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*Original article (Orijinal araştırma)*

# Persistence and insecticidal efficacy of a diatomaceous earth formulation, Inert-PMS, in stored wheat grain against *Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman, *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst)

Diatomlu bir toprak formülasyonu olan Inert-PMS'in, depolanmış buğday tanelerinde *Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman, *Rhyzopertha dominica* (F.) ve *Tribolium castaneum* (Herbst)'a karşı kalıcılığı ve insektisit etkinliği

**Misbah ASHRAF[1] Waqas WAKIL[2*] Faisal HAFEEZ[1] Muhammad FAROOQ[1]**

## Summary

Experiment was conducted to determine the persistence and insecticidal efficacy of a new enhanced diatomaceous earth, Inert-PMS, in wheat against four stored grain insect pests [*Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman, *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst)] under the laboratory conditions at 50, 75 and 100 mg/kg, for intervals of 24 h, 4 and 7 d at 28ºC and 65% RH. For persistence, Inert-PMS was applied to stored grain for 0, 30, 60, 90 and 120 d. The results demonstrated that adult mortality was directly proportional to the dose and exposure interval. While the efficacy remained constant up to 60 d, it fell after 90 and 120 d of storage. *L. paeta* and *C. ferrugineus* were the most susceptible to Inert-PMS (100% mortality) followed by *R. dominica* (81%) and *T. castaneum* (72%) at 75 mg/kg after 4 d. Inert-PMS also suppressed the reproduction at lower dose rates. Inert-PMS is an eco-friendly formulation that not only interferes with the growth and development of stored grain insects but is also cheap and free from ill effects.

**Keywords:** *Cryptolestes ferrugineus*, *Liposcelis paeta*, *Rhyzopertha dominica*, *Tribolium castaneum*, Inert-PMS, new enhanced diatomaceous earth

## Özet

Bu çalışma, yeni geliştirilmiş diatomlu bir toprak olan Inert-PMS'in dört farklı depolanmış buğday zararlısına [*Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman, *Rhyzopertha dominica* (F.) ve *Tribolium castaneum* (Herbst)] karşı kalıcılığı ve insektisit etkilerini belirlemek amacıyla, 24 saat, 4 ve 7 gün aralıklarla 50, 75 ve 100 mg/kg dozları uygulanarak 28ºC sıcaklık ve % 65 orantılı neme sahip laboratuvar koşullarında gerçekleştirilmiştir. Kalıcılığını belirlemek için, inert PMS'in etkisi 0, 30, 60, 90 ve 120 gün süresince depolanan buğdaylarda değerlendirilmiştir. Sonuçlar, ergin ölümünün doz ve maruz kalma aralığı ile doğru orantılı olduğunu göstermiştir. Etki, 60 günlük depolamada sabit devam ederken, 90 ve 120 günlük depolamada ise düşmüştür. 75 mg/kg uygulama dozunda 4 gün sonununda, *L. paeta* ve *C. ferrugineus* Inert-PMS'ye en hassas türler olmuş (%100 ölüm), bunu *R. dominica* (%81) ve *T. castaneum* (%72) izlemiştir Inert-PMS, ayrıca düşük dozlarda da üremeyi baskılamıştır. Çevre dostu bir formülasyon olan Inert-PMS, depolanmış buğday zararlılarında sadece büyüme ve gelişmeye müdahale etmekle kalmayıp, aynı zamanda ucuzdur ve olumsuz etkileri de yoktur.

**Anahtar sözcükler:** *Cryptolestes ferrugineus*, *Liposcelis paeta*, *Rhyzopertha dominica*, *Tribolium castaneum*, Inert-PMS, yeni geliştirilmiş diatomlu toprak

[1] Entomological Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan
[2] Department of Entomology, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan
[*] Corresponding author (Sorumlu yazar) e-mail: arid1972@yahoo.com
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## Introduction

Stored grain insect pests cause 5-15% damage to stored oilseeds, cereals and pulses (Padin et al., 2002). These insects cause both quantitative and qualitative losses to stored commodities (Bello et al., 2001; Michalaki et al., 2007). Among them, the lesser grain borer, *Rhyzopertha dominica* (F.)*,* is most destructive internal feeders of stored grains. Its female lays eggs outside the grain. After hatching, the new larvae bore into the grain and complete their life cycle within the grain (Arbogast, 1991). Likewise, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens)*,* feeds directly on the grain germ, increases mold growth and excretes hydroxyquinone compounds that contaminate and damage to the grain (Assie et al., 2007). Also, the red flour beetle, *Tribolium castaneum* (Herbst), regarded as a secondary pest, feeds on whole cereal grains (Aitken, 1975), and the tropical psocid, *Liposcelis paeta* Pearman, commonly (Rajendran, 1994) reaches damaging population densities under favorable conditions (McFarlane, 1982; Turner, 1994).

All these insects are associated with stored grain, including household grain storage through to large commercial facilities. The economic threshold for these insects under storage conditions is zero (Flinn et al., 2007). So measures for control of stored grain insects must be applied at the time storage. Since 1950, insecticide application has been the most common control practice in Pakistan (Subramanyam & Hagstrum, 1995). However, the efficacy and effectiveness has declined due to resistant insect populations, increased costs, pesticide residues in animal and human food, and environmental pollution. These problems compelled pest management specialists to search alternatives, such as diatomaceous earth (DE), that are more specific and free from all ill effects (Lorini & Galley, 1999, 2000). DE has become more important in chemical control over the last decade and is now regarded as key component in integrated pest management (IPM) for stored grains (Korunic, 1999). DE is a soft rock composed of fossilized residues of unicellular algae called diatoms. According to geological sources; it is pure amorphous silicon dioxide and nontoxic to mammals (IARC, 1997). DE absorbs cuticular waxes of the insects, causing death by to desiccation (Rigaux et al., 2001) and it also abrades the cuticles of insects (Ebeling, 1971).

The efficacy of DE against stored grain pests is dependent on the commodity, the insect species, temperature and grain moisture content (Fields & Muir, 1995; Fields & Korunic, 2000). The researchers working in different parts of the world have found that variation in DE formulation greatly effects its efficacy, such as for Insecto and SilicoSec (Vayias & Athanassiou, 2004; Kavallieratos et al., 2005). Optimizing the DE application rate is as important factor in this context (Jackson & Webley, 1994; Korunic et al., 1996; Korunic & Ormesher, 1996; Korunic, 1997). Also, promising and persuasive results have been obtained when plant-based mixtures in conjunction with DE were applied to stored grain insects (Athanassiou et al*.,* 2008).

The present study was designed to assess the persistence and efficacy of a new enhanced formulation of DE, Inert-PMS, as surface treatment in stored wheat grain, by measuring its effect on reproduction of *C. ferrugineus*, *L. paeta, R. dominica* and *T. castaneum*.

## Materials and Methods

### Grains

Untreated, clean, pest-free soft wheat was used. The grain moisture contents was 10.5-11.5% measured by mini Gac Plus (Dickey-John Crop., Auburn, IL, USA) grain moisture meter (Athanassiou & Kavallieratos, 2005).

### Rearing of insect pests

The four most important insect pests of stored grain, *C. ferrugineus*, *L. paeta, R. dominica* and *T. castaneum,* were obtained from infested wheat samples and populations were established and maintained at the stored grain laboratory, University of Agriculture, Faisalabad, Pakistan. The insect populations were reared on clean, pest-free wheat in plastic jars. Beetle cultures were maintained at 25-28ºC and 65±5% relative humidity (Kavallieratos et al., 2012), and the psocid population at 30ºC and 75±5% relative humidity (Opit & Throne, 2008).

### Diatomaceous earth formulation and bioassay

The DE formulation used, Inert-PMS (Biofa GmbH Münsingen, Germany), is a new enhanced formulation of DE containing of the insecticides, spinosad and pirimiphos-methyl. Test was conducted with dosage rates of 0, 50, 75 and 100 mg/kg. One kg of wheat was prepared for each treatment and placed in cylindrical jars. Inert-PMS was mixed with the grain and 50 g of grain transferred to vials. Thirty mixed-sex adults of *C. ferrugineus*, *L. paeta, R. dominica* and *T. castaneum* were introduced into separate vials. Each treatment was replicated nine times in a completely randomized design, with untreated vials as a control. The jars were incubated at 28ºC and 65% RH. The humidity was maintained in the incubators by using saturated solutions of sodium chloride (Greenspan, 1977). The number of dead and live adults was counted after 24 h, 4 d and 7 d, and progeny data was also assessed. The residual bioassays of Inert-PMS were conducted after 0, 30, 60, 90 and 120 d exposure by the procedure described above. After 7 d, all adults (dead and alive) were removed from the vials and the vials retained for an additional period of 63 d for the beetles and 30 d for psocid under the same conditions. Finally, the vials were opened and the number of adult progeny counted.

### Data analysis

Control mortality was corrected by using the Abbott's formula (Abbot, 1925). The data were analyzed by two-way ANOVA, using the GLM procedure of MINITAB 13.2 (Minitab 2002 Software Inc., Northampton, MA, USA). The means were separated by Tukey-Kramer HSD test at 5% significant level (Sokal & Rohlf, 1995).

## Results

### *Cryptolestes ferrugineus*

Similar mortality trend was obtained when *C. ferrugineus* were exposed to different dose rates and exposure intervals. However, higher mean mortality (94.1%) was obtained at 120 d at 100 mg/kg (Figure 1). Likewise, mortality decreased after 60 d, resulting in negative impact on progeny production of *C. ferrugineus*. The mean number of progeny from treated grain was significantly lower (0.33) at 100 mg Inert-PMS/kg (Figure 5) in the initial bioassay.

