osmia caerulescens* (L., 1758), *Osmia cornuta* (Latreille, 1805) and *Osmia melanura* Morawitz, 1871] have been recorded from the orchards, as well as many species belonging to other solitary bee families (Özbek, 2008; Güler & Dikmen, 2013).

The prominent fruit production in the provinces investigated in this study are pome fruit, sour cherry and sweet cherry in Ankara, and plum and cherry in the Çankırı (TÜİK, 2019). Among the pome fruits, the two fruits most commonly produced in both provinces are apple and pear (TÜİK, 2019). All of these fruits, except for sour cherry, are not self-fertile. They need pollinators to ensure the exchange of pollen between their cultivars, and to increase fruit set (Klein et al., 2012; Martins et al., 2015; Eeraerts et al., 2019a). Hansted et al. (2012, 2014) recommend the use of pollinators even in sour cherry orchards to obtain quality products. In practice, the honey bee is the main pollinator used in orchards. However, frequent unfavourable weather conditions, especially in early spring, lead to low fruit set production, and thus, economic losses. Also, such solitary bees complement honey bee in the pollination of various other plant species.

Given these circumstances, the main purpose of this study was to determine the presence and density of the red mason bee, which is one of several the species shown as an alternative, or supportive to the honey bee and has a manageable potential with trap-nests in Ankara and Çankırı orchards.

## Materials and Methods

Field studies were conducted in the orchards in Ankara and Çankırı Provinces of Turkey from 2014 to 2016. These were mixed orchards consisting of apple, sweet and sour cherry, peach, pear, almond, and plum trees. The smallest orchard had 100 trees, and the largest had 2350 trees. All orchards were located in intensively agricultural areas. The distance between orchards ranged from 2 to 106 km.

In 2014, the presence of the red mason bee was determined by Malaise traps in eleven orchards. The traps were installed during the budding period of stone fruits (late-March and mid-April) and were removed during the green fruit period (mid- to late-May). The traps were checked on a weekly basis and the specimens in the killing bottles recorded.

In addition, the artificial nests were used to determine the occurrence and abundance of the red mason bee in 8 of 11 orchards with Malaise traps in 2014. The trap-nests were hung on the south-facing branches or posts at about 1.5 m above the ground in all the orchards on the same day as the installation of the Malaise traps. Each nesting site was consisted of at least 25 reeds with an inner diameter of 6-9 mm and a length of 15-25 cm, inserted into PVC pipes. Also, traps were set in the orchard of the Plant Protection Central Research Institute in Yenimahalle, Ankara, which had apple, sweet cherry, pear, and mulberry trees. These traps were to determine the abundance of red mason bees with the artificial nests, in that orchard as the bees had previously been detected in that orchard (unpublished data). In 2015, trap-nests were only hung to four orchards (Eldivan 1, 2, 3 and Yenimahalle) to determine the change in red mason bee abundance from the previous year. At the end of the flowering period, all reeds were collected from the orchards and kept in ventilated cylindrical plastic containers (20 cm diameter, 27 cm long) under laboratory conditions until autumn. In October of 2014 and 2015, the reeds were separated based on whether they contained red mason bees. During this process, all reeds were also checked for parasitoids, cleptoparasites, predators and other nesting hosts. The cocoons of *O. bicornis* in the reeds were removed (Figure 1) and stored in the climate chamber under controlled conditions at 4-6ºC and 50-60% RH for hibernation. To achieve the synchronization of the adult *O. bicornis* emergence with the flowering period of fruit trees, the temperature was increased incrementally to 10, 15 and 20ºC starting from mid-March 2015 and 2016 (Bosch & Kemp, 2004; Krunić & Stanisavljević, 2006b). The number of adults and their sex were recorded daily and then released to the orchards.

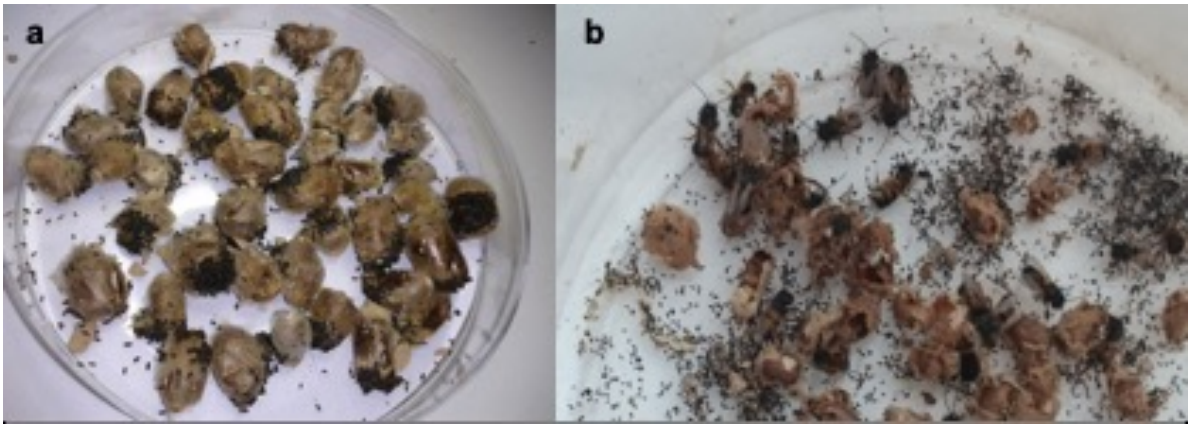


Figure 1. Red mason bee a) cocoons, and b) adults.

## Results

The study focused on red mason bee because this is the only species of *Osmia* recorded in both Malaise traps and trap-nests. Results from the samples obtained from the Malaise traps showed that *O. bicornis* was present in all orchards except one in the Ankara Province. Given that the bees were first recorded between 27 and 28 March 2014 in Malaise traps, it is concluded that their flight activity began between in late-March to early-April in the orchards of Ankara and Çankırı Provinces (Figure 2). Males were active before females and their numbers were generally higher (total of 46 males and 27 females from all traps). Adult flight activity continued until early-May (Figure 2). Within this period, the stone fruits and the early cultivars of the pome fruits were flowering. Since all the fruit cultivars in these orchards were in the green fruit stage in early-May, it is concluded that the mason bee may be effectively pollinate of these cultivars.

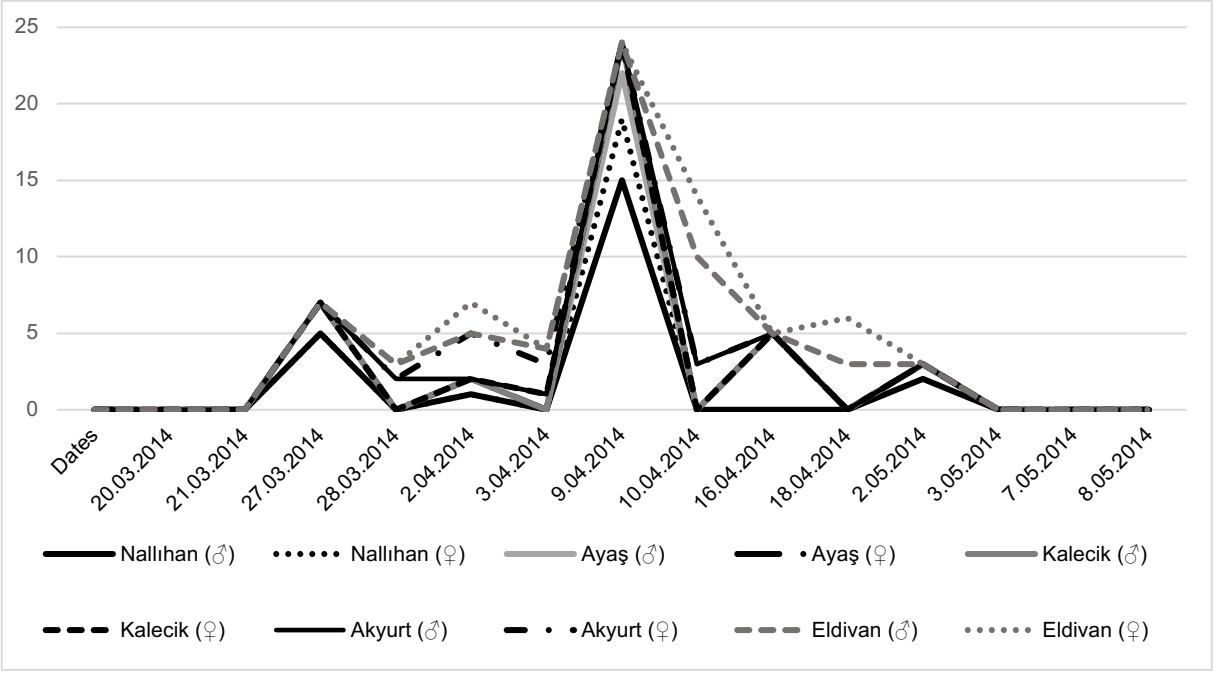


Figure 2. The activity of the red mason bee, determined by Malaise traps installed in 11 orchards in Ankara and Çankırı Provinces in 2014.

The nesting successes, the number of the cocoons, the percentage of the adult emergences, and the sex ratios of *O. bicornis* from 2014 to 2015 are shown in Tables 1 and 2. As a result of the dissected reeds, the nesting activity of *O. bicornis* was recorded in three of eight orchards placed the artificial nests as well as in the garden of the Institute in Yenimahalle in 2014 (Table 1). Therefore, the study was continued in only four orchards (Eldivan 1, 2, 3 and Yenimahalle) in 2015. In 2014, the highest number of cocoons (78) was obtained from the orchard of the Institute known to have a resident population of *O. bicornis* (Table 1). While the number of cocoons was limited to 18 in the same area, the highest number (122) was recorded from the Eldivan 2 orchard in 2015. Furthermore, *Ancistrocerus parietum* (L., 1758), *Ancistrocerus claripennis* subsp. *claripennis* Thomson, 1874 (Hymenoptera: Vespidae: Eumeninae) and *Chrysis* spp. (Hymenoptera: Chrysididae) were encountered in the reeds both with low-nesting and without nesting in both years (Table 1). *Ancistrocerus parietum* was recorded from the nesting in several locations in Ankara, but *A. claripennis* subsp. *claripennis* was found only in Eldivan.

Table 1. Results obtained by dissection of the reeds in artificial nests of the red mason bee in 2014 and 2015

| Orchards | 2014 | | | 2015 | | |
|---|---|---|---|---|---|---|
| | Cocoons | *Ancistrocerus* spp. | *Chrysis* sp. | Cocoons | *Ancistrocerus* spp. | *Chrysis* sp. |
| Eldivan 1 | 14 | 2 | 3 | 3 | 4 | 0 |
| Eldivan 2 | 42 | 0 | 1 | 122 | 0 | 1 |
| Eldivan 3 | 1 | 9 | 0 | 0 | 5 | 2 |
| Yenimahalle (Institute) | 78 | 0 | 0 | 18 | 0 | 0 |
| Ayaş* | 0 | 16 | 0 | - | - | - |
| Akyurt* | 0 | 7 | 0 | - | - | - |
| Kalecik* | 0 | 4 | 0 | - | - | - |
| Nallıhan* | 0 | 19 | 0 | - | - | - |
| Total | 135 | 57 | 4 | 143 | 9 | 3 |

* In these orchards, the study was not continued in 2015 because no nesting was recorded in 2014.