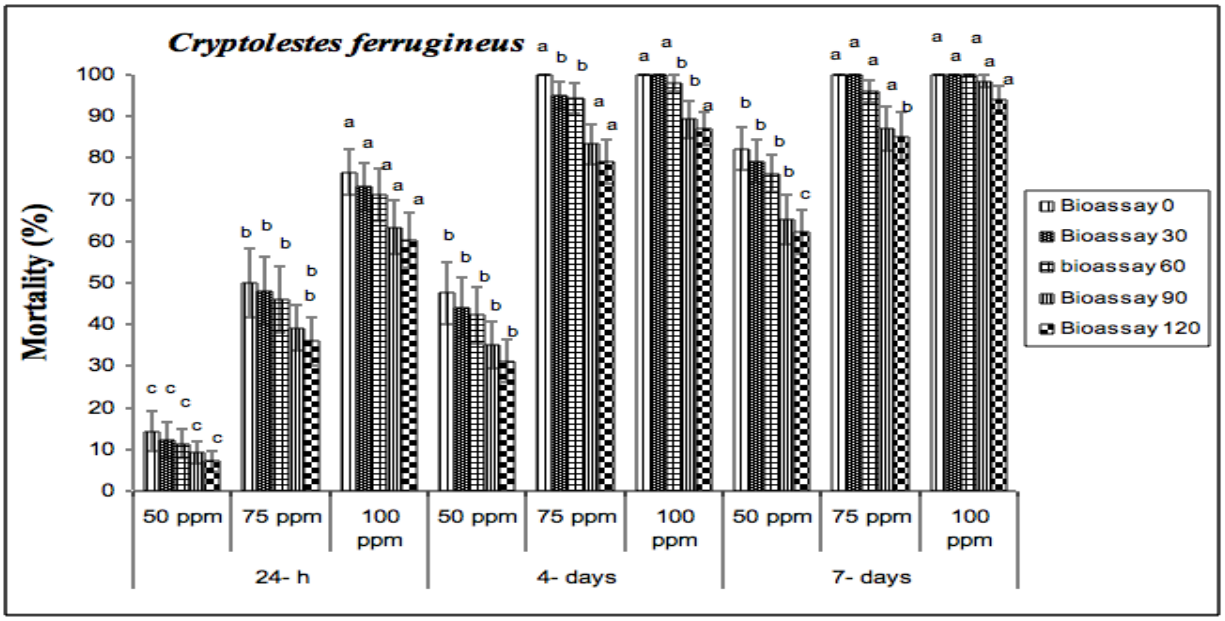


Figure 1. Mean mortality (% ± SE) of *Cryptolestes ferrugineus,* exposed for 24 h in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by the same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

### *Liposcelis paeta*

Mortality of *L. paeta* increased with increase of exposure interval and dose rate. The lowest mortality rate (10.2%) occurred at 50 mg Inert-PMS/kg after 24 h following 120 d of storage. Increasing the exposure interval from 24 h to 4 d and 7 d, increased adult mortality to 44.0 and 71.4% (Figure 2). A similar mortality occurred until 60 d, but mortality decreased rapidly after 60 d. Moreover, progeny production after the exposure to Inert-PMS was generally low and decreased with the increasing dose rate. For the initial bioassay, mean number of progeny was 0.41 at 50 mg/kg and 0.25 at 100 mg/kg. However, mean number of progeny from the untreated stored grains were always higher than from treated ones (Figure 5).

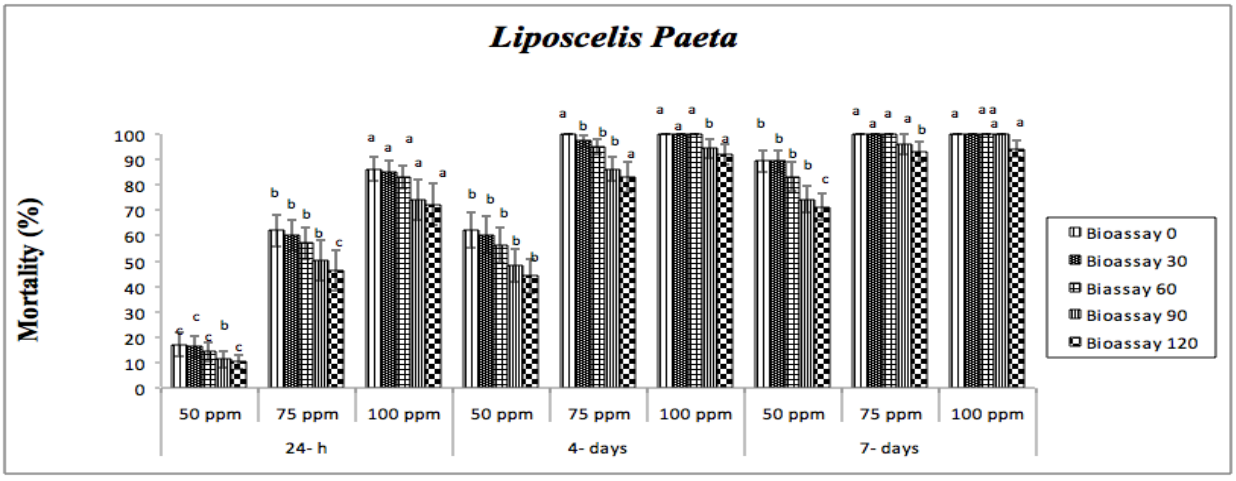


Figure 2. Mean mortality (% ± SE) of *Liposcelis paeta*, exposed for 24 h in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

### *Rhyzopertha dominica*

Inert-PMS gave 12.3% mortality at 0 d storage, after 24 h, followed by 39.1 and 72.2% after 4 d and 7 d, respectively (Figure 3), at the same dose rate and storage. However, progeny production for *R. dominica* was adversely affected by the Inert-PMS dose rate. Newly emerged young progeny had mostly died in treated grains; progeny number was 0.41 at 100 mg Inert-PMS/kg (Figure 5) in the initial bioassay.

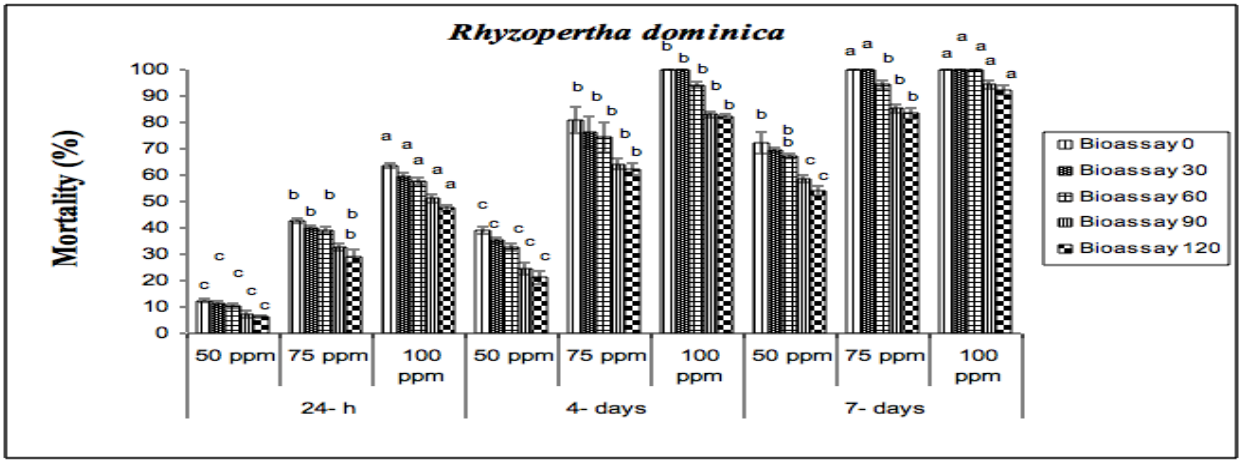


Figure 3. Mean mortality (% ± SE) of *Rhyzopertha dominica* exposed for 4 d in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by the same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

***Tribolium castaneum***

Mortality of *T. castaneum* increased with the exposure intervals and dose rate. Minimum mortality (4.90%) occurred at 50 mg/kg after 24 h following 120 d of storage. However, increasing the exposure interval, increased the adult mortality to 38.1 and 46.2% after 4 d and 7 d, respectively (Figure 4), at 50 mg/kg. A similar level of the mortality occurred for 60 d storage, but mortality levels decreased after 60 da. Progeny emergence (Figure 5) after exposure of this beetle was generally low (0.83 mean numbers of emerged adults) at 50 mg/kg and decreased with increase of dose (0.58 mean numbers of emerged adults) at 100 mg/kg in the initial bioassay.

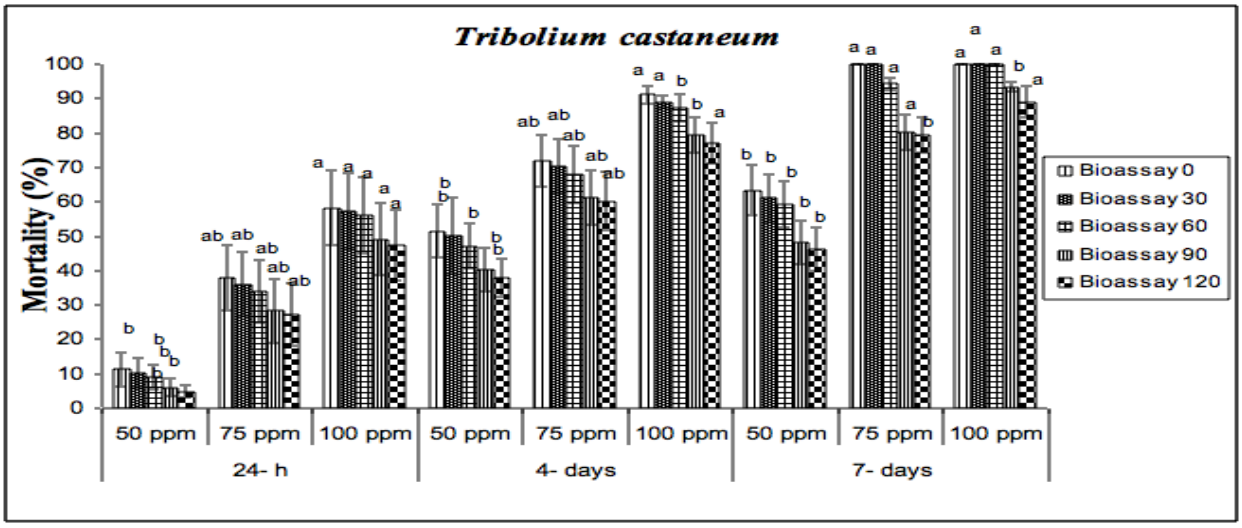


Figure 4. Mean mortality (% ± SE) of *Tribolium castaneum* exposed for 7 d in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by the same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

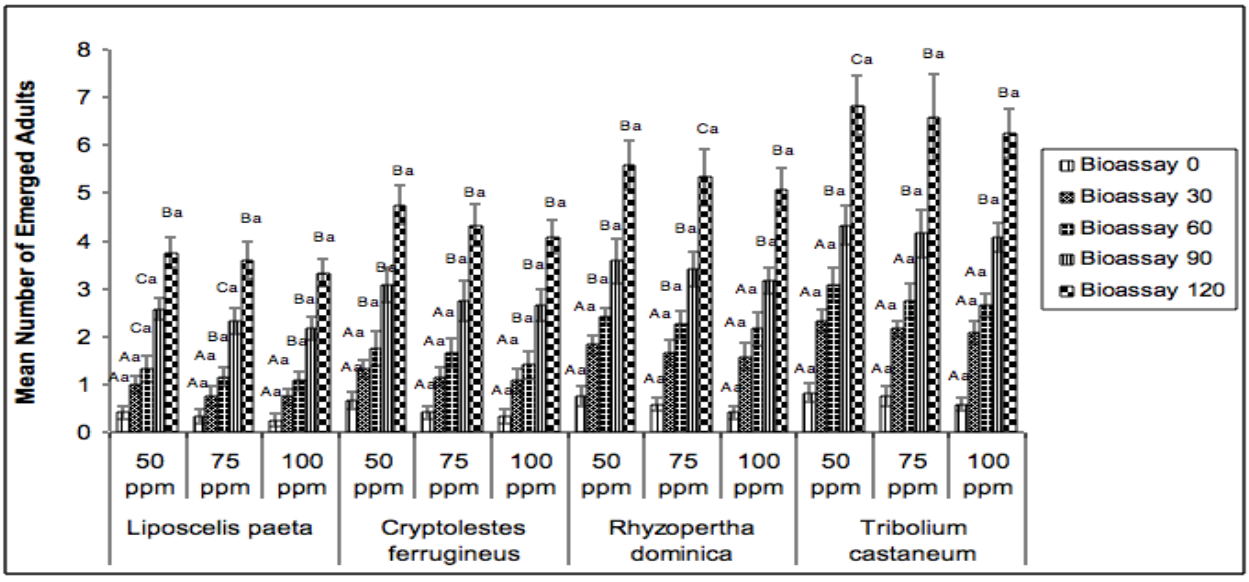


Figure 5. Mean number of emerged adults (±SE) of beetles, *Liposcelis paeta*, *Cryptolestes ferrugineus and Rhyzopertha dominica,* 63 d after the removal of adult insects from grain treated with Inert-PMS at three doses, and a psocid, *Tribolium castaneum,* after 30 d after the removal of adult insects. Means followed by the same lowercase letters (within each dose) and uppercase letters (within each bioassay) are not significantly different from each other as indicated by the HSD test at 0.05%.

## Discussion

Earlier studies have shown that stored grain insect pests can be controlled by commercially available DE formulations. For example, Kavallieratos et al. (2005) used DEs Insecto and SilicoSec on eight different grain commodities, at 750 mg/kg and recorded mortality after 14 d of exposure ranging from 63 to 97%. Similarly, Vardeman et al. (2006) reported that Protect-It at the same exposure found that 400 mg/kg gave 85% adult mortality for *R. dominica*. However, our findings are consistent with the results reported in other studies (Fields & Korunic, 2000; Athanassiou & Kavallieratos, 2005). In addition to this, the dose rates used in the above studies were considerably higher. Korunic et al. (1998) noted that 500 mg DE/kg caused an 8% reduction in the bulk density of grain, when it was mixed with the whole grain mass. DE may be useful as a surface treatment to reduce the contact upon grain bulk density (Subramanyam et al., 1994). Our results clearly revealed that Inert-PMS can be used at doses that are significantly lower that older DEs, which in some cases, would be a reduction to 50 mg/kg. The increased efficacy of Inert-PMS can be attributed to the presence of the chemicals, spinosad and pirimiphos-methyl, included in its formulation. Our results indicated that Inert-PMS applied to stored grain is most effective against *L. paeta,* followed by *C. ferrugineus*, *R. dominica* and *T. castaneum*.

Athanassiou et al. (2006) reported 100% mortality of the exposed *R. dominica* adults after 14 d in wheat with 75 mg DEBBM (a diatomaceous earth formulation enhanced with bitterbarkomycin)/kg used as powder. However, our study indicated that Inert-PMS gave 100% mortality at 75 mg/kg after 4 d against all insects tested.

From our findings, Inert-PMS can be used successfully in stored wheat against stored grain insect pests, but its effectiveness could be influenced by several factors, such as the type of commodity, the application rate and the exposure interval. Treatment or wheat grain gave promising results, with the DE tested persisted for 60 d. It appears that the decline in persistence occurs gradually. This decline in persistence might be due to environmental conditions during the experiment as well as to kernel oil absorption by DE particles. According to Subramanyam & Roesli (2000), DEs provide safeguard to the grain as long as they remain dry and DEs are generally less effective under humid conditions (Arthur, 2000). For instance, Vayias & Athanassiou (2004) reported that SilicoSec was more effective against *Tribolium confusum* Jacquelin du Val adults and larvae at 55% than at 65% RH. Insects can moderate water loss under humid conditions, and this indirectly reduces DE efficacy (Subramanyam & Roesli, 2000; Mewis & Ulrichs, 2001).

In our study, Inert-PMS was effective in wheat for all insect species tested. Mortality was low at short exposure intervals (24 h) and increased with longer exposure. This shows that the chance of insects coming into contact with DE particles is improved with increasing exposure time leading to increased efficacy (Athanassiou et al., 2003, 2005). Our study shows that Inert-PMS is effective and could offer long-term protection of stored grain against the pest species tested.

DEs are generally slower acting than other grain protectants (Golob, 1997; Korunic, 1998), which may permit adults of insects to oviposit before dying and persist to cause grain damage (Subramanyam & Roesli, 2000). Hence, even if 100% parental mortality occurs, progeny emergence needs to be prevented due to have a zero threshold level for this pest (Athanassiou et al., 2003; Vardeman et al., 2006). With Inert-PMS parental mortality was high even after 4 d of exposure and this resulted in reduced progeny production. Furthermore, we observed that most of the individuals exposed to Inert-PMS during the first exposure interval had little or no activity.

In summary, Inert-PMS is effective even at low dose rates and potentially could become a vital element in an IPM-compatible strategy. However, there are numerous DEs that contain low to high concentrations of insecticides that cause both progeny suppression and adult mortality (Athanassiou et al*.,* 2004, 2006). Additional experimental work is needed to determine if Inert-PMS and other DE formulations are useful for on-farm insect management.

## References

Abbot, W. S., 1925. A method of computing effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Aitken, A. D., 1975. Insect travellers, Coleoptera, Technical Bulletin 31, HMSO, London.

Arbogast, R. T., 1991. Beetles: Coleoptera. Ecology and Management of Food Industry Pests. FDA Technical Bulletin 4, Association of Official Analytical Chemist, Arlington, VA, pp. 131–176.

Arthur, F. H., 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) effects of temperature and relative humidity. Journal of Economic Entomology, 93: 526–532.

Assie, K. L., F. Francis, N. Gengler & E. Haubruge, 2007. Response and genetic analysis of malathion-specific resistant *Tribolium castaneum* (Herbst) in relation to population density. Journal of Stored Products Research, 43: 33-44.

Athanassiou, C. G., N. G. Kavallieratos & N. A. Andris, 2004. Insecticidal effect of three diatomaceous earth formulations against adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) on oat, rye and triticale. Journal of Economic Entomology*,* 97: 2160–2167.

Athanassiou, C. G., N. G. Kavallieratos, F. C. Tsaganou, B. J. Vayias, C. B. Dimizas & C. Th. Buchelos, 2003. Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Protect*.*, 22: 1141–1147.

Athanassiou, C. G., B. J. Vayias, C. B. Dimizas, N. G. Kavallieratos, A. S. Papagregoriou & C. Th. Buchelos, 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research, 41: 47–55.

Athanassiou, C. G., N. G. Kavallieratos, B. J. Vayias & V. K. Stephou, 2008. Evaluation of a new enhanced diatomaceous earth formulation for use against *Rhyzopertha dominica* (Coleoptera: Bostrychidae). International Journal of Pest Management, 54: 43–49.

Athanassiou, C. G., Z. Korunic, N. G. Kavallieratos, G. G. Peteinatos, M. C. Boukouvala & N. H. Mikeli, 2006. "New Trends In The Use of Diatomaceous Earth Against Stored Grain Insects. 730–740." In: 9th International Conference on Stored-Prod. Protect. Proc. 15–18 October 2006 (Eds: I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D. A. Faroni, L. O. F. de Bortolini, M. R. Sartori, M. C. Elias, R. N. C. Guedes, R. G. da Fonseca& V. M. Scussel). Brazilian Post Harvest Association, Campinas, Brazil. 1359 pp.