Table 2. Nesting successes, adult emergence and sex ratios in the artificial nests of the red mason bee in 2014 and 2015

| Orchards | The nesting success (%) | | The adult emergence ratio | The sex ratio (♀:♂) | |
|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2014 | 2015 |
| Yenimahalle (Institute) | 47.8 | 8.9 | 94.9 | 1:2.4 | 1:3.2 |
| Eldivan 1 | 6.0 | 3.7 | 35.7 | 1:4.0 | 1:3.0 |
| Eldivan 2 | 28.0 | 24.2 | 69.1 | 1:2.2 | 1:1.5 |

The high degree of the nesting success and the adult emergence ratio in 2014 were obtained from the reeds of the orchard of the Institute (Table 2). The nesting success in all orchards in 2015 was dramatically lower compared to 2014. Although there was 143 pupae collected in 2015 in incubation, adult emergence was not recorded. Therefore, the adult emergence ratio of 2015 could not be calculated. The sex ratio of *O. bicornis* in 2015 was calculated by dissection of the cocoons.

The sex ratios of *O. bicornis* varied between orchards and years (Table 2). The highest female number among three orchards was obtained in Eldivan 2 with a ratio of 1:1.5-2.2. While the sex ratio of the red mason bee in the orchard of the Institute in Yenimahalle changed had an increased proportion of males in 2015, the ratios in the other orchards moved in favor of females.

## Discussion

The artificial nests have been used for *Osmia* spp. in the orchard of the Plant Protection Central Research Institute in Yenimahalle, Ankara since 2009 but only a few records were kept. It was noted that especially the population of nesting *O. bicornis* increased gradually year by year (unpublished data). Therefore, a 48% nesting success recorded in 2014 was not surprising. The continued development toward the creation of a resident population was destroyed in 2015 due to an insecticide application on 17 April 2015 to the orchard of the Institute (without our knowledge) at the time of the flight activity of *O. bicornis*. It is concluded that this a possible reason for the significant decrease in the number of the cocoons of 2015 (Table 1). In Eldivan 2, which was recorded as the second orchard having a high abundance of cocoons (42) in 2014, this number increased in 2015 (122 cocoons). This rise can be explained by the fact that the location of the orchard is more suitable for the resident population of the red mason bee compared to other orchards of Eldivan (Table 1). The upper side of Eldivan 2 was adjacent to a meadowland (Figure 3) instead of being surrounded by other orchards as in Eldivan 1 and 3. The meadowland was covered with the plant species belonging to Asteraceae (including *Senecio vernalis* Waldst. & Kit., *Taraxacum* sp. and *Sonchus* sp.), Boraginaceae [including *Asperugo procumbens* L. and *Buglossoides arvensis* (L.) I. M. Johnst.], Brassicaceae [including *Alyssum desertorum* Stapf., *Camelina hispida* Boiss., *Capsella bursa- pastoris* (L.) Medik., *Lepidium draba* L., *Microthlaspi perfoliatum* (L.) F. K. Mey.] and Lamiaceae (including *Lamium amplexicaule* L. and *Lamium orientale* E. H. L. Krauser) families which attract many bee species. Meadowlands, which have a high diversity and abundance of flowering plants, provide support to bee populations for supplying both alternative nesting areas and alternative food sources (Öckinger & Smith, 2007). It is known that increased landscape heterogeneity enhances pollinator richness and abundance, as well as pollination service (Steckel et al., 2014). The connection of species and landscape in bees is stronger than other insect groups due to the mutualistic relationship between bees and flowering plants. Thus, comparable to the results of Steffan-Dewenter (2003) and Kremen (2008) the densities of bee populations, especially in standard commercial farms such as all the orchards in this study, are more influenced by habitat connectivity. Consequently, *Osmia* spp., just like other bees, do not tend to disperse from the nesting areas if they have access to suitable food sources (Vicens & Bosch, 2000a). They prefer to nest in older nests, both for more efficient use of their energy and to avoid the higher pressure of natural enemies.

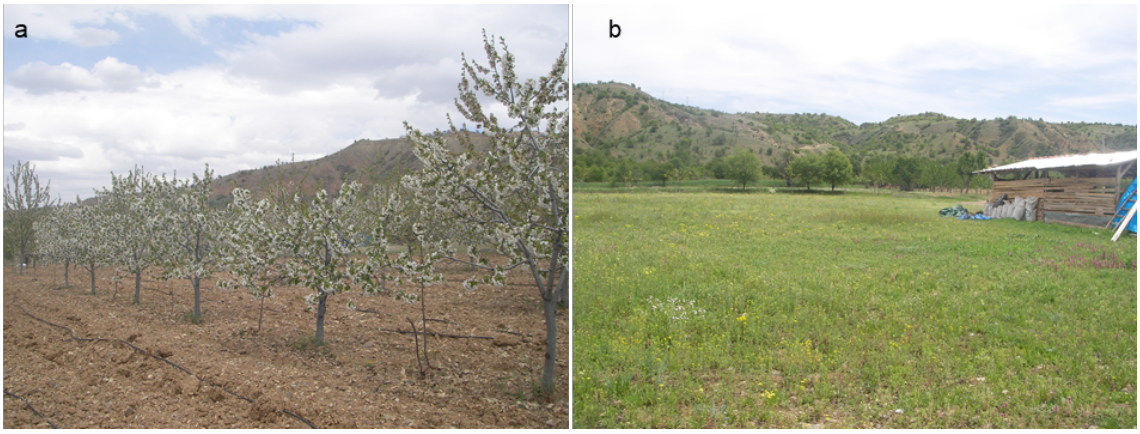


Figure 3. a) The flowering period of the Eldivan 2 orchard, and b) the meadowland covered with flowering plants that attract bees.

In a study by Krunić & Stanisavljević (2006a) that aimed to increase the population of the red mason bee in Serbia, the adult emergence rate ranged between 83-94%. The ratios in this study were 36, 69 and 95% (Table 2) in Eldivan 1, Eldivan 2, and the Institute orchard, respectively. Danks (1971) reported that the preadult mortality rates in solitary bees nesting in dry stalks are 50-60% and these mortality rates can be evaluated as a relatively low when compared with animals, which are parental care, such as bird and mammal and social insects. Solitary bees have a relatively long preadult period in which they are vulnerable to many biotic and abiotic factors. The basic biotic factors are predators, parasitoids, cleptoparasites and pathogens. Güler (2012) reported that the parasitoid *Melittobia acasta* (Walker, 1839) (Hymenoptera: Eulophidae) and the nest destroyer *Trogoderma versicolor* (Creutzer 1799) (Coleoptera: Dermestidae) from the artificial nests in the sweet cherry orchards of Sultandağı (Afyonkarahisar). In that study, the parasitoid was found in 25-74% of all nests while the nest destroyer was found in 33%. In this study, however, these species were not encountered, but a total of 66 *Ancistrocerus* spp. as predator and seven *Chrysis* sp. as cleptoparasite were recorded from some of the reeds (Table 1). The members of the Eumeninae subfamily, which includes *Ancistrocerus* spp., actually nest in empty reeds. However, they destroy the nests of other species such as *Osmia*, if they cannot find empty reeds (Krunić et al., 2005). Moreover, the flight activity of the first offspring of *Ancistrocerus* sp. synchronizes with that of *O. bicornis* females and their nesting needs overlap with 53-97% of the preferences of the red mason bee (Budriene et al., 2004). These species also construct linear nests in reeds of 4-10 mm diameter and use mud to separate cells (Boesi et al., 2005). In this study, *Chrysis* spp. was not only determined from the nests of the wild bee, but also found in the nests of *Ancistrocerus* spp. (Table 1). They have typically a cleptoparasite behavior; their eggs are deposited inside nests of other bee and wasp species, consuming pollen provided by the host, the development of their larvae is more rapid than the host larvae (Krunić et al., 2005).

The most important abiotic factors that affect the flight activity of bees are temperature and precipitation. Between 15 March and 15 May, when the red mason bee is active, meteorological data for Yenimahalle (location at the Institute) and Eldivan (location at Eldivan 1 and 2 orchards) towns was analyzed. The number of days within this period in 2014 with an average temperature of less than 10ºC was 16 d in Yenimahalle increasing in 2015 to 22 d (Figure 4). In Eldivan, the days of average temperature in 2014 was 22 increasing to 33 d in 2015 (Figure 5). In both locations, the number of days when the temperature dropped below zero was only 3 and 11 d in 2014, respectively. In 2015, however, the number of days below zero increased by two to three times; 10 d in Yenimahalle and 20 d in Eldivan. The number of days with precipitation at both locations did not change much between years; 24 d in 2014 and 23 d in 2015 in Yenimahalle, and 24 and 19 d in Eldivan. These climatic conditions suggest that temperature might have been responsible for both the decline in nesting success and the emergence of adults from cocoons in 2015. In addition, the pesticide application in April 2015 may have had an impact on all data from Yenimahalle.

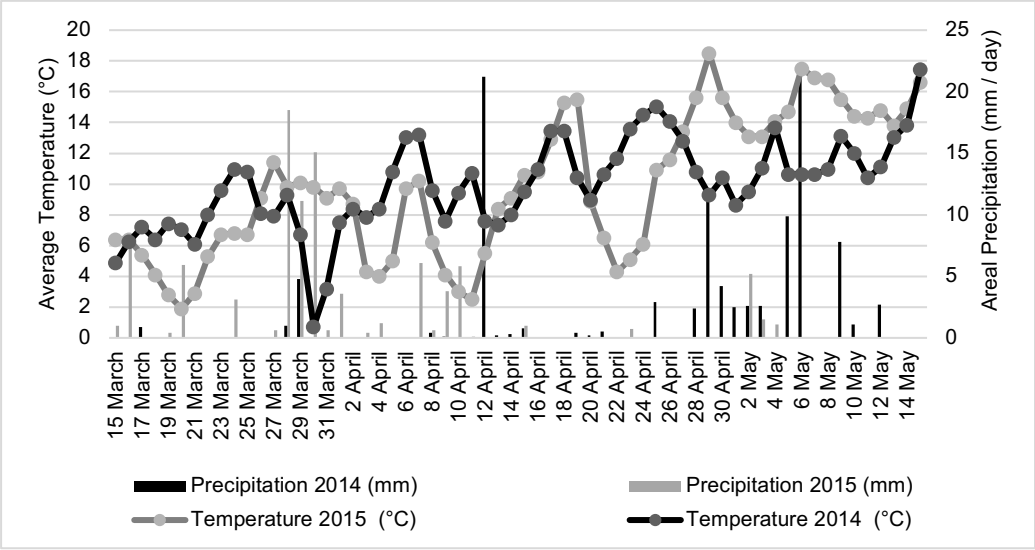


Figure 4. Average temperature and precipitation in Yenimahalle (Ankara) during the orchard flowering periods of 2014 and 2015.

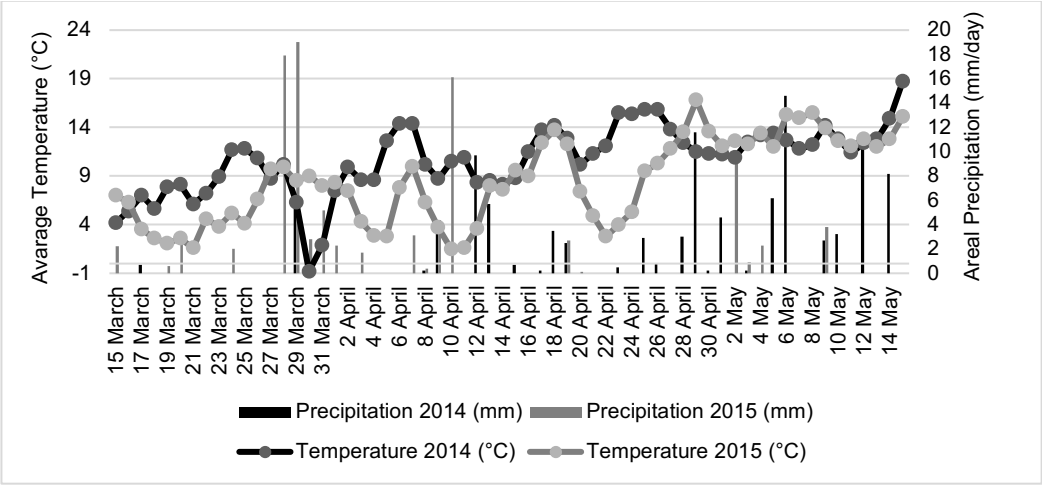


Figure 5. Average temperature and precipitation in Eldivan (Çankırı) during the orchard flowering periods of 2014 and 2015.