Athanassiou, C. G. & N. G. Kavallieratos, 2005. Insecticidal effect and adherence of PyriSec in different grain commodities. Crop Protection 27: 703–710.

Bello, G. D., S. Padin, C. Lopez-Lastra & M. Fabrizio, 2001. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. Journal of Stored Products Research, 37: 77-81.

Ebeling, W., 1971. Sorptive dusts for pest control. Annual Review of Entomology 16, 123–158.

Fields, P. & Z. Korunic, 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research, 36:1–13.

Fields, P. G. & W. E. Muir, 1995. "Physical Control, 195–221" In: Integrated Management of Insects in Stored Products. (Eds: B. Subramanyam, & D. W. Hagstrumm) Marcel Dekker Inc., New York. 432 pp.

Flinn, P. W., D. W. Hagstrum, C. R. Reed & T. W. Phillips, 2007. Stored grain advisor pro: Decision support system for insect management in commercial grain elevators. Journal of Stored Products Research, 43: 375-383.

Golob, P., 1997. Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research, 33: 69–79.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. Journal of research of the National Bureau of Standards, Section A, Physics and Chemistry. 81: 89-96.

IARC (International Agency for the Research of Cancer), 1997. Silica, some silicates, coal dust and para-aramid fibrils. IARC Working Group on the Evaluation of Carcinogenic Risks to Human, Lyon, France.

Jackson, K. & D. Webley, 1994. "Effects of Dryacide on The Physical Properties of Grains, Pulses and Oilseeds. 635–637" In: Proceedings of the Sixth International Working Conference on Stored-product Protection. 17–23 April 1994, (Eds: E. Highley, E.J. Wright, H.J. Banks, B.R. Champ ) Canberra, Australia, CAB International, Oxford, UK (1994), 1274 pp.

Kavallieratos, N. G., C. G. Athanassiou, F. G. Pashalidou and N. S. Andris & Z. Tomanovic, 2005. Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science., 61: 660–666.

Kavallieratos, N. G., C. G. Athanassiou, F. H. Arthur & J. E. Throne, 2012. Lesser grain borers, *Rhyzopertha dominica*, select rough rice kernels with cracked hulls for reproduction*.* Journal of Insect Science, 12:38.

Korunic, Z., S. Cenkovski & P. Fields, 1998. Grain bulk density as affected by diatomaceous earth and application method. Postharvest Biology and Technology, 13: 81–89.

Korunic, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. Journal of Stored Products Research, 33: 219–229.

Korunic, Z., 1999. Enhanced diatomaceous earth: an alternative to methyl bromide. Australian Journal of Technology, 2: 95–104.

Korunic, Z. & P. Ormesher, 1996. "Diatomaceous Earth-An Alternative To Methyl Bromide, 1–10" In: Workshop on Alternative to Methyl Bromide Diatomaceous Earth and Stored Product Pests. Proc. (Eds: W. Quarles & P.S. Winn) Ontario, 30–31 May. 1996. Toronto.

Korunic, Z., P. G. Fields, M. I. P. Kovacs, J. S. Noll, O. M. Lukov, C. Demianyk & K. J. Shibley, 1996. The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology. 9: 373–378.

Lorini, I. & D. J. Galley, 1999. Deltamethrin resistance in *R. dominica* (Coleoptera: Bostrychidae) a pest of stored grain in Brazil. Journal of Stored Products Research, 35: 37-46.

Lorini, I. & D. J. Galley, 2000. Estimation of realized heritability of resistance to deltamethrin insecticide in selected strain of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Journal of Stored Products Research, 36: 119-124.

McFarlane, J. A., 1982. Damage to milled rice by psocids. Tropical Stored Products Information. 44: 3–10.

Mewis, I. & Ch. Ulrichs, 2001. Action of amorphous diatomaceous earth against different stages of the stored product pests *Tribolium confusum* Du val (Coleoptera: Tenebrionidae), *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), *Sitophilus granarius* L. (Coleoptera: Curculionidae) and *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). Journal of Stored Products Research, 37:153–164.

Michalaki, M. P., C. G. Athanassiou, T. Steenberg & C. Th. Buchelos, 2007. Effect of *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocerales) alone or in combination with diatomaceous earth against *Tribolium confusum* jacquelin du Val (Coleopteran: Tenebrionidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidea). Biological Control, 40: 280-285.

Opit, G. P. & J. E. Throne, 2008. Effects of Diet on Population Growth of Psocids *Lepinotus reticulates* and *Liposcelis entomophila.* Journal of Economic Entomology, 101(2): 616-622.

Padin, S. B., G. D. Bello & M. Fabrizio, 2002. Grain losses caused by *Tribolium castaneum, Sitophlus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. Journal of Stored Products Research, 38: 69-74.

Rajendran, S., 1994. Psocids in food commodities and their control. Pestology, 28: 14–19.

Rigaux, M., E. Haubruge & P. G. Fields, 2001. Mechanisms for tolerance to diatomaceous earth between strains of *Tribolium castaneum.* Entomologia Experimentalis Applicata, 101: 33–39.

Sokal, R. R. & F. J. Rohlf, 1995. Biometry, third ed. Freedman and Company, New York.

Subramanyam, B. & R. Roesli, 2000. "Inert Dusts. 321–380" In: Alternatives to Pesticides in Stored Product IPM. (Eds: B. Subramanyam & D. W. Hagstrum) Dordrecht: Kluwer Academic Publishers. 437pp.

Subramanyam, B. & D. W. Hagstrum, 1995. "Resistance Measurement and Management. 331–398" In: Integrated Management of Insects in Stored Products (Eds: B. Subramanyam & D. W. Hagstrum). Marcel Dekker Inc., New York. 432 pp.

Subramanyam, Bh., C. L. Swanson, N. Madamanchi & S. Norwood, 1994. Effectiveness of Insecto, a New Diatomaceous Earth Formulation in Suppressing Several Stored-Grain Insect Species. 650–659. In: Proceedings of the Sixth International Working Conference on Stored-product Protection. 17–23 April 1994, (Eds: E. Highley, E.J. Wright, H.J. Banks, B.R. Champ ) Canberra, Australia, CAB International, Oxford, UK (1994), 1274 pp.

Turner, B. D., 1994. *Liposcelis bostrychophila* (Psocoptera: Liposcelididae), a stored food pest in the U.K. International Journal of Pest Management, 40: 179–190.

Vardeman, E. A., F. H. Arthur, J. R. Nechols & J. F. Campbell, 2006. Effect of temperature, exposure interval and depth of diatomaceous earth on distribution, mortality and reproduction of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera : Bostrichidae) in stored wheat. Journal of Economic Entomology, 99:1017–1024.

Vayias, B. J. & C. G. Athanassiou, 2004. Factors affecting efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused beetle *Tribolium confusum* du Val (Coleoptera: Tenebrionidae). Crop Protection, 23:565–573.

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***Original article (Orijinal araştırma)***

# Effect of Fungatol and Gamma-T-ol from *Melaleuca alternifolia* (Maiden & Betche) Cheel on *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae)[1]

*Aphis gossypii* Glover (Hemiptera: Aphididae) ve *Tetranychus urticae* Koch (Acari: Tetranychidae) üzerine *Melaleuca alternifolia* (Maiden & Betche) Cheel'dan elde edilen Fungatol ve Gamma-T-ol'ün etkisi

**İsmail KASAP[2]*** **Şahin KÖK[2]** **Errol HASSAN[3]**

## Summary

In this study, the effect of Fungatol and Gamma-T-ol extracted from *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae) on *Aphis gossypii* Glover and *Tetranychus urticae* Koch was determined using leaf dipping method. In laboratory tests, the leaf discs (50 mm diameter) taken from bean (*Phaseolus vulgaris* L.) and eggplant (*Solanum melongena* L.) and were dipped in five different concentrations of Fungatol (1.25, 1.90, 2.20, 2.50 and 3.50%) and Gamma-T-ol (0.25, 0.50, 1.00, 1.50 and 3.60%) for 5 s. The tests were repeated five times. Mortality was recorded after 1, 24, 48 h in tests with *A. gossypii* adult females and after 1, 24, 48, 72 h in tests with *T. urticae* adult females. After 1, 24 and 48 h, the highest concentrations of Fungatol (3.50%) and Gamma-T-ol (3.60%) had caused 0, 18, 42% and 0, 20, 48.9% mortality of *A. gossypii*, respectively. After 1, 24, 48 and 72 h the same concentrations of these extracts had caused 0, 52, 74, 94% and 0, 52, 78, 93.3% mortality of *T. urticae* adult females, respectively. The results showed that Fungatol and Gamma-T-ol extracts offer good potential to be used to control *A. gossypii* and *T. urticae*. Their use in pest management could be considered after validation in the field.

**Keywords:** *Aphis gossypii, Tetranychus urticae,* Fungatol, Gamma-T-ol, botanical extract

## Özet

Bu çalışmada *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae)'dan elde edilen Fungatol ve Gamma-T-ol ekstraktlarının *Aphis gossypii* Glover ve *Tetranychus urticae* Koch üzerine etkisi yaprak daldırma yöntemi kullanılarak belirlenmiştir. Laboratuvar testlerinde patlıcan (*Solanum melongena* L.) ve fasulye (*Phaseolus vulgaris* L.) bitkilerinden alınan 50 mm çapındaki yaprak diskleri Fungatol'un %1.25, 1.90, 2.20, 2.50, 3.50; Gamma-T-ol'ün %0.25, 0.50, 1.00, 1.50, 3.60'lık konsantrasyonlarına 5 sn daldırılmıştır. Testler 5 tekerrürlü olarak yapılmıştır. Ölüm kontrolü *A. gossypii* ergin dişileriyle yapılan testlerde 1, 24, 48 saat ve *T. urticae* ergin dişileriyle yapılan testlerde 1, 24, 48, 72 saat sonra yapılmıştır. Fungatol (%3.50) ve Gamma-T-ol (%3.60)'ün en yüksek dozları *A. gossypii*'de 1, 24 ve 48 saat sonra sırasıyla %0, 18, 42 ve %0, 20, 48.9 oranında ölüme sebep olmuştur. Ekstraktların aynı konsantrasyonları *T. urticae* ergin dişilerinde 1, 24, 48, 72 saat sonra sırasıyla %0, 52, 74, 94 ve % 0, 52, 78, 93.3 ölüm meydana getirmiştir. Sonuçlar Fungatol ve Gamma-T-ol ekstraktlarının *A. gossypii* ve *T. urticae* ile mücadelede iyi bir potansiyele sahip olduğunu göstermektedir. Zararlılar ile mücadelede bu ekstraktların kullanımı arazi çalışmalarından sonra değerlendirilebilir.