The sex ratios varied between 1:1.5 and 1:4 (♀:♂; Table 2). This ratio for *O. bicornis* population in the cherry orchards of Sultandağı (Afyonkarahisar) was previously recorded as 1:3.7 and 1:4 (Güler, 2012). Krunić & Stanisavljević (2006a) reported that the sex ratios of the same species changed between 1:1.35 and 1:2.68. It is known that the sex ratio is a parameter which changes according to weather and trophic conditions (Ivanov, 2006; Sampson et al., 2009). The ratio favors males under unfavorable conditions. As in all bees, *Osmia* females have the ability to control the number of males in a ratio of 1:2 or 1:3 (Sampson et al., 2009).

## Conclusion

The red mason bee, one of the commercially used pollinator species in many countries, is found naturally under Ankara and Çankırı ecological conditions, although the farmers are not aware of it like other wild bees. The main pollinator in many agricultural and natural areas is undoubtedly the honey bee (Kremen, 2008; Aslan et al., 2016; Hung et al., 2018). However, it has a limitation because pollination effectiveness decreases seriously under unfavorable weather conditions (days below the 12.8°C, raining, or windy blowing over 32 to 40 kph) (Spivak & Mader, 2010). However, some wild bee species are more

effective pollinators of some crops such as stone and pome fruits, and forage plants than *Apis mellifera* (Maeta & Kitamura, 1981; Tepedino, 1997; Bosch & Kemp, 2000; Vicens & Bosch, 2000c; Richards, 2020). Therefore, pollination service is usually provided by the activity of more than one bee species (Garibaldi et al., 2013). Wild bee species are insurance for the pollination system in cases of colony collapse or adverse weather conditions for the honey bee. In order to provide a sustainable pollination services, natural bee populations need to be protected and supported. Identifying these species and creating conditions to support their populations will bring a healthier environment and a higher quality product. As the data from Eldivan 2 shows, if the artificial nests including reeds of appropriate diameter are placed and alternative sources of food are found in the environment, it is possible to increase the population of the red mason bee in the orchard. Importantly, at this point, farmers should be informed about bees and pollination services, and be encouraged to permanently support red mason bee populations in their orchards at minimal cost.

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*Original article (Orijinal araştırma)*

# Identification and prevalence of potato cyst nematodes and root-knot nematodes in the potato production areas of İzmir Province, Turkey[1]

İzmir İli (Türkiye) patates üretim alanlarında patates kist nematodları ve kök-ur nematodlarının teşhisi ve yaygınlığı

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## Abstract

*Globodera* spp.and *Meloidogyne* spp. were identified using morphological and molecular methods. Also, the distribution and population densities of these nematodes were determined in potato cultivation areas of İzmir (Turkey) in 2015. Two hundred and twenty-three soil samples were collected during the survey and 32 samples were found to be infested with *Globodera* spp. and 41 samples with *Meloidogyne* spp. The identification of nematodes was made morphologically using perennial patterns of cyst/female individuals and morphometrics of second stage juveniles. Also, species specific primers were used for molecular identification. Only *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) were found in the samples that contained cyst nematodes. Also, the root-knot nematodes, *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980, *Meloidogyne hapla* (Chitwood, 1949), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) were found. The prevalence rates of *Meloidogyne* species were determined as 2.4, 12.2, 61.0 and 24.4%, respectively. In terms of the number of individuals in soil, all *G. rostochiensis* (10 eggs/g soil) and *M. chitwoodi* (1 juvenile/250 $cm^3$ soil) population levels were detected above the economic damage thresholds for potato production. Also, two populations of *M. incognita* (0.5-2 juvenile/250 $cm^3$ soil) were found above the specified threshold levels.

**Keywords:** *Globodera* spp., *Meloidogyne* spp., molecular identification, potato, Turkey

## Öz

*Globodera* spp. ve *Meloidogyne* spp. morfolojik ve moleküler yöntemler kullanılarak tanımlanmıştır. Ayrıca, 2015 yılında İzmir (Türkiye) patates üretim alanlarında bu nematodların dağılımı ve popülasyon yoğunlukları tespit edilmiştir. Sürvey sırasında toplam 223 toprak örneği alınmış ve 32 örneğin *Globodera* spp. ile 41 örneğin *Meloidogyne* spp. ile bulaşık olduğu tespit edilmiştir. Nematodların tür teşhisi, kist/dişi bireylerin anal kesitleri ve ikinci dönem larvaların morfometrik ölçümleri kullanılarak morfolojik olarak yapılmıştır. Aynı zamanda moleküler tür teşhislerinde türe özgü spesifik primerleri kullanılmıştır. Kist nematodları ile bulaşık olan tüm örneklerde sadece *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) türü bulunmuştur. Ayrıca, kök-ur nematodlarından *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980, *Meloidogyne hapla* (Chitwood, 1949), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 ve *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) türleri tespit edilmiştir. *Meloidogyne* türlerinin yaygınlık oranları sırasıyla %2.4, 12.2, 61.0 ve 24.4 olarak belirlenmiştir. Topraktaki birey sayıları açısından, tüm *G. rostochiensis* (10 yumurta/g toprak) ve *M. chitwoodi* (1 larva/250 $cm^3$ toprak) popülasyon yoğunlukları patates üretimi için ekonomik zarar eşiklerinin üzerinde tespit edilmiştir. *Meloidogyne incognita*'ya ait iki popülasyon (0.5-2 larva/250 $cm^3$ toprak) da belirtilen eşik seviyelerinin üzerinde bulunmuştur.

**Anahtar sözcükler:** *Globodera* spp., *Meloidogyne* spp., moleküler tanılama, patates, Türkiye

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## Introduction

Potato (*Solanum tuberosum* L.) is a tuberous plant in the Solanaceae family that has been spread to various climatic regions around the world from its native origin in South America. It is one of the most produced crops after corn, rice and wheat (Günel et al., 2005). World potato production was about 390 Mt in 2016 (FAO, 2017). In Turkey, annual production is around 5 Mt from an area of 142 Kha. İzmir is one of the most important provinces for potato production in Turkey (TÜİK, 2017). However, there are many pests and diseases that negatively affect potato production. Among these, plant parasitic nematodes are important and some of them are on the quarantine list. In particular, cyst nematodes, *Globodera* spp. Skarbilovich, 1959 (Tylenchida: Heteroderidae) and root-knot nematodes, *Meloidogyne* spp. Göeldi, 1892 (Tylenchida: Meloidogynidae) are on the top of the quarantine list in Europe (EPPO, 2020a, b).

One of the most important plant parasitic nematode genera that causes economic losses in potato is *Globodera* spp. (Van Riel & Mulder, 1998). Although tomato, eggplant, other *Solanum* plants and hybrids are important hosts of *Globodera* spp., potato is a crucial host for these nematodes (Anonymous, 2019). Typical symptoms of *Globodera* spp. especially aboveground are weakly developed plants and yellowing, which is similar to water and nutrient deficiencies, growth retardation, wilting under heat stress, and also reduced size and number of tubers (EPPO, 2017).

*Globodera* spp. was recorded for the first time in potato cultivation areas of Bolu Province in Turkey and was reported to be quarantined in the area (Enneli & Öztürk, 1996). Also, Özarslandan et al. (2009) found that potato production areas of Niğde Province were infested with *Meloidogyne chitwoodi* Golden, O'Bannon, Santos & Finley, 1980. Yıldız et al. (2009) reported that the infestation rate of *M. chitwoodi* was 37.9% in the potato areas of the Ödemiş District of İzmir. Moreover, Ulutaş (2010) reported that *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 infestation in Aegean Region potato production areas was 17.5 and 61.7% in Ödemiş District of İzmir.

*Meloidogyne* spp., which has more than 90 described species, is the most destructive, damaging and economically important plant parasitic nematode genera that affects most of the agricultural crops around the world (Hunt & Handoo, 2009; Jones et al., 2013). *Meloidogyne* spp. can cause yellowing, stunting, wilting and brown spots in potato plants. Also, these nematodes can lead to physical and chemical deformations in the tubers which can reduce their market value (Vovlas et al., 2005). *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne chitwoodi* Golden, O'Bannon, Santos & Finley, 1980, *Meloidogyne fallax* (Karssen, 1996), *Meloidogyne hapla* (Chitwood, 1949), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae), which cause economic crop losses, have been identified in potato cultivation areas in tropical and subtropical regions (Brodie et al., 1993; Molendijk & Mulder, 1996; Vovlas et al., 2005; Moens et al., 2009; Wesemael et al., 2011; Jones et al., 2013; Okendi & Moleleki, 2013). Although there are many studies conducted with plant parasitic nematodes on different crops in Turkey (Yüksel, 1966, 1967, Elekcioğlu & Uygun, 1994; Kaşkavalcı & Öncüer, 1999; Kepenekci et al., 2002; Özarslandan et al., 2009; Aydınlı et al., 2013; İmren et al., 2014; Aydınlı, 2018), only a few recent studies have examined potato cultivation areas in İzmir Province. Therefore, this survey was performed to identify *Globodera* and *Meloidogyne* spp. in İzmir Province using the morphological and molecular methods. Reliable and quick identification of nematode species is crucial for choosing appropriate integrated management strategies.

## Materials and Methods

### Materials

The main material of the study consisted of soil samples that were collected from potato production areas in İzmir Province, and *Meloidogyne* and *Globodera* spp. individuals obtained from the soil. In addition,

primers, consumables, chemicals and equipment were used in molecular studies. Besides, the GPS was used to obtain the coordinates was used.

**Survey**

The survey studies were conducted during the potato production season of 2015, in two cultivation periods (May-June and September-November) in potato production areas in İzmir Province. One hundred and fifteen soil samples were collected from Bayındır, Dikili, Kiraz, Ödemiş and Tire Districts of İzmir Province during the spring potato production period (May-June 2015) and 108 soil samples from Ödemiş during the autumn potato production season (September-November 2015).

Fenwick can (Fenwick, 1940) was used to extract *Globodera* spp. from soil samples, and motile plant parasitic nematodes were extracted using a modified Baermann funnel method (Hooper, 1986), specifically for the second stage juveniles of *Meloidogyne* spp. The prevalence of nematodes in these districts were determined as proportion of *Globodera* and *Meloidogyne* spp. in infested soil samples to the total number of the samples. Moreover, *Globodera* and *Meloidogyne* spp., the number of cysts and juveniles were counted under microscope in order to investigate the density of each population. The soil samples infested with *Meloidogyne* spp. were placed separately in plastic pots and one susceptible tomato (SC2121) seedling was planted in each pot in a greenhouse at 25°C and 60% RH. After 3 months, the infected tomato plants were harvested and single egg masses were inoculated into a hole 2-3 cm deep near sterile tomato seedlings within 7 d after transplanting. These pure cultures form *Meloidogyne* spp. populations were used for identification.

**Morphological identification**

**Perineal patterns**

For identification of *Globodera* spp., the perineal regions of vulva-anus parts of cysts were examined. The vulva region of the 8-10 cysts was cut with the help of a scalpel and kept in 15% $H_2O_2$ to ensure decoloration. The vulva parts of the cysts were placed into glycerin on a slide, covered with coverslip and sealed with nail-polish (Hooper, 1986). For identification of *Meloidogyne* spp., 5-6 female individuals were taken and permanent preparations were made using the perineal specimens as described by Taylor & Netscher (1974) and developed by Hartman & Sasser (1985). For the permanent preparations, vulval regions of mature females were cut in 45% lactic acid, then transferred into glycerin and were mounted on slides under a Leica DM4000B stereo microscope.

**Morphometric measurements of second stage juveniles**

Fixation and permanent preparation of second stage juvenile of *Globodera* and *Meloidogyne* spp. were performed. Fixation was performed according to the method developed by Hooper (1986). The fixed nematodes were taken into pure glycerin according to the Seinhorst (1959) method. Wax ring method was used in making the preparation (Hooper, 1986). *Globodera* and *Meloidogyne* spp. were identified by morphological and morphometric characters according to Siddiqi (2000).

**Molecular identification**

**Nematode DNA extraction**

For molecular identification of *Globodera* spp. (32 populations) and *Meloidogyne* spp. (41 populations), DNA isolation was performed using five cysts and five egg masses from each population, respectively, using a Qiagen DNA isolation kit (DNeasy Blood & Tissue Kit).

### Polymerase chain reaction

*Globodera* and *Meloidogyne* specimens, specific primers were identified using primers from White et al. (1990), Bulman & Marshall (1997), Zijlstra et al. (2000), Wishart et al. (2002) and Tesarova et al. (2003) (Table 1).

Table 1. Primers used in the PCR to identify the species of *Meloidogyne* and *Globodera* spp.

| Species | Primers | Primer sequences (5'-3') | Fragments (bp) | Reference |
|---|---|---|---|---|
| *G. rostochiensis*, *G. pallida* | ITS5 | 5'-GGAAGTAAAAGTCGTAACAAGG-3' | - | White et al., 1990 |
| | PITSp4 | 5'-ACAACAGCAATCGTCGAG -3' | 264 | Bulman & Marshall, 1997 |
| | PITSr3 | 5'-AGCGCAGACATGCCGCAA -3' | 434 | Bulman & Marshall, 1997 |
| *M. chitwoodi*, *M. fallax*, *M. hapla* | JMV1 | 5'-GGATGGCGTGCTTTCAAC-3' | 540 | Wishart et al., 2002 |
| | JMV2 | 5'-TTTCCCCTTATGATGTTTACCC-3' | 540 | Wishart et al., 2002 |
| | $JMV_{hapla}$ | 5'-AAAAATCCCCTCGAAAAATCCACC-3' | 440 | Wishart et al., 2002 |
| *M. incognita* | Sec-1f | 5'-GGGCAAGTAAGGATGCTCTG-3' | 502 | Tesarova et al., 2003 |
| | Sec-1r | GCACCTCTTTCATAGCCACG | 502 | Tesarova et al., 2003 |
| *M. javanica* | Fjav | 5'-GGTGCGCGATTGAACTGAGC-3' | 720 | Zijlstra et al., 2000 |
| | Rjav | 5'-CAGGCCCTTCAGTGGAACTATAC-3' | 720 | Zijlstra et al., 2000 |
| *M. arenaria* | Far | 5'-TCGGCGATAGAGGTAAATGAC-3' | 420 | Zijlstra et al., 2000 |
| | Rar | 5'-TCGGCGATAGACACTACAAACT-3' | 420 | Zijlstra et al., 2000 |

The PCR mixture was prepared in 25 µl (2 µl DNA sample, 12.5 µl PCR master mix, 7.5 µl water and 1 µl specific primers) for each sample. For the PCR cycle, the parameters specified in Tables 2 and 3 were applied.

Table 2. Parameters used in the PCR study to determine the species of *Meloidogyne* spp.

| Time & Degree | Time & Degree | | Time & Degree | Time & Degree | |
|---|---|---|---|---|---|
| | | 45 cycles | | | |
| | | 50°C (194/195) | | | |
| | | 61°C (Far/Rar) | | | |
| | | 64°C (Fjav/Rjav) | | | |
| 94°C | 94°C | 50°C (JMV primers) | 72°C | 4°C | |
| 2 min | 30 s | 30 s | 7 min | ∞ | |
| | | 35 cycles | | | |
| 94°C | 94°C | 50°C (Sec-1f/Sec-1r) | 72°C | 72°C | 4°C |
| 3 min | 30 s | 30 s | 90 s | 10 min | ∞ |

Table 3. Parameters used in the PCR study to determine the species of *Globodera* spp.

| Time & Degree | Time & Degree | | Time & Degree | Time & Degree | |
|---|---|---|---|---|---|
| | | 35 cycles | | | |
| 94°C | 94°C | 60°C (ITS5/PITSp4/PITSr3) | 72°C | 72°C | 4°C |
| 2 min | 30 s | 30 s | 30 s | 10 min | ∞ |

PCR products were processed with 1.5% agarose gel prepared with tris-borate-EDT buffer and then stained with ethidium bromide and imaged under UV.

## Results and Discussion

### Morphological identification

As a result of the surveys conducted in potato fields of İzmir Province, only *G. rostochiensis* was found in the samples infested with cyst nematodes. Also, *Meloidogyne* spp. were detected in 41 soil samples and all populations were cultured. From the morphological identification, the specimens were identified as *M. chitwoodi* (1 sample), *M. hapla* (5 samples), *M. incognita* (25 samples) and *M. javanica* (10 samples).

Tail length, hyaline terminus, stylet length, body diameter at anus, greatest body diameter, body length (L), a, c and c' of juvenile are the main morphometric properties for identification of *Globodera* and *Meloidogyne* spp. (Whitehead, 1968; Stone, 1973; Hesling, 1978; Eisenback et al., 1981; Golden, 1986; Jepson, 1987; Karssen, 2002; EPPO, 2017). The mean morphometric measurements of the second stage juveniles of *G. rostochiensis,* and *M. chitwoodi, M. hapla, M. incognita* and *M. javanica* are shown in Table 4.

Table 4. Morphometric data for second stage juveniles of potato cyst and root-knot nematode specimens collected from potato fields in İzmir Province, Turkey

| Morphometric characters | J2 measurements (µm) mean±standard error (min-max) | | |
|---|---|---|---|
| *Globodera rostochiensis* | This study (n=96) | Salgut (2017) | Tirchi et al. (2016) |
| Stylet length | 22.1±0.8 (16.2-25.8) | 21.4±0.4 (19.5-23.6) | 21.5±0.7 (20.0-22.8) |
| Tail length | 46.0±2.7 (23.9-74.3) | 54.1±1.6 (42.0-60.0) | 45.8±4.9 (37.0-54.7) |
| Body diameter at anus | 12.1±0.6 (8.8-14.4) | 15.2±0,6 (12.0-17.0) | 12.0±0.6 (10.5-12.5) |
| Body diameter | 18.4±1.1 (11.1-23.3) | 22.8±1.1 (18.0-28.0) | 19.4±1.4 (16.2-21.7) |
| Hyaline terminus | 22.8±1.7 (10.2-35.1) | 27.5±1.2 (22.8-33.2) | 27.1±2.8 (22.8-31.9) |
| Body length (L) | 415.7±10.2 (334.7-464.1) | 496.8±14.0 (445.0-585.0) | 422.5±40.9 (371.1-502.5) |
| a | 22.9±1.5 (16.7-36.3) | - | 21.9±2.4 (18.6-26.0) |
| c | 9.2±0.5 (4.9-12.0) | - | 9.3±1.0 (8.1-11.4) |
| c' | 3.8±0.2 (2.9-5.4) | - | 3.8±0.3 (3.2-4.4) |
| *Meloidogyne chitwoodi* | This study (n=3) | Evlice & Bayram (2016) | Golden et al. (1980) |
| Stylet length | 15.7±1.5 (13.4-18.5) | 10.0±0.4 (9.3-10.8) | 9.9±0.3 (9.0-10.3) |
| Dorsal oesophageal gland (DGO) | 2.8±0.1 (2.7-3.0) | 3.0±0.2 (2.6-3.3) | 3.2±0.2 (2.6-3.9) |
| Body diameter at S-E pore | 14.0±0.7 (12.6-15.0) | 12.4±0.4 (11.5-13.4) | - |
| S-E pore/Stylet length | 2.8±0.7 (1.5-3.9) | - | - |
| Tail length | 65.7±12.3 (43.9-86.3) | 43.0±2.1 (40.2-47.9) | 43.0±1.8 (39.0-47.0) |
| Body diameter at anus | 12.7±2.0 (9.2-16.1) | 9.6±0.5 (8.8-10.8) | - |
| Anus primordium | 88.8±20.4 (66.0-129.6) | 89.2±6.3 (80.4-97.8) | - |
| Body diameter at vulva | 14.5±1.4 (11.8-16.5) | - | - |
| Greatest body diameter | 15.5±1.7 (12.3-18.0) | 13.1±0.4 (12.5-13.9) | - |
| Hyaline terminus | 11.5±0.7 (10.3-12.6) | 10.4±0.9 (8.8-12.2) | 11.0±1.0 (8.6-13.8) |
| Body length (L) | 356.4±16.3 (324.3-377.6) | 366.8±11.3 (352.3-390.0) | 390.0±16.0 (336.0-417.0) |
| a | 23.6±3.1 (20.0-29.9) | 28.1±0.8 (26.0-29.1) | 27.5±1.2 (24.5-29.8) |
| b' | 5.8±1.1 (3.8-7.1) | 7.6±0.4 (7.0-8.3) | - |
| c | 5.9±1.3 (4.4-8.4) | 8.5±0.4 (7.4-9.0) | 8.9±0.4 (7.9-9.6) |
| c' | 5.1±0.2 (4.8-5.4) | 4.5±0.2 (4.1-4.7) | - |
| (S-E pore/L) X100 | 11.1±3.1 (5.9-16.7) | 18.3±0.6 (17.5-19.3) | - |
| *Meloidogyne hapla* | This study (n=15) | Uysal et al. (2017) | Whitehead (1968) |
| Stylet length | 15.1±1.2 (11.5-18.4) | 12.4±0.8 (11.2-13.6) | 9.7±0.92 (7.9-10.9) |
| Dorsal oesophageal gland (DGO) | 3.1±0.4 (2.1-4.7) | 4.8±1.7 (3.2-6.4) | - |
| Body diameter at S-E pore | 14.6±0.8 (11.2-17.1) | - | - |
| S-E pore/Stylet length | 3.3±0.3 (2.0-4.2) | - | - |
| Tail length | 53.6±3.5 (31.2-67.8) | 49.5±2.9 (44.8-56.0) | 43.0±4.0 (33.0-48.0) |
| Body diameter at anus | 11.3±0.7 (7.2-14.4) | - | - |
| Anus primordium | 68.7±4.9 (54.2-92.1) | - | - |
| Body diameter at vulva | 14.3±0.7 (10.1-17.6) | - | - |
| Greatest body diameter | 15.3±0.8 (11.3-18.9) | - | - |
| Hyaline terminus | 11.4±1.7 (4.8-17.4) | 13.1±2.1 (11.2-17.6) | - |
| Body length (L) | 358.1±7.9 (319.1-420.6) | 380.5±23.7 (328-412.8) | 337.0±11.4 (312.0-355.0) |
| a | 23.8±1.1 (19.5-30.9) | 29.2 | 23.9±1.7 (20.1-26.6) |
| b' | 4.9±0.4 (4.0-6.7) | - | - |
| c | 6.9±0.6 (4.8-8.0) | 7.7 | 7.9±0.2 (7.3-10.2) |
| c' | 4.8±0.3 (3.6-6.0) | - | - |
| (S-E pore/L) X100 | 13.8±1.4 (8.0-17.3) | - | - |