**Anahtar sözcükler:** *Aphis gossypii, Tetranychus urticae,* Fungatol, Gamma-T-ol, bitkisel ekstrakt

[1] Some parts of this research was previously presented on International Conference of Biopesticides 7 (19-25 October 2014, Antalya, Turkey)

[2] Department of Plant Protection, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[3] School of Agriculture and Food Sciences, The University of Queensland Gatton, Queensland, 4343, Australia
* Corresponding author (Sorumlu yazar) e-mail: ikasap@comu.edu.tr
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## Introduction

Chemical application is the most widely used method for control in agricultural production throughout the world, due to its simplicity and effectiveness. However, synthetic pesticides used in agriculture have caused serious short and long-term damage to the environment and human health. Long-term use of synthetic pesticides has caused water and soil pollution leading to serious health problems such as abortion, diarrhea, hepatitis A and typhoid in humans exposed to this pollution (Cutler & Miller, 2005; Grant et al., 2012).

Botanical pesticides can provide a viable alternative to synthetic chemicals. The first control using plant extracts dates back to the ancient Romans who used *Helleborus* plants and olive oil as natural insecticides (Smith & Secoy, 1975). Likewise, *Balanites* plant extracts were used by the ancient Egyptians against species belong to the Diptera family (Thacker, 2002). By 2005, about 2000 botanical extracts had been reported to have insecticidal effects on agricultural pests (Koul, 2005). Tea tree, *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae), is a native to Australia. Tea tree oil has become important in recent years due to its antimicrobial and anti-inflammatory properties (Carson et al., 2006). Furthermore, this oil has toxic and repellent effects on agricultural pests (Halbert et al., 2009). Tea tree oil has repellent and fumigant effects on sheep blowfly [*Lucilia cuprina* (Wiedemann)] and sheep lice [*Bovicola ovis* (Schrank)] (Callander & James, 2012). Both andiroba, *Carapa guianensis* Aubl. and *M. alternifolia* showed insecticidal activity against adult flies of *Haematobia irritans* L. and *Musca domestica* L. (Klauck et al., 2014). Common thyme, cinnamon, lemongrass, geranium, peppermint, tea tree and sweet basil caused >94% mortality against *Tetranychus urticae* Koch 10 µl/l air at 25°C (Lim, 2011).

Aphids not only suck plant sap but also excrete honeydew on plant leaves. As a result, saprophytic fungi grow the leaves impeding photosynthesis. Furthermore, aphids carry phytopathogenic viruses (Uygun et al., 2010). The cotton aphid, *Aphis gossypii* Glover, is a polyphagous pest of cultivated cotton and other hosts (Minks & Harrewijn, 1987). This aphid damages plants by feeding on fruits and leaves (Blackman & Eastop, 2000). The two-spotted spider mite, *T. urticae* is a serious pest on many crops and ornamental plants. This mite feeds on more than 150 species of host plants, including most deciduous fruit trees and many vegetables (Jeppson et al., 1975; Zhang et al., 2003). Control of *A. gossypii* and *T. urticae* is mainly dependent on the application of pesticides due to their ease of application and effectiveness. However, these pests can become resistant to the pesticides used to control them, resulting in lower efficacy.

The aim of this study was to determine the insecticidal effect of Fungatol and Gamma-T-ol, products based on extracts of *M. alternifolia,* on *A. gossypii* and *T. urticae*.

## Materials and Method

### Botanical extracts

Fungatol and Gamma-T-ol, the botanical products used in this study, were derived from *M. alternifolia*. The Fungatol is a commercial blend of three mono terpene alcohols solubilised in nonionic surfactants. The Gamma-T-ol is a commercial blend of three non oxygenated monoterpenes solubilised in nonionic surfactants. Both material produce from the steam distilled essential oil of *M. alternifolia* using super critical carbon dioxide extraction. Fungatol and Gamma-T-ol were supplied by BioAust Pty Ltd (Brisbane, Qld, Australia).

### Pest rearing

*Aphis gossypii* Glover adults used in the experiments were reared on eggplant (*Solanum melongena* L.) plants in laboratory conditions (25±2°C, 65±10% humidity and 16L:8D photoperiod). *Tetranychus urticae* adults were reared similarly on bean (*Phaseolus vulgaris* L.) plants.

**Leaf dipping method**

The tests were carried out in 50 mm diameter Petri dishes. A layer of moistened cotton was placed in the dishes under a sheet of blotting paper, and after treatment 50 mm diameter disc of eggplant or bean leaf placed on top. A 10-mm-diameter hole was made in each dish lid in order to prevent moisture accumulation inside and these holes were covered with insect net. Fungatol and Gamma-T-ol were diluted with distilled water to concentrations of 1.25, 1.90, 2.20, 2.50, 3.50% and 0.25, 0.50, 1.00, 1.50, 3.60%, respectively (Iramu, 2012). Leaf discs were dipped in these solutions for 5 s. The leaf discs were allowed to dry for about 15 min. Leaf discs were then placed into the dishes. Distilled water was used as a control. Ten adult females (5-6 d old) of *A. gossypii* and *T. urticae* were placed on the leaf discs in each dish. Assessment was performed 1, 24, 48 and 72 h after treatment and the number of alive and dead individuals recorded. In experiments with *A. gossypii* adults, the mortalities were recorded after 1, 24 and 48 h only, because the products had caused phytotoxic effects on eggplant leaf discs by 72 h. The tests were repeated five times.

Statistical analyses were performed on data obtained from the tests using SAS software package (SAS Institute, 1998). Mortalities were calculated by dividing the initial number of individuals by the number alive at the assessment time. Mortalities were corrected by the Abbott's formula (Abbott, 1925). Analysis of variance was applied to the Abbott values, and different concentrations of each product were evaluated in compared with each other. Duncan's Test was performed to compare mean values. Probit analysis was performed by Polo-PC™ software using concentrations of Fungatol and Gamma-T-ol, total number of insects and number of dead insects obtained from tests and $LC_{50}$ values were determined (LeOra Software, 1994). Probit analysis was only done for the tests with 20-80% mortality (Yu, 2008). This study was carried out at Çanakkale Onsekiz Mart University in 2015.

## Results and Discussion

For both Fungatol and Gamma-T-ol, the lowest mortality of *Aphis gossypii* Glover occurred at the lowest concentration of the products and the highest mortality at the highest concentrations. For Fungatol, the highest mortality occurred at a concentration of 3.50% after 48 h and the lowest at 1.25%, likewise after 48 h. For Gamma-T-ol, the highest mortality occurred at a concentration of 3.60%, while the lowest mortality occurred at 0.50% also after 48 h. For both products mortality increased with increasing product concentration (Table 1).

Table 1. Mortality of *Aphis gossypii* caused by Fungatol and Gamma-T-ol (mean ± SE)

| Products | Concentration (%) | 24 h Mortality (%) | | 48 h Mortality (%) | |
|---|---|---|---|---|---|
| Fungatol | 1.25 | 4.0±2.45 | a* | 8.9±4.16 | a |
| | 1.90 | 10.0±3.16 | ab | 18.0±3.74 | ab |
| | 2.20 | 12.0±4.90 | ab | 22.0±5.83 | ab |
| | 2.50 | 16.0±2.45 | b | 26.7±4.44 | b |
| | 3.50 | 18.0±3.74 | b | 42.0±4.90 | c |
| Gamma-T-ol | 0.25 | 6.0±2.45 | a | 12.0±2.00 | a |
| | 0.50 | 6.0±2.45 | a | 16.0±5.10 | a |
| | 1.00 | 8.0±2.00 | a | 20.0±4.47 | a |
| | 1.50 | 12.0±2.00 | a | 22.2±6.09 | a |
| | 3.60 | 20.0±3.16 | b | 48.9±5.67 | b |

*Means in the same column followed by the same letters are not significantly different (P<0.05).

For *A. gossypii* adult females, there was no statistically significant difference in mortality after 1 h at any concentration of either product. However, the difference between mortalities after 24 and 48 h for Fungatol and Gamma-Tol were statistically significant at all concentrations (P<0.05).

Similarly for *Tetranychus urticae,* Fungatol and Gamma-T-ol caused the lowest mortality at the lowest concentration and the highest mortality at the highest concentration. As with *A. gossypii*, as concentration of both products increased, mortality increased (Table 2). $LC_{50}$ values of Gamma-T-ol for *T. urticae* after 48 and 72 h are presented in Table 2.