Table 4. Continued

| Morphometric characters | J2 measurements (µm) mean±standard error (min-max) | | |
|---|---|---|---|
| *Meloidogyne incognita* | This study (n=75) | Uysal et al. (2017) | Whitehead (1968) |
| Stylet length | 14.4±0.6 (11.6-22.0) | 13.3±0.7 (12-14.4) | 10.5±0.59 (9.6-11.7) |
| Dorsal oesophageal gland (DGO) | 2.9±0.3 (1.5-4.7) | 3.4±0.5 (3.2-4.8) | |
| Body diameter at S-E pore | 13.7±0.8 (10.6-18.4) | - | - |
| S-E pore/Stylet length | 4.4±0.3 (3.3-6.3) | - | - |
| Tail length | 52.6±3.5 (37.3-105.0) | 57.6±5.5 (50.4-68.8) | 46.0±3.0 (38.0-55.0) |
| Body diameter at anus | 10.8±0.7 (6.7-14.7) | - | - |
| Anus primordium | 100.1±7.4 (69.7-181.0) | - | - |
| Body diameter at vulva | 14.1±0.7 (10.6-18.2) | - | - |
| Greatest body diameter | 15.2±0.8 (11.4-21.2) | - | - |
| Hyaline terminus | 10.6±1.1 (4.50-18.90) | 11.6±2.2 (6.4-16.0) | - |
| Body length (L) | 361.9±5.6 (308.3-403.0) | 409.7±22.4 (360.0-441.6) | 371.0±16.0 (337.0-403.0) |
| a | 24.2±1.3 (17.1-30.1) | 29.5 | 28.3±1.7 (24.9-31.5) |
| b | - | - | 2.4±0.3 (2.0-3.1) |
| b’ | 4,5±0.2 (2.9-6.2) | - | 7.1±0.4 (6.4-8.4) |
| c | 7,1±0.5 (3.5-10.2) | 7.1 | 8.1±0.7 (6.9-10.6) |
| c’ | 4,9±0.3 (3.7-7.8) | - | - |
| (S-E pore/L) X100 | 17,5±1.2 (12.3-22.7) | - | - |
| *Meloidogyne javanica* | This study (n=30) | Uysal et al. (2017) | Whitehead (1968) |
| Stylet length | 15.3±0.8 (11.7-18.7) | 14.0±0.5 (13.6-14.4) | 10.4±0.5 (9.4-11.4) |
| Dorsal oesophageal gland (DGO) | 3.1±0.3 (2.1-4.4) | 3.4±0.4 (3.2-4.0) | - |
| Body diameter at S-E pore | 13.8±0.7 (10.4-17.1) | - | - |
| S-E pore/Stylet length | 3.7±0.3 (2.5-4.8) | - | - |
| Tail length | 50.3±2.8 (27.9-62.3) | 55.2±3.8 (52.8-60.8) | 49.0±4.0 (36.0-56.0) |
| Body diameter at anus | 10.2±0.5 (8.0-11.9) | - | - |
| Anus primordium | 90.1±2.6 (59.8-112.2) | - | - |
| Body diameter at vulva | 13.6±0.8 (8.8-16.4) | - | - |
| Greatest body diameter | 14.7±0.7 (10.4-18.4) | - | - |
| Hyaline terminus | 11.7±1.6 (4.7-23.8) | 14.4±2.3 (12.8-17.6) | - |
| Body length (L) | 387.9±10.5 (326.5-439.3) | 448.0±16.3 (427.2-465.6) | 417.0±22.0 (387.0-459.0) |
| a | 26.7±1.7 (21.0-37.7) | 32.0 | 30.6±2.0 (27.1-35.9) |
| b’ | 5.4±0.4 (3.9-7.8) | - | 7.5±0.2 (7.1-8.0) |
| c | 7.9±0.5 (6.2-10.8) | 8.1 | 8.5±0.7 (7.3-11.1) |
| c’ | 4.9±0.2 (3.5-5.8) | - | - |
| (S-E pore/L) X100 | 14.6±0.9 (11.6-19.5) | - | - |

The morphological and morphometric data of populations identified as *G. rostochiensis* (Figure 1), and *M. chitwoodi*, *M. hapla*, *M. incognita* and *M. javanica* (Figure 2) were confirmed according to the original definition (Siddiqi, 2000).

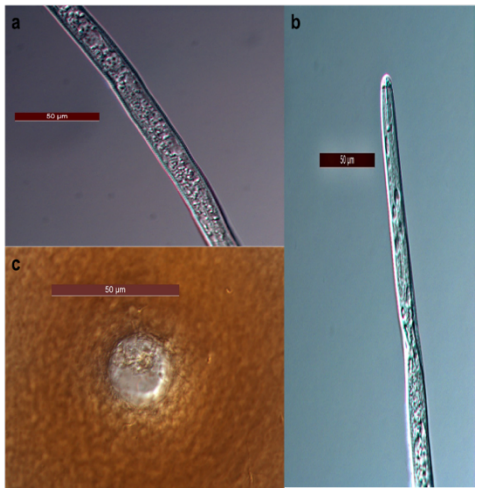


Figure 1. *Globodera rostochiensis* isolates collected from İzmir Province: a) midbody region view of second stage juvenile; b) anterior view of second stage juvenile; and c) perineal pattern.

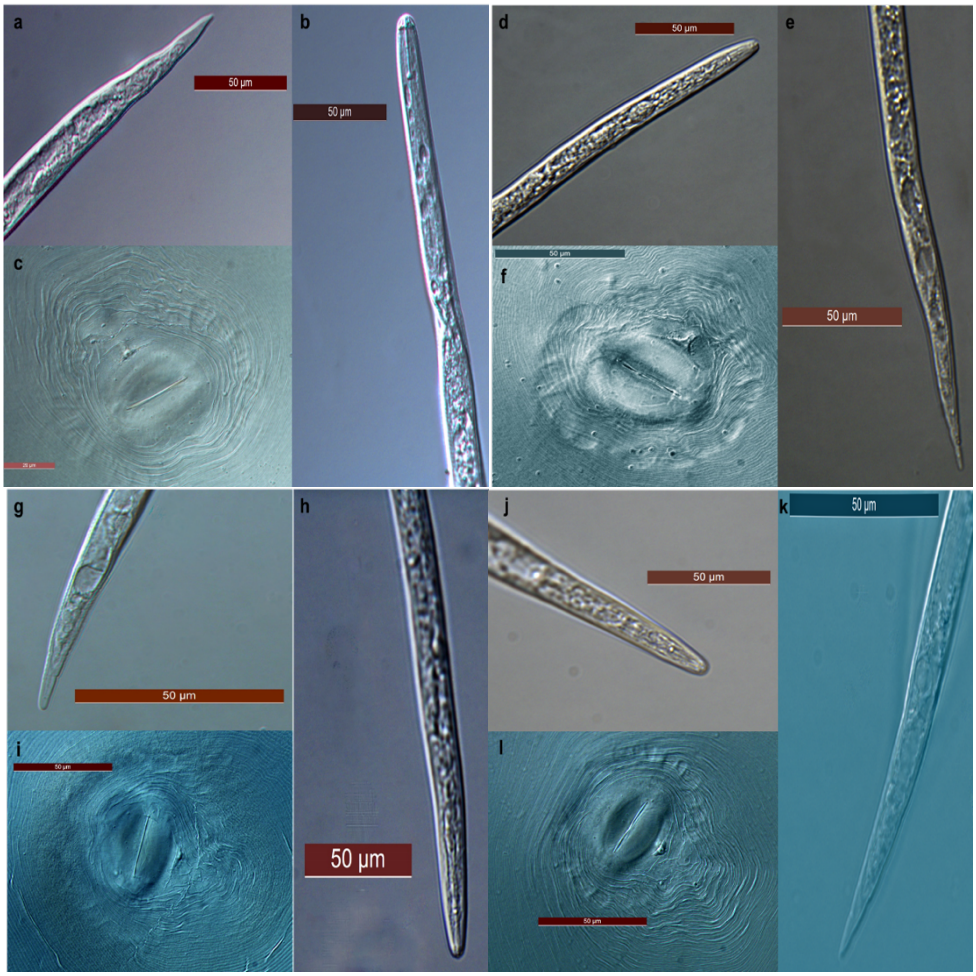


Figure 2. *Meloidogyne* spp. isolates collected from İzmir Province: a) tail view of second stage juvenile of *M. incognita*; b) anterior view of second stage juvenile of *M. incognita*; c) perineal pattern of *M. incognita*; d) anterior view of second stage juvenile of *M. javanica*; e) tail view of second stage juvenile of *M. javanica*; f) perineal pattern of *M. javanica*; g) tail view of second stage juvenile of *M. hapla*; h) anterior view of second stage juvenile of *M. hapla*; i) perineal pattern of *M. hapla*; j) anterior view of second stage juvenile of *M. chitwoodi*; k) tail view of second stage juvenile of *M. chitwoodi*; and l) perineal pattern of *M. chitwoodi*.

### Molecular identification

PCR studies were conducted using primer set PITSr3/PITSp4/ITS5 (Bulman & Marshall, 1997) for the determination of *G. rostochiensis* species and a band was obtained at 434 bp for all *G. rostochiensis* specimens (Figure 3). Ulutaş et al. (2012) had determined *G. rostochiensis* with the same primers in potato cultivation areas of Aegean Region of Turkey.

All *M. chitwoodi* and *M. hapla* specimens were diagnosed using JMV1/JMV2/JMV$_{hapla}$ primer set, with 440 and 540 bp DNA bands, respectively (Figure 3), as described by Wishart et al. (2002) and these results are compatible with the results of earlier studies in Turkey (Devran et al., 2009; Akyazı et al., 2012; Evlice & Bayram, 2016; Uysal et al., 2017). For *M. javanica*, specific SCAR primers (Zijlstra et al., 2000) produced 720 bp DNA bands (Figure 3), and this finding is in agreement with previous studies which conducted on different crops in Turkey (Devran & Söğüt, 2009; Devran, 2013; Özarslandan & Elekcioğlu, 2010; Devran et al., 2017; Uysal et al., 2017). SEC-1F/SEC-1R primers (Tesarova et al., 2003) were used and a DNA band was obtained at 502 bp for all *M. incognita* populations (Figure 3). These results are similar to previous studies in Turkey (Devran & Söğüt, 2009; Akyazı & Felek, 2013; Devran, 2013; Uysal et al., 2017). The perineal patterns, measurements of the second stage juveniles and molecular definitions overlapped for all nematode species.

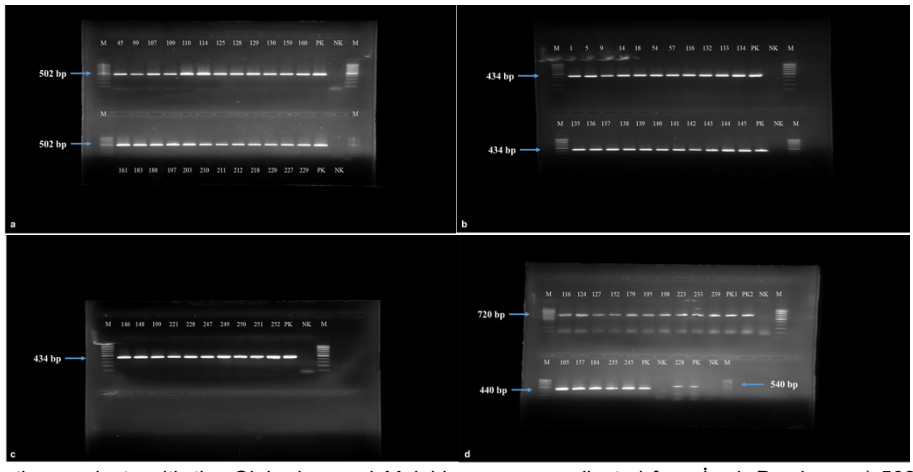


Figure 3. Amplification products with the *Globodera* and *Meloidogyne* spp. collected from İzmir Province: a) 502 bp PCR product obtained from *M. incognita* samples using SEC 1-F/SEC 1-R primers; b-c) 434 bp PCR product obtained from *G. rostochiensis* samples using PITSr3/PITSp4/ITS5 primers; d) 720 bp PCR product obtained from *M. javanica* samples using Fjav/Rjav, 440 bp PCR product obtained from *M. hapla* samples and 540 bp *M. chitwoodi* samples using JMV1/JMV2/JMV$_{hapla}$ primers (PK, positive control; NK, negative control; M, molecular marker (100-1000 bp Thermo).

### Occurrence of *Globodera* spp. and *Meloidogyne* spp. in districts of İzmir

*Meloidogyne* spp. were detected in 41 soil samples from Bayındır (3 samples), Dikili (3 samples), Kiraz (1 sample) and Ödemiş (34 samples) Districts. No plant parasitic nematodes were found in potato cultivation areas Tire District. *Globodera* sp. was detected only in soil samples taken from 32 different fields in Ödemiş District. As a result, *Meloidogyne* spp. (18.4%) were found more widely distributed than *Globodera* spp. (14.4%) in İzmir potato cultivation areas in 2015 (Figure 4).

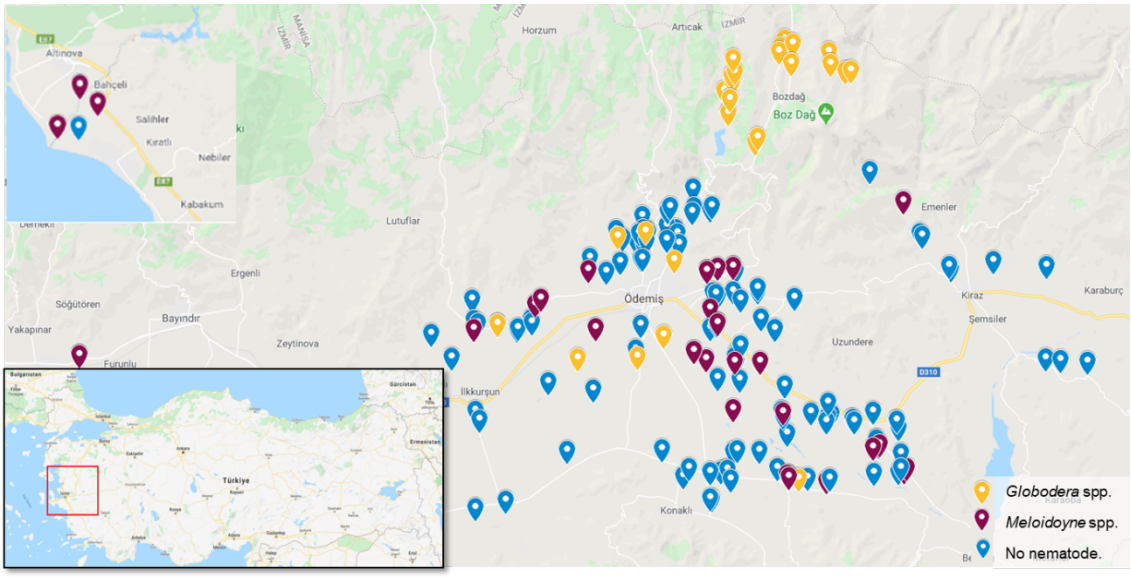


Figure 4. Surveyed area and the locations of nematode species found in potato fields in İzmir Province (Anonymous, 2020).

From the morphological and molecular identification studies, only *G. rostochiensis* was found in samples infested with cysts. Also, 61.0% of samples contained *M. incognita*, 24.4% *M. javanica*, 12.2% *M. hapla* and 2.4% *M. chitwoodi*.

Several nematode species that cause significant yield losses are found in potato cultivation areas. The main nematode species associated with potatoes are the yellow potato cyst nematode *G. rostochiensis*, the white potato cyst nematode, *Globodera pallida* (Stone, 1973) Behrens, 1975 and also the root-knot nematodes *Meloidogyne* spp. (Vovlas et al., 2005; Medina et al., 2016). In this study, *G. rostochiensis*, *M. chitwoodi, M. hapla M. incognita*, and *M. javanica* have been found in different parts of İzmir potato cultivation areas. In contrast, *G. pallida* was not detected in the surveyed areas of İzmir Province. In Turkey, many studies had been carried out to determine the nematode species on different crops. *Globodera* spp. was determined for the first time in Turkey in potato cultivation areas of Bolu Province (Enneli & Öztürk, 1996) and in the following decade, this nematode was found in Afyon, İzmir (Ödemiş-Bozdağ), Konya and Sivas Provinces (Anonymous, 2008). Ulutaş (2010) reported that *G. rostochiensis* prevalence in the Aegean Region potato production areas at 17.5% and also in Ödemiş District of İzmir at 61.7%. In İzmir, potato is cultivated both in high lands and low lands in two different growing seasons. In this study, while *G. rostochiensis* was found in all potato growing areas of the high lands, in the low lands infestation rate was only 6.82%.

Unfavorable environmental conditions cause the death of the eggs in the cyst and annual mortality rates are more than 50% for temperate regions and 75% in warmer climates (Marks & Brodie, 1998). Due to the high egg mortality of *Globodera* spp. in warmer climates, these species may better adapt to subtropical regions and its cycle is interrupted at temperatures above 28°C (Lima et al., 2018). Moreover, *G. rostochiensis* can develop well between 15-25°C (EPPO, 2017).

In addition, *M. incognita* and *M. javanica* have been found to be the predominant species and also *M. arenaria, Meloidogyne artiella* Franklin, 1961, *Meloidogyne ethiopica* Whitehead, 1968, *Meloidogyne exiqua* Goeldi, 1892, *M. hapla, Meloidogyne luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno & Karssen, 2014, and *Meloidogyne thamesi* (Chitwood, 1952) were identified in different cultivation areas of Turkey (Yüksel, 1966, 1967, Elekcioğlu & Uygun, 1994; Kaşkavalcı & Öncüer, 1999; Kepenekçi et al., 2002; Özarslandan et al., 2009; Aydınlı et al., 2013; İmren et al., 2014; Aydınlı, 2018). Also, Yıldız et

al. (2009) reported that *M. chitwoodi* was found in 37.9% of potato areas of İzmir. In Central and East Anatolia Region, only *M. chitwoodi* has been detected in potato cultivation areas in different studies (Özarslandan & Elekçioğlu, 2010; Özarslandan et al., 2013; Evlice & Bayram, 2016). With this study, *M. incognita* and *M. javanica* were found to be the widely distributed nematodes, but only one sample was infested with *M. chitwoodi*. These results may be explained due to the fact some nematode species have been found with low population levels for years. Furthermore, climate change could be affecting population density of plant parasitic nematode species in a way that the cool climate species such as *M. chitwoodi* and *M. hapla* are being replaced by species with high adaptability to higher temperatures such as *M. incognita* and *M. javanica* (Gözel & Elekcioğlu, 1996; Da Conceiçao et al., 2009).

**Distribution of *Globodera* and *Meloidogyne* spp. in İzmir**

*Globodera* and *Meloidogyne* spp. were detected in 14.4% and 18.4% of samples. When grouped in terms of the number of individuals in 100 $cm^3$ soil, for *Globodera* species, 1-50 individuals were detected in 37.5%, 51-250 individuals in 46.9% and 251-500 individuals in 15.6% of the samples. For *Meloidogyne* species 1-50 individuals were found in 85.4% of the samples 51-250 individuals were detected in 14.6% of the samples (Table 5).

There are many factors such as initial population density, host plants, crop rotation, season and soil type that can affect the economic damage caused by *Globodera* spp. and *Meloidogyne* spp. (Greco et al., 1992; Greco, 1993; Potter & Olthof, 1993). The economic damage threshold levels of potato cyst nematodes are considered as 10 eggs/g soil (Phillips et al., 1991). In this study, all the samples which were infested with *G. rostochiensis* were determined above the economic damage levels for potato production. Damage thresholds have been established for several crops, where the average is about 0.5-2 juveniles/g of soil for *Meloidogyne* species. Also, it is reported that the economic damage threshold of *M. chitwoodi* is 1 juvenile/250$cm^3$ of soil (Brodie et al., 1993). Although *M. chitwoodi* detected in this study was above the economic damage threshold, it should be noted that this species was found only in one sample. Considering the population densities of the 41 infested samples, only three populations (*M. chitwoodi*, 1 population; *M. incognita*, 2 populations) were found above the specified threshold levels.

Table 5. Rate of nematode detections in a survey in the potato production areas in İzmir in 2015 by population density (%)

| The presence of nematodes according to the number of individuals (%) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Nematode species | Positive samples | Proportion positive samples by genera (%)* | Total availability rate (%) | Population density (individuals/100 $cm^3$ soil) (%) | | | |
| | | | | 1-50 | 51-250 | 251-500 | ≥ 500 |
| *Globodera rostochiensis* | 223 | 32 | 100.00 | 37.50 | 46.88 | 15.62 | - |
| *Meloidogyne incognita* | 223 | 25 | 60.97 | 80.00 | 20.00 | - | - |
| *Meloidogyne javanica* | 223 | 10 | 24.39 | 100.00 | - | - | - |
| *Meloidogyne hapla* | 223 | 5 | 12.20 | 100.00 | - | - | - |
| *Meloidogyne chitwoodi* | 223 | 1 | 2.44 | - | 100.00 | | |

* 32 and 41 positive samples for *Globodera* spp. and *Meloidogyne* spp., respectively, of a total of 223 samples.

The main factors affecting the degree of damage caused by nematodes on potato tubers include the population density of the existing species and the duration of the plant in the soil. During planting time, if there is a high *Globodera* spp. or *Meloidogyne* spp. population present in the soil, these plants are affected more quickly (Lima et al., 2018).

The importance of these nematode species varies depending on their adaptation to the geographical region (local climate) and host plant species. Although nematode species have their own biology and behavior, it is often difficult to control or eliminate when they infect an area. Also, morphological similarities make it difficult to identify the nematodes.

Reliable identification of *Globodera* spp. and *Meloidogyne* spp. and determination of the population densities in potato cultivation areas are crucial to selection of effective control methods and to apply quarantine regulations (Lima et al., 2018). The differences between temperate and tropical *Meloidogyne* spp. and their distribution in Turkey indicate that different strategies are needed in various geographical regions.