Table 2. Mortality of *Tetranychus urticae* caused by Fungatol and Gamma-T-ol (mean ± SE)

| Products | Concentration (%) | 24 h Mortality (%) | | 48 h Mortality (%) | | 72 h Mortality (%) | |
|---|---|---|---|---|---|---|---|
| Fungatol | 1.25 | 2.0±2.00 | a* | 32.0±3.74 | a | 44.4±4.97 | a |
| | 1.90 | 14.0±7.48 | ab | 52.0±5.83 | b | 62.2±10.30 | b |
| | 2.20 | 26.0±5.10 | b | 66.0±5.10 | c | 75.6±2.22 | bc |
| | 2.50 | 44.0±5.10 | c | 68.0±3.74 | c | 88.9±3.51 | cd |
| | 3.50 | 52.0±3.74 | c | 74.0±4.00 | c | 94.0±2.45 | d |
| Gamma-T-ol | 0.25 | 0.0±0.00 | a | 10.0±5.48 | a | 28.0±3.74 | a |
| | 0.50 | 4.0±2.45 | a | 20.0±4.47 | a | 53.3±8.17 | b |
| | 1.00 | 4.0±2.45 | a | 42.0±5.83 | b | 64.0±7.48 | b |
| | 1.50 | 18.0±3.74 | b | 60.0±4.47 | c | 66.7±6.09 | b |
| | 3.60 | 52.0±3.74 | c | 78.0±3.74 | d | 93.3±4.44 | c |
| $LC_{50}$ Values | | | | 1.3±0.14 | | 0.8±0.23 | |

*Means in the same column followed by the same letters are not significantly different (P<0.05).

In tests with *T. urticae* adult females, there was no statistically significant difference in mortality after 1 h at any concentration. However, the difference between mortalities after 24, 48 and 72 h for Fungatol and Gamma-Tol were statistically significant for all concentrations (P<0.05; Table 2).

Mortalities of *A. gossypii* and *T. urticae* at 3.50% concentration of Fungatol and 3.60% concentration of Gamma-T-ol were compared. For 3.50% Fungatol, the mortality of *T. urticae* was higher than *A. gossypii*. Likewise, 3.60% Gamma-T-ol was more effective against *T. urticae* (Table 3).

In tests with *A. gossypii* and *T. urticae* adult females, there was no statistically significant difference in mortality after 1 h at any concentration. However, the difference between mortalities of *A. gossypii* and *T. urticae* after 24 and 48 h for 3.50% Fungatol and 3.60% Gamma-T-ol were statistically significant at all concentrations (P<0.05; Table 3).

Table 3. Mortality of *Aphis gossypii* and *Tetranychus urticae* caused by Fungatol (3.50% concentration) and Gamma-T-ol (3.60% concentration) (mean ± SE)

| Product | Pests | 24 h | | 48 h | |
|---|---|---|---|---|---|
| | | Mortality (%) | | Mortality (%) | |
| Fungatol (3.50%) | *Aphis gossypii* | 18.0±3.74 | a* | 42.0±4.90 | a |
| | *Tetranychus urticae* | 52.0±3.74 | b | 74.0±4.00 | b |
| Gamma-T-ol (3.60%) | *Aphis gossypii* | 20.0±3.16 | a | 48.9±5.67 | a |
| | *Tetranychus urticae* | 52.0±3.74 | b | 78.0±3.74 | b |

*Means in the same column followed by the same letters are not significantly different (P<0.05).

Although botanical extracts can be used as effective pest control, the effects of Fungatol and Gamma-T-ol on agricultural pests have not been fully investigated. Only a single study has investigated the effect of Fungatol and Gamma-T-ol on *A. gossypii* (Iramu, 2012). It showed that Fungatol, Gamma-T-ol, Fungatol + neem and Gamma-T-ol + neem were toxic to *A. gossypii* under laboratory conditions. Fungatol + neem and Gamma-T-ol + neem $LC_{50}$ rates were 2.78 and 0.76%, respectively, using the leaf dipping method. Furthermore, it was reported that these products had no effect on development and reproduction of *A. gossypii*. Bayindir et al. (2015) examined the effects of Gamma-T-ol, Fungatol, Fungatol + neem spray (50.0-001) and Fungatol + neem spray (50.0-002) on the third or fourth stages of tomato leaf miner using the dipping method under laboratory conditions. They reported Fungatol + neem spray (50.0-001) was the most effective combination against *Tuta absoluta* (Meyrick). Kök & Kasap (2015) investigated toxic effects of Fungatol and Gamma-T-ol on adult females of *Myzus persicae* Sulzer under laboratory conditions. They reported that concentrations of 3.50 and 3.60% of caused mortality of 72 and 80%, respectively after 72 h. They argued that further studies of Fungatol and Gamma-T-ol on *M. persicae* under field conditions are needed before these products can be recommended for pest control in agricultural production.

Birgücü et al. (2015) investigated the effects of extracts derived from *Cassia angustifolia* Vahl, *Melia azedarach* L., *Ocimum basilicum* L., *Satureja hortensis* L., *Schinus molle* L. and *Thevetia peruviana* L., on *A. gossypii* and *Bemisia tabaci* (Gennadius). They reported that extracts of *C. angustifolia*, *O. basilicum*, *T. peruviana* were potentially useful. Durmuşoğlu et al. (2011) examined efficacy of commercial products made from annona (*Annona squamosa* L.), karanj [*Derris indica* (Lam.)], neem (*Azadirachta indica* A. Juss), alone and in mixtures, on larvae of *T. absoluta* using the leaf dipping method. They concluded that annona, karanj and neem, alone and in mixtures, can be used as alternative pesticides. Durmuşoğlu et al. (2003) tested mortality of NeemAzal T/S and neem oil on different stages of *Nezara virudula* (L.) under laboratory conditions. They reported that 0.5% NeemAzal T/S and 2% neem oil were highly effective in controlling nymphs of *N. virudula*. Balcı et al. (2014) tested the effect of unprocessed and nanoformulated of neem oil and tea tree oil, and two commercial neem formulations on larvae of *T. absoluta* under laboratory conditions. Pavela (2016) examined the acaricidal effect of aqueous extracts obtained from 28 plant species on *T. urticae*. Twenty four of these extracts showed effects higher than 50% and 16 of them demonstrated mortality higher than 90%. The toxic and repellent effects of leaf, flower and seed extracts of *Prunus laurocerasus* L. on *T. urticae* were investigated by Akyazı et al. (2015) under laboratory conditions. They reported that the mortality from seed extracts was higher than from flower and leaf extracts. Akyazı et al. (2014a) investigated the ovicidal effect of a tobacco (*Nicotiana tabacum* L.) leaf extract on *T. urticae* eggs under laboratory conditions.

They reported that the tobacco extract at 10% was highly effective on *T. urticae* eggs for a period of 10 d after treatment. Akyazı et al. (2014b) examined ovicidal effect of garlic bulb extract, soft soap and garlic + soft soap mixture on *T. urticae* eggs using the leaf dipping method under laboratory conditions. They reported that garlic extract (1, 5 and 10 ml) and garlic + soft soap mixture (25 g soft soap + 125 ml garlic stock solution) had an ovicidal effect on *T. urticae* eggs. The efficacy of 8-hydroxydihydrochelerythrine and 8-methoxydihydrosanguinarine, the principal components isolated from *Macleaya cordata* (Willd.), on *A. gossypii* was investigated by Baek et al. (2013) under laboratory conditions. They indicated that these alcohol extracts caused mortalities of 76.1 and 73.6% in *A. gossypii*, respectively. Tunç & Şahinkaya (1998) investigated the insecticidal effect of *Eucalyptus camaldulensis* Dehnh., *Cuminum cyminum* L., *Origanum syriacum* var *bevanii* L. and *Pimpinella anisum* L., extracts on *Tetranychus cinnabarinus* Koch and *A. gossypii*. They reported that a concentration of 0.5% for the first three extracts caused 99% mortality of the pest 2-3 d after application.

New control methods that are less harmful to the nature and human health are needed to replace chemicals used in agriculture. The importance of natural insecticides is increasing year by year as negative effects on both human health and environment of synthetic chemicals used in agriculture are uncovered. In conclusion, Fungatol and Gamma-T-ol show moderate to high level insecticidal effect under laboratory conditions against *A. gossypii* and *T. urticae.* If these effects can be confirmed in field experiments, the use of these products in pest management should be considered.

## References

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

Akyazı, R., M. Soysal & D. Eminoğlu, 2014a. "Effects of the aqueous tobacco (*Nicotiana tabacum*) leaf extract on the hatchability of *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) eggs, 127". International Conference on Biopesticides (October 19-25, Side-Antalya, TURKEY), 168 pp.

Akyazı, R., M. Soysal & D. Eminoğlu, 2014b. "Garlic (*Allium sativum* L.) bulb extract and soft soap's effect on hatchability of *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) eggs, 112". XIV International Congress of Acarology (July 13-18, Kyoto, Japan), 166 pp.

Akyazı, R., M. Soysal & E. Hassan, 2015. Toxic and repellent effects of *Prunus laurocerasus* L. (Rosaceae) extracts against *Tetranychus urticae* Koch (Acari: Tetranychidae). Turkish Journal of Entomology, 39 (4): 367-380.

Baek, M.Y., H. J. Park, G. M. Kim, D. Y. Lee, G. Y. Lee, S. J. Moon, E. M. Ahn, G. S. Kim, M. H. Bang & N. I. Baek, 2013. Insecticidal alkaloids from the seeds of *Macleaya cordata* on cotton aphid (*Aphis gossypii*). Journal of the Korean Society for Applied Biological Chemistry, 56, 135-140.

Balcı, H., E. Durmuşoğlu & E. Hassan, 2014. "The effects of some classical and nano-formulated plant-based insecticides on *Tuta absoluta* (Lepidoptera: Gelechiidae) under laboratory conditions, 24". International Conference on Biopesticides (October 19-25, Side-Antalya, TURKEY), 168 pp.

Bayindir, A., Ş. Özger, İ. Karaca, A. K. Birgücü & E. Hassan, 2015. Effects of some plant extracts on *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) under laboratory conditions. Advances in Food Sciences, 37(3): 132-137.

Birgücü, A. K., S. Satar & İ. Karaca, 2015. Effects of some plant extracts on *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius) Takahashi (Hemiptera: Aleyrodidae). Asian Journal of Agriculture and Food Sciences, 3(2): 149-154.

Blackman, R. L. & V. F. Eastop, 2000. Aphids on the World's Crops: An Identification and Information Guide. Second Edition, John Wiley & Sons Ltd., U.K., 414pp.

Callander, J. T. & P. J. James, 2012. Insecticidal and repellent effects of tea tree (*Melaleuca alternifolia*) oil against *Lucilia cuprina*. Veterinary Parasitology, 184, 271-278.

Carson, C. F., K. A. Hammer & T. V. Riley, 2006. *Melaleuca alternifolia* (Tea Tree) Oil: a Review of antimicrobial and other medicinal properties. Clinical Microbiology Reviews, 19, 50–62.

Cutler, D. M. & G. Miller, 2005. The role of public health improvements in health advances: the 20th century United States. Demography, 42(1): 1-22.

Durmuşoğlu, E., A. Hatipoğlu & H. Balcı, 2011. Bazı bitkisel kökenli insektisitlerin laboratuvar koşullarında *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) larvalarına etkileri. Turkish Journal of Entomology, 35 (4): 651-663.

Durmuşoğlu, E., Y. Karsavuran, I. Özgen & A. Güncan, 2003. Effects of two different neem products on different stages of *Nezara viridula* (L.) (Heteroptera, Pentatomidae). Anz. Schadlingskunde Jornal of Pest Science 76, 151–154.

Grant, S., J. Saphores, D. Feldman, A. Hamilton, T. D. Fletcher, P. Cook, M. Stewardson, B. Sanders, L. Levin, R. Ambrose, A. Deletic, R. Brown, S. Jiang, D. Rosso, W. Cooper & I. Marusic, 2012. Taking the "waste" out of "wastewater" for human water security and ecosystem sustainability. Science, 337, 681–686.

Halbert, S. E., D. Corsini, M. Wiebel & S. F. Vaughn, 2009. Plant-derived compounds and extracts with potential as aphid repellents. Annals Applied Biology, 154, 303–307.

Iramu, E. T., 2012. A Critical Evaluation of the Effects of Plant Extract Formulations against two Generalized Insect Pests of *Abelmoschus manihot* (L.) Medik (Family: Malvaceae). School of Agriculture and Food Sciences, the University of Queensland. PhD Thesis, 198p.

Jeppson, L. R., H. H. Keifer & T. W. Baker, 1975. Mites Injurious to Economic Plants. University of California Press, Berkeley, CA, PP. 370–376.

Klauck, V., R. Pazinato, L. M. Stefani, R. C. Santos, R. A. Vaucher, M. D. Baldissera, R. Raffin, A. Boligon, M. Athayde, D. Baretta, G. Machado & A. S. Dasilva, 2014. Insecticidal and repellent effects of tea tree and andiroba oils on flies associated with livestock. Medical and Veterinary Entomology, 28, 33-39.

Kök, Ş. & İ. Kasap, 2015. "Natural insecticides: effects of two different plant extract on green peach aphid, *Myzus persicae* Sulzer 1776 (Hemiptera: Aphididae), 59-60". International Participation Turkey Natural Nutrition and Lifelong Health Summit (May 20-23, Bilecik,Turkey), 780pp.

Koul, O., 2005. Insect Antifeedants. CRC Press, Florida, 1005pp.

LeOra Software, 1994. Polo-PC a User's Guide to Probit or Logit Analysis, 1119, Shattuck Avenue, Berkeley, CA, 94707.

Lim, E.G., H. S. Roh, T. A. Coudron & C. G. Park, 2011. Temperature-dependent fumigant activity of essential oils against twospotted spider mite (Acari: Tetranychidae). Journal of Economic Entomology, 104(2): 414-419.

Minks, A. K. & P. Harrewijn, 1987. Aphids: Their Biology, Natural Enemies and Control (Volume A). Elsevier Science Publishers B. V., Amsterdam, 364pp.

Pavela, R., 2016. Acaricidal properties of extracts of some medicinal and culinary plants against *Tetranychus urticae* Koch. Plant Protection Science, 52(1): 54–63.

SAS Institute, 1998. User's Manuel version 7.0. SAS, Institute, Cary, N.C.

Smith, A. E. & D. M. Secoy, 1975. Use of plants in control of agriculture and domestic pests. Journal of Agricultural and Food Chemistry, 23: 1050-1051.

Thacker, J. M. R., 2002. An introduction to Arthropod Pest Control. Cambridge University Press, Cambridge, 343pp.

Tunç, I. & Ş. Şahinkaya, 1998. Sensitivity of two greenhouse pests to vapours of essential oils. Entomologia Experimentalis et Applicata, 86: 183–187.

Uygun, N., M. R. Ulusoy, İ. Karaca & S. Satar, 2010. Meyve ve Bağ Zararlıları. Çukurova University Publications, Özyurt Ltd., Adana-Turkey. 347p.

Yu, S. J., 2008. The Toxicology and Biochemistry of Insecticides. CRC press, London, 276p.

Zhang, Q. H., F. Schylter, A. Battisti, G. Birgersson & P. Anderson, 2003. Electrophysiological responses of *Thaumetopoea pityocampa* females to host volatiles: Implications for host selection of active and inactive terpenes. Journal of Pest Science, 76: 103-107.

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***Original article (Orijinal araştırma)***

# First record of the egg parasitoids of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in Turkey using DNA barcoding

## Türkiye'de *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae)'un yumurta parazitoidlerinin DNA Barkodu kullanılarak belirlenmiş ilk kaydı

**Feza CAN CENGIZ[1]* Kamuran KAYA[2] Başak ULAŞLI[1] Jérôme MORINIÈRE[3]**

## Summary

*Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is an invasive insect species attacking maize (*Zea mays* L.) and other cereal crops causing important yield losses. The occurrence of this insect in Turkey was first reported in maize growing areas of some provinces in the East Mediterranean region of Turkey in 2014. Chemical or other pest control methods do not always provide acceptable control of this pest, so biological control is considered an important alternative. However, for a successful biological control, the first step is to reliably identify the natural enemies of a target pest, which is difficult to achieve using methods based on morphology. Recent developments in molecular techniques allow more reliable identification of insect species and their parasitoids. Therefore, the aim of this study was to identify the egg parasitoids of *C. partellus* by molecular methods. Parasitized eggs were collected from maize fields in Hatay province, Turkey, from September to October 2014 and in September 2015. Eggs were maintained in the laboratory and emerging adult parasitoids were subjected to molecular analysis. Using DNA barcoding, two native natural enemies, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) and *Telenomus busseolae* (Gahan) (Hymenoptera: Platygastridae) were identified as egg parasitoids of *C. partellus* for the first time in Turkey.

**Keywords:** *Trichogramma brassicae, Telenomus busseolae*, COI, spotted stem borer

## Özet

*Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) mısır ve diğer tahıl ürünlerinde zararlı olan ve önemli ürün kayıplarına neden olan istilacı bir türdür. Bu böceğin Türkiye'deki varlığı ilk kez 2014 yılında Türkiye'nin Doğu Akdeniz Bölgesi'nin bazı illerinin mısır alanlarında kaydedilmiştir. Bu zararlı için insektisitler veya diğer zararlı mücadele metodları her zaman yeterli kontrolü sağlamamakta, biyolojik mücadele ise önemli bir alternatif olarak düşünülmektedir. Ancak biyolojik metodların başarılı bir şekilde uygulanması için zararlının doğal düşmanlarının ilk adımda iyi tanımlanmış olması gerekmektedir, Morfolojiye dayanan klasik teşhis metodlarıyla bunu gerçekleştirmek oldukça zordur. Moleküler tekniklerdeki son gelişmeler, böcek türlerinin ve onların parazitoidlerinin daha doğru tanılanmasına olanak vermektedir. Dolayısıyla bu çalışmanın amacı *C. partellus'* un yumurta parazitoidlerini, moleküler metodlar kullanarak tanılamaktır. Bu zararlının parazitlenmiş yumurtaları, 2014 yılının eylül ve ekim ayları ile 2015 yılı eylül ayında Türkiye'nin Hatay ilindeki mısır alanlarından toplanmıştır. Yumurtalar daha sonra laboratuvar koşullarında kültüre alınmış ve çıkış yapan erginlerin moleküler analizleri yapılmıştır. DNA barkod ile İki yerel doğal düşman, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) ve *Telenomus busseolae* (Gahan) (Hymenoptera: Platygasteridae) *C. partellus'*un yumurta parazitoidleri olarak ilk kez kaydedilmiştir.

**Anahtar sözcükler:** *Trichogramma brassicae, Telenomus busseolae*, COI, benekli gövdekurdu

[1] Mustafa Kemal University, Agricultural Faculty, Plant Protection Deparment, Entomology Section 31040 Hatay-Turkey
[2] Mustafa Kemal University, Agricultural Research and Application Center 31040 Hatay-Turkey
[3] SNSB - Bavarian State Collection of Zoology (ZSM), Münchhausenstrasse 21; 81247 München-Germany
* Corresponding author (Sorumlu yazar) e-mail: cezafan_onurcan@hotmail.com
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## Introduction

Maize (*Zea mays* L.) is a globally important cereal crop providing staple food in many countries. It takes the third place among the most common cereal crops after wheat and rice (FAO-STAT, 2014), and in Turkey after wheat and barley (TÜİK, 2014). Maize production is the main income source of farmers in developing countries (Tagne et al., 2008). Despite the importance of maize production, a large number of lepidopteran pests continue to cause significant economic loss. The invasive pest, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), commonly known as spotted stem borer, is one of the most serious pests of cereal crops, especially maize and sorghum in Asia and South Africa. *Chilo partellus* has a broad host range, including both wild and cultivated plants (Khan et al., 1997; Rebe et al., 2004), but is of most concern as a pest of maize. *Chilo partellus* has been reported to cause severe yield losses in maize throughout its geographical distribution. In some studies, maize yield losses attributable to *C. partellus* ranged from 24 to 75% (Kumar & Mihm, 1995; Kumar, 2002). Farid et al. (2007) reported 10 to 50% damage in the Peshawar Valley, Pakistan. Yield losses caused by stem borers in Africa have been as high as 80 to 88% in maize (Van den Berg, 2009; Kfir, 1990) and as much as 88% in sorghum (Seshu Reddy, 1988). This pest was recorded for the first time in maize fields in some provinces in the East Mediterranean region of Turkey in 2014 and 2015 (Sertkaya et al., 2014; Bayram & Tonğa, 2015).