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*Original article (Orijinal araştırma)*

# Identification and genetic diversity of the Mediterranean cereal cyst nematode, *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae) in cereal production areas of Northern Cyprus

Kuzey Kıbrıs arpa ve buğday üretim alanlarında Akdeniz tahıl kist nematodu, *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae)'un tanımlanması ve genetik çeşitliliği

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## Abstract

The Mediterranean cereal cyst nematode, *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae) is a destructive plant-parasitic nematode on cereal crops in particularly wheat and barley. It has a global distribution with a severe negative impact on yield quantity. In this study, a survey was conducted to identify plant-parasitic nematodes in cereal-growing areas in Cyprus. Forty-five samples including roots and soil from the root zone of plants were collected from cereal fields located in Gazimağusa, Girne, Güzelyurt and Lefkoşa Provinces before crop harvesting from late-May and early-June in 2017. Cyst-forming nematodes were determined by Fenwick's flotation and decanting techniques from 37 soil samples (82%). The internal transcribed spacer (ITS) regions of the ribosomal DNA of isolates were amplified and sequenced and subjected to a BLASTn search of the NCBI database for species identification, and the analyses showed that all samples were identified as *H. latipons*. Phylogenetic analyses based on ITS sequences revealed that *H. latipons* isolates from Northern Cyprus were closely related to isolates obtained from Morocco, Russia, Syria and Turkey. Data of this study demonstrated for the first time the presence of *H. latipons* in the cereal fields of Gazimağusa, Girne, Güzelyurt and Lefkoşa Provinces, where the nematode most likely causes serious economic problems in the cereal production. These results were the most up-to-dated analyses on the occurrence of *H. latipons* in cereal fields of Northern Cyprus and provided basic data for breeding programs to improve the resistant levels in the local cultivars.

**Keywords:** *Heterodera latipons*, heterogeneity, ITS, phylogeny

## Öz

Akdeniz tahıl kist nematodu, *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae) özellikle buğday ve arpada olmak üzere tahıllarda önemli zararlara neden olan bir bitki paraziti nematod olup, dünyada tahıl yetiştirilen alanların büyük çoğunluğunda tespit edilmiştir. Bu çalışmada, Kıbrıs'ın tahıl alanlarındaki patojen nematodları belirlemek için bir sürvey yapılmıştır. Gazimağusa, Girne, Güzelyurt ve Lefkoşa tahıl üretim alanlarından toplam 45 adet toprak ve kök örneği 2017 yılının mayıs ayı sonu-haziran ayı başı arasında tahıl hasadı öncesinde alınmıştır. Toprak ve kök örneklerinden Fenwick yöntemii kullanılarak kistler toplanılmış, 37 adet örnekte (%82) kiste rastlanılmış olup, tüm örnekler ribozomal DNA'nın ITS bölgesi kullanılarak, tahıl kist nematodu *H. latipons* moleküler düzeyde tanımlanarak belirlenmiştir. Çalışmada Kuzey Kıbrıs arpa ve buğday alanlarından elde edilen *H. latipons* örneklerine ait ITS bölgesi sekans dizilerinin Fas, Rusya, Suriye ve Türkiye'den elde edilen izolatlara oldukça benzerlik gösterdiği tespit edilmiştir. Kuzey Kıbrıs'ın Gazimağusa, Girne, Güzelyurt ve Lefkoşa illeri tahıl alanlarında *H. latipons*'un ilk olarak tespit edildiği bu çalışma, nematodun yaygınlığını ortaya koyan ve zararlının mücadelesinde başta dayanıklı çeşit kullanımı olmak üzere diğer mücadele yöntemlerinin uygulanmasında temel oluşturacak verileri içermektedir.

**Anahtar sözcükler:** *Heterodera latipons*, heterojenite, ITS, filogenetik

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## Introduction

Plant-parasitic nematodes are considered economically important biotic stress factors that cause severe damage to the global cereal production system. These nematodes are responsible for an annual global loss of $125 billion (Chitwood, 2003). The foliar symptoms associated with plant-parasitic nematodes infestation are similar to those caused by other soil borne pathogens, therefore, losses caused by plant-parasitic nematodes can be overlooked. Cyst-forming nematodes (*Heterodera* spp.) form a highly specialized group infesting cereal crops and causing economic damage to their production (Greco et al., 2002). *Heterodera* spp. are obligate sedentary endoparasites, and their life cycles depend on the invasion of root tissues of susceptible hosts. The feeding structures stimulated by the nematodes are called syncytia and provide a constant source of food for them to become reproductive females (Kyndt et al., 2013). Mediterranean cereal cyst nematode is a species that is occurs in Cyprus, Israel, Italy, Lebanon, Libya, Syria, Tunisia, and Turkey (Franklin, 1969; Saxena et al., 1988).Yellowing of cereal stands, ranging from mild to severe, have been observed in the early stage of *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae) infestation, while the infested fields show patchy plant growth due to poor tillering and shorter spikes in the affected area in the later stages (Dababat & Fourie, 2018). The nematode population generally expands from the initial location increasing the number of affected plants, which results in enlargement of patches. These symptoms may be combined with other biotic or abiotic stresses that increase disease severity. Before the flowering stage, white and lemon-shaped females can be easily seen on the infested roots (Greco et al., 2002). Infested plants also tend to wilt during the warmer parts of the day (McDonald & Nicol, 2005).

More than 60 species have been described in the genus *Heterodera*, which has been extensively studied, especially those associated with cereals and grasses crops. Although many *Heterodera* species infest cereals, the most prevalent species is *Heterodera avenae* Wollenweber, 1924 which is considered a complex (*H. avenae* group) containing *H. avenae*; *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984; *Heterodera arenaria* Cooper, 1955, *Heterodera pratensis* Gäbler, Sturhan, Subbotin & Rumpenhorst, 2000, *Heterodera aucklandica* Wouts & Sturhan, 1995, *Heterodera mani* Mathews, 1971, *Heterodera ustinovi* Kirjanova, 1969 and *Heterodera australis* Subbotin, Sturhan, Rumpenhorst & Moens, 2002 species (Wouts & Sturhan, 1995; Gabler et al., 2000; Sturhan & Krall, 2002; Subbotin et al., 2002). *Heterodera latipons* is considered to form a distinct species complex within the *H. avenae* group, which discriminated by molecular analyses (Figure 1) (Subbotin et al., 2003).

*Heterodera latipons*, known as the Mediterranean cereal cyst nematode, has been identified in Asia and Europe, this species, however, is predominantly distributed in the countries in the Mediterranean basin (Abidou et al., 2005; Smiley & Nicol, 2009). *Heterodera latipons* has been noted to attack several species such as; oat in Israel (Cohn & Ausher, 1973), barley in Libya (Franklin, 1969), wheat in China (Peng et al., 2007), barley and wheat in Turkey (Rumpenhorst et al., 1996; Imren et al., 2012) and Morocco (Mokrini et al., 2017). The Mediterranean cereal cyst nematode as well as the lesion nematode, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae*)* have been determined in Cyprus (Philis, 1988b, 1995) and are assumed to be the most damaging plant-parasitic nematode species on barley and wheat. Also, Sikora (1988) reported that the Mediterranean cereal cyst nematode could be one of the most important constraints on cereal production in the temperate semiarid regions, such as Cyprus. Significant yield loss in cereal production by this nematode has been also reported by Philis (1988a) in Cyprus. However, there was limited information on the distribution and genetic structure of the Mediterranean cereal cyst nematode in the cereal fields in the northern part of the island.

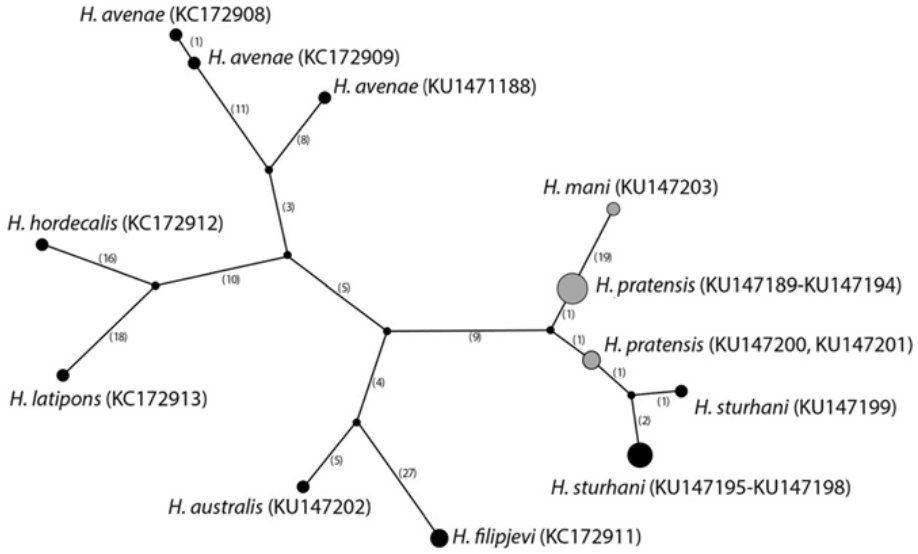


Figure 1. The phylogenetic relationship among COI haplotypes of *Heterodera avenae* group species. Small black circles represent missing haplotypes (Subbotin et al., 2003) (A number of changes are indicated in brackets).

Characterization of the cyst nematode populations molecularly at intra- or interspecies level can be vital information for the selection of appropriate and efficient management strategies (Ganguly & Rao, 2003). The increasing number of species in *H. avenae* complex has resulted in difficulties in morphological and morphometric identification and requires specialized taxonomists (Subbotin et al., 2003). Due to the minor morphological and morphometric differences within this genera complex; sufficient criteria to discriminate the species from each other would not be applicable (Subbotin et al., 1999). Therefore, molecular diagnostic techniques have provided clues to solve taxonomic problems associated with the conventional species identification (Szalanski et al., 1997; Al-Banna et al., 2004; Subbotin et al., 2010). The internal transcribed spacer (ITS) region of ribosomal DNA exhibits considerably high variations among nematode populations and is commonly used to identify species and reveal phylogenetic relationship among nematode populations at species level (Subbotin et al., 2003; Madani et al., 2004; Smiley et al., 2008). Philis (1988a) examined the morphological and morphometric characteristics of *H. latipons* obtained from Cyprus. No previous information on the genetic structure about the Mediterranean cereal cyst nematode in Cyprus is available. To fill the informative gap which is necessary to understand population structures of the Mediterranean cereal cyst nematode, surveys were conducted in the main cereal-growing areas of Cyprus. The main objectives of this study were to (a) identify nematode species using molecular tools based on their ITS sequences and (b) determine phylogenetic relationships among the nematode populations obtained in this study and representative isolates from the other countries.

## Materials and Methods

### Nematode populations

A comprehensive survey was carried out in 2017 for the identification, distribution, and estimation of genetic variation of *H. latipons*, from Northern Cyprus. Forty-five samples including soil and roots of plant were collected from wheat and barley fields located in Gazimağusa, Girne, Güzelyurt and Lefkoşa Provinces before the harvesting time, between the end of May and the beginning of June 2017 (Table 1). Cysts were extracted from soil and roots by flotation and decanting techniques (Fenwick, 1940). Extracted cysts were firstly classified to genus level under a V20 stereomicroscope (Zeiss, Jena, Germany). At least 20 full cysts were selected and handpicked with a needle from each sample and stored at 4°C to be used in the molecular analysis.

Table 1. List of samples with their geographical locations and *Heterodera* species identified from this study

| No | Province | Location | Crop | Cyst infestation | *Heterodera* species | Accession Nos. |
|---|---|---|---|---|---|---|
| 1 | Gazimağusa | Çayönü-I | Barley | Absent | - | |
| 2 | | Çayönü-II | Barley | Present | *H. latipons* | MK431040 |
| 3 | | Merkez | Barley | Present | *H. latipons* | MN621871 |
| 4 | | Türkmenköy | Barley | Absent | - | |
| 5 | | Yıldırımköy | Barley | Absent | - | |
| 6 | | Gelincik Iskele-I | Barley | Present | *H. latipons* | MK431035 |
| 7 | | Gelincik Iskele-II | Barley | Absent | - | |
| 8 | | Atlılar | Barley | Absent | - | |
| 9 | | Derince-I (Iskele) | Barley | Present | *H. latipons* | MK431036 |
| 10 | | Derince-II | Barley | Present | *H. latipons* | MK431037 |
| 11 | | Derince-III | Wheat | Present | *H. latipons* | MK431038 |
| 12 | | Birşen Iskele | Barley | Present | *H. latipons* | MN621872 |
| 13 | | Çetereisi | Barley | Present | *H. latipons* | MN621877 |
| 14 | | Gelincik | Barley | Present | *H. latipons* | MN621878 |
| 15 | | Tarfo | Barley | Present | *H. latipons* | MN621880 |
| 16 | | Yeşilköy-I | Barley | Present | *H. latipons* | MK431027 |
| 17 | | Yeşilköy-II | Wheat | Present | *H. latipons* | MN621870 |
| 18 | | Yeşilköy-III | Barley | Absent | - | |
| 19 | | İncirlik | Barley | Present | *H. latipons* | MK431032 |
| 20 | | Kumyalı | Barley | Present | *H. latipons* | MN621875 |
| 21 | | Çayırova | Barley | Present | *H. latipons* | MN621876 |
| 22 | | Kurtuluş | Barley | Present | *H. latipons* | MN621879 |
| 23 | | Beyarmudu-I | Barley | Present | *H. latipons* | MN621881 |
| 24 | | Beyarmudu-II | Barley | Present | *H. latipons* | MN621882 |
| 25 | | Çanakkale Mah. | Barley | Present | - | |
| 26 | | Tatlısu | Barley | Present | *H. latipons* | MK431039 |
| 27 | Güzelyurt | Güneşköy | Barley | Present | *H. latipons* | MK431029 |
| 28 | | Meteoroloji Station | Barley | Absent | - | |
| 29 | | Taşpınar-I | Barley | Present | *H. latipons* | MK431030 |
| 30 | | Taşpınar-II | Wheat | Present | *H. latipons* | MK431024 |
| 31 | | University | Barley | Present | *H. latipons* | MK431025 |
| 32 | | Tepebaşı-I | Barley | Present | *H. latipons* | MN621868 |
| 33 | | Tepebaşı-II | Barley | Present | *H. latipons* | MN621869 |
| 34 | | Gazievren | Barley | Present | *H. latipons* | MN621873 |
| 35 | | Zümrütköy | Barley | Present | *H. latipons* | MN621874 |
| 36 | | Aydınköy | Barley | Present | *H. latipons* | MN621883 |
| 37 | | Bostancı-I | Barley | Present | *H. latipons* | MN621884 |
| 38 | | Bostancı-II | Barley | Present | *H. latipons* | MN621885 |
| 39 | | Doğancı | Barley | Present | *H. latipons* | MN621886 |
| 40 | | Ergazi İskele | Barley | Present | *H. latipons* | MN621887 |
| 41 | Lefkoşa | Yılmazköy | Barley | Present | *H. latipons* | MK431026 |
| 42 | | Clup Mexico | Barley | Present | *H. latipons* | MK431031 |
| 43 | | Mehmetçik Iskele | Wheat | Present | *H. latipons* | MK431034 |
| 44 | Girne | Kormacit | Barley | Present | *H. latipons* | MK431028 |
| 45 | | Çamlıköy | Wheat | Present | *H. latipons* | MK431033 |

**Molecular identification**

The genomic DNA was extracted from a single mature cyst following the method described by Subbotin et al. (2001). The cyst was crushed, and the juveniles (J2s) were moved into 10 µl of double-distilled water. The J2s were homogenized via a micro-homogenizer, then the entire suspension was put into a 1.5 ml Eppendorf tube. A 10 µl of 1xPCR reaction mix [75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20] and 2 µl of proteinase K (600 µg/ml; Qiagen GmbH, Hilden, Germany) were added to the lysate. The tube was exposed to incubation at 60°C for 30 min, and a further 5 min at 95°C to dispose of proteinase K. The tube was centrifuged at 16,000 rpm. The supernatant was carefully removed without disturbing the pellet, transferred into another Eppendorf tube, and stored at -20°C until further use.

PCR amplification to produce the ITS fragments of the isolates, including the 5.8S ribosomal gene as well as parts of the 18S and 28S genes, was performed with AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') and TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') primers using a T100 thermal cycler (Bio-Rad, Hercules, CA, USA) (Subbotin et al., 2001). PCR reactions were conducted in a 50 µl reaction mixture containing 5 µl 10× PCR reaction buffer, 0.4 µM of each primer, 2 µl template DNA, 200 µM of each dNTPs, and 1.25-unit *Taq* DNA polymerase (New England BioLabs, Ipswich, MA, USA). The thermal cycler program for amplifying the ITS region was: 3 min for an initial denaturation step at 94°C, followed by 35 cycles with 1 min denaturation at 94°C, 1 min annealing at 55°C, and 2 min extension at 72°C and a 10 min final extension at 72°C. Negative control (no DNA template) was used to ensure that there was no contamination in the reaction mix. The amplification products were evaluated on 1.5% agarose gel (100 V; 60 min) using a G: BOX F3 gel doc system (Syngene, Cambridge, UK) after ethidium bromide staining.

The PCR products were cut and eluted from the gel and purified using a QIAquick PCR purification kit (cat no 28106; Qiagen GmbH, Hilden, Germany). The purified products were subjected to bidirectional sequencing by a commercial company (Macrogen Inc., Seoul, Korea). The sequences were aligned with Clustal W (Thompson et al., 1994), which was a multiple sequence alignment method and then identified using the BLASTn algorithm on the NCBI website (NCBI, 2019). The sequences derived from this study were deposited into the GenBank database with the accession numbers shown in Table 1.

**Phylogenetic analysis**

The ITS sequences of the isolates were used to reveal intraspecific genomic variability of *H. latipons* populations and to determine phylogenetic relationships among themselves and the sequences of representative *H. latipons* isolates from different countries available in the GenBank database. A total of 43 nucleotide sequences was involved in the analysis. The evolutionary history was inferred using the neighbor-joining method, based on evolutionary distances computed using the Tamura-Nei method (Tamura & Nei, 1993). Gaps were treated as missing data. One sequence belonging to *Heterodera schachtii* Schmidt, 1871 isolate (accession AY166438) obtained from Belgium was used as an outgroup to root the trees and to characterize polarization. Bootstrap support was calculated for all analyses using 1000 replicates.

## Results and Discussion

*Heterodera latipons* is one of the damaging biotic stresses reported to cause yield loss in wheat production systems around the world (Dababat & Fourie, 2018). In this study, the Mediterranean cereal cyst nematode was determined in 37 fields out of 45 cereal fields (82%) surveyed in cereal-growing areas of Northern Cyprus (Table 1). In Cyprus, the Mediterranean cereal cyst nematode was first identified by Philis (1988a) and is considered as the most destructive plant-parasitic nematode species on cereals. *Pratylenchus thornei*, root lesion nematode, was determined to often co-occurred with *H. latipons* in the same area, which can also cause substantial yield losses of barley and wheat in Cyprus (Philis, 1988a, 1995).


Figure 2. Phylogenetic tree (neighbor-joining) constructed based on the ITS sequence alignment from the 37 populations of *Heterodera latipons* including their accession numbers and strain numbers. Bootstrap values are given for the appropriate clades.

This study demonstrated that surveyed cereal-growing areas were predominantly infested with the Mediterranean cereal cyst nematode, which complies with reports that found a relatively high density of the Mediterranean cereal cyst nematode in the samples obtained from cereal fields by Imren et al. (2018) and Mokrini et al. (2017) in Turkey and Morocco, respectively.

The phylogenetic relationship among the cyst nematode populations was compared to international genotypes (Figure 2). The phylogenetic tree generated from 1000 bootstrapped sequence alignments were subjected to global rearrangement with random replications. *H. latipons* populations showed intraspecific polymorphism and were clustered into two distinct groups. The first group comprised *H. latipons* populations from Gazimagusa, Girne, Güzelyurt and Lefkosa Provinces and the second group consisted

of only isolates from Gazimagusa. Generally, the phylogenetic analyses showed that *H. latipons* isolates from Northern Cyprus were closely related to the Moroccan, Syrian and Turkish isolates. Also, Northern Cyprus cyst samples were grouped with Iran and Russia populations of *H. latipons.*

The sequence analysis of the ITS regions was frequently used for discrimination of nematode species, as well as the species of *Heterodera* genus (Subbotin et al., 2003; Baklawa et al., 2015; Imren et al., 2015; Mokrini et al., 2017). This was the first study where the intraspecific polymorphism based on the ITS sequences among *H. latipons* populations of Northern Cyprus was used. The results agree with many other studies revealing the existence of intraspecific polymorphism among *H. latipons* populations obtained from different countries (Subbotin et al*.,* 1999; Rivoal et al., 2003; Madani et al., 2004; Imren et al., 2015; Mokrini et al., 2017). Based on the results of this study, variation in nucleotide sequences observed among the nematode populations and were found to be close to Syrian and Turkish populations of *H. latipons* than Iranian and Russian populations. İmren et al. (2015) showed intraspecific differentiation between the Turkish *H. latipons* populations and the Moroccan and Syrian populations. Madani et al. (2004) and Rivoal et al. (2003) also showed intraspecific variations among populations of *H. latipons* using the PCR-RFLP method. Also, Mokrini et al. (2017) reported that *H. latipons* populations collected from different cereal-growing areas of Morocco were grouped into the same group with high similarity. In the present study, the phylogenetic tree clustered the populations of 37 distant sites at species level based on the ITS sequences as shown in Figure 2.

High prevalence of *H. latipons* was revealed in barley and wheat-growing areas of Northern Cyprus, confirming the observations that cyst nematodes species are the most important pathogens of barley, wheat and other cereals (Abidou et al., 2005; Sahin et al., 2010; Imren et al., 2012, 2015; Toktay et al., 2015). The density of these populations mostly exceeded or approached the threshold level for economic loss. CCN populations were reduced to levels below the economic damage threshold of 5 eggs/g soil under cereal monocultures (Gair et al., 1969; Dababat & Fourie, 2018). Further evaluations are necessary to determine the virulence of *H. latipons* populations to barley and wheat cultivars widely grown in Northern Cyprus and to identify the pathotypes of the *H. latipons* populations from Gazimağusa, Girne, Güzelyurt and Lefkoşa cereal-growing areas, as well as to include suitable resistance sources to cereal breeding programs. Appropriate and applicable management strategies, such as the use of resistant cultivars, fallowing and rotation with non-host crops, might be the most effective cultural methods to keep the nematode population densities below damaging levels in Cyprus. Also, several sources of resistance to cyst nematode have been identified in domestic cereals and have been recommended for inclusion in breeding programs (Dababat et al., 2015). The effectiveness of the use of resistant cultivars might not be guaranteed due to the formation of new pathotypes that show variation in the virulence, however, the cultivation of these cultivars, which have valuable traits in their genome provide resistance to populations of *H. latipons*, should be recommended.

Based on previous information, the presence of *H. latipons* in Northern Cyprus was first confirmed using molecular tools. These results demonstrated the strongest analysis to date on the existence and distribution of *H. latipons* in the main barley and wheat-producing areas of Northern Cypruss, serving a basis for more specific resistance breeding, as well as other management practices. In conclusion, an attempt was made to understand the diversity of cyst nematode species of Northern Cyprus. Additional studies are needed to explain the cyst nematode species in cereal cropping areas in Northern Cyprus.

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