There are many chemical formulations which have been applied in the field for the management of *C. partellus*. Research has shown that a wide range of insecticide in various formulations is effective against *C. partellus*. However, the use of insecticides for pest control is not only expensive for small farm holders, but also has undesirable consequences such as resistance development, secondary pest outbreaks, environmental pollution and risk to operators. Furthermore, stem borer larvae are difficult spray targets as they are hidden within the plant stem, which reduces the efficiency of insecticides by preventing non-systemic chemicals from reaching the larvae. Moreover, insecticides have a negative impact on beneficial fauna. Therefore, biological control agents are preferable for control of this important pest and different development stages of the pest, such as eggs, should be targeted. A number of parasitoids of stem borer lepidopteran pests in cereal crops have been recorded around the globe. Hymenopteran parasitoids are one of the most species-rich groups of animals, potentially accounting for more than 20% of the world insects (LaSalle & Gauld, 1991). Most of these parasitoid species belong to the families Braconidae, Chalcidoidae, Eulophidae, Ichneumonidae, Platygastridae and Trichogrammatidae.

Many egg, larval and pupal parasitoids of *C. partellus* have been reported from different countries. *Trichogramma chilonis* Ishii and *Trichogrammatoidea lutea* Girault (Trichogrammatidae) were found to be important egg parasitoids of *C. partellus* (Neupane et al., 1985; Kfir, 1990). Jalali & Singh (2006), reported that the parasitism rates by *T. chilonis* on *C. partellus* eggs on fodder maize were up to 75.2 and 62.6% in the first generation when parasitoids were released at 3 and 5 d intervals, respectively. In the second generation, parasitism rates were 90.4 and 78.4% at 3 and 5 d release intervals, respectively. In the study of Kfir (1990), one egg parasite, seven larval parasites (two of them egg-larval parasite) and two pupal parasites were recorded from parasitized *C. partellus* collected from maize and grain sorghum in South Africa. *Apanteles sesamiae* Cameron (Braconidae) was shown to be the most abundant larval parasite followed by an *Iphiaulax* sp. (Braconidae). *Dentichasmias busseolae* Heinrich (Ichneumonidae) and *Pediobius furvus* (Gahan) (Eulophidae) were the most abundant pupal parasites, while other species were found to be uncommon larval or pupal parasites. Kfir (1990) also reported *Chelonus curvimaculatus* Cameron and an *Chelonus* sp., (Braconidae) as egg-larval parasites of *C. partellus*. In another study, *Cotesia flavipes* Cameron, *Cotesia sesamiae* (Cameron) (Braconidae) and *Psilochalsis soudanensis* (Steffan) (Chalcidoidae) were determined to be larval parasitoids, and *P. furvus* and *D. busseolae* to be pupal parasitoids of *C. partellus* in Uganda (Rwomushana et al. 2005). Divya et al. (2009) recorded two larval parasitoids, one braconid (*C. flavipes*) and one tachinid (*Sturmiopsis inferens* Townsend), and one eulophid (*Tetrastichus* sp.) pupal parasitoid.

*Trichogramma* spp. (Trichogrammatidae) and *Telenomus* spp. (Platygastridae) are tiny parasitoid wasps that are important biological control agents of lepidopterous insect pests. Some of them have been used successfully in the control of crop pests (Borror et al., 1981). *Trichogramma* spp. are also effective biocontrol agents, because they can control the pest in the egg stage (Bournier, 1982; Somchoudhury & Dutt, 1988) and they are cost effective. This is an ideal choice for releases against crop borers because of its ease of propagation and application (Farid et al., 2007). The species of genus *Telenomus* are eggs parasites of a wide range of hosts, but they preferably attack Lepidoptera and Heteroptera (Masner & Johnson, 1979). *Telenomus* spp. are important in the natural control of insect pest populations (Yuliarti et al., 2002).

A reliable identification of effective parasitoid species is the first and most important step for a successful biological control program. Identification of species of these wasps has been exclusively based on the morphology of the male genitalia (Sorokina, 1993). In the cases where males are not found or are in low numbers, identification becomes even more difficult (Aeschlimann, 1990). However, the small size of these parasitoids (<1 mm long) and lack of clear diagnostic features make then difficult to identify, and this confusion can impede the implementation of effective biological control programs (Smith & Hubbes, 1986; Pinto et al., 1989; Pinto & Stouthamer 1994). Given the difficulties in distinguishing species of the genera, *Trichogramma* and *Telenomus*, molecular studies have gained importance in recent years (Yuliarti et al., 2002; Sümer et al., 2009; Poorjavad et al., 2012; Nasir et al., 2013). Therefore, with the advent of molecular techniques, DNA-based approaches have been used to generate molecular markers that are useful for the characterization of closely related or cryptic species for biological control work (Landry et al., 1993; Hoy et al., 2000; Chang at al., 2001). The general ease of species diagnosis reveals one of the great values of a DNA-based approach for identification. Newly encountered species will be recognized by their genetic divergence from known members of the assemblage (Hebert et al., 2003). Since standard morphological methods are not always sufficiently precise to differentiate micro-hymenopteran to species level (Landry et al., 1993) genetic means have become the tools of choice for the routine identification of *Trichogramma* spp. (Sümer et al., 2009). The use of a standardized DNA region for fast and reliable species identification, such as cytochrome c oxidase I (COI), has proven to be efficient in hyperdiverse groups for which morphological identification is difficult or impossible (Valentini et al., 2009).

The present study aimed to determine and identify *C. partellus* and its egg parasitoids in Turkey using DNA barcoding.

## Materials and Methods

Egg masses of *C. partellus* were collected during maize field surveys in Reyhanlı-Hatay Province in September to October 2014 and September 2015. Each egg mass was put into a glass tube with parts of the plant. These egg masses were incubated at 26±1°C, 70±5% RH, 16L:8D h photoperiod, until larval or pupal formation, or adult emergence of any possible natural enemies (i.e. egg parasitoids) occurred. Any emerged egg parasitoids were collected and preserved in 96% ethanol for molecular analysis. Larvae of *C. partellus* were cultured in plastic containers to obtain adults. Emerged adults of *C. partellus* were identified based on male genitalia by the first author. All specimens of the pest were deposited in the Museum of Mustafa Kemal University, Hatay, Turkey.

Amplification of the 5' COI barcoding region was undertaken by first extracting DNA from dry legs of eight *C. partellus* specimens and from the whole specimen for four micro hymenopterans (three *Trichogramma* and one *Telenomus*). DNA extraction was performed using the DNEasy tissue kit following the manufacturer's protocol (Qiagen, Hilden, Germany).

Polymerase chain reactions were performed following standard protocols of the entomology lab of the Bavarian State Collection of Zoology (http://zsm-entomology.de/wiki/Coleoptera#The_Beetle_DNA_Lab) using the primers dgHco 5'-TAAACTTCAGGGTGACCAAARAAYCA-3' and mLCOIintF 5'-GGWACWGGWTGAACWGTWTAYCCYCC-3' (Leray et al., 2013), which amplify 300- to 350-bp

fragments targeting the center of the 5' region of COI. PCR products were purified and processed for sequencing, using BigDye v3.1 (Applied Biosystems, Foster City, CA, USA). The assembly and editing of the sequences was performed using Sequencher 4.10.1 (Gene Codes, Ann Arbor, MI, USA). In order to screen for pseudogenes, successfully amplified sequences were aligned with reference sequences in MEGA v6.0 (Tamura et al., 2013) and the coding frame was checked for stop codons. Successfully sequenced PCR products were identified using BLAST searches with the identification tool on the BOLD system (http://www.boldsystems.org, Ratnasingham & Hebert, 2007). Sequences of *C. partellus* were successfully identified with >98% similarity to published *C. partellus* specimens [genbank accessions: KP233794, KP233796; BIN (Cluster ID) BOLD:AAN5677]. *Trichogramma brassicae* Bezdenko sequences were identified with >99% similarity (KC488653, KC488655 and KC488656; BOLD:AAD6262). The single sequence of *Telenomus busseolae* (Gahan) was identified with >95% similarity (DQ888418).

## Results and Discussion

Two native natural enemies, *T. busseolae* belonging to Platygastridae and *T. brassicae* belonging to Trichogrammatidae were identified from parasitized eggs of *C. partellus* from maize collected from Reyhanlı of Hatay Province of the eastern Mediterranean region of Turkey on 15 September and 15 October 2014, respectively. *C. partellus* was present in both years. *Chilo partellus* was recorded recently for the first time from some provinces in the eastern Mediterranean region of Turkey, but natural enemies of this pest were not determined in these studies (Sertkaya et al., 2014; Bayram & Tonğa, 2015). When new insect species are introduced into a region, some can become invasive and cause significant economic damage. The success or failure of invasion of a species may depend on the its life history parameters, its response to climatic conditions, the competition with native species and the impact of natural enemies (Grabenweger et al., 2010). Natural enemies are considered to be the most important and practical option for keeping populations of invasive insects under pressure, thereby minimizing spread. Indigenous natural enemies may be able to reduce stem borer populations in the field (Bonhof, 2000; Midega et al., 2004). To control the stem borer lepidopteran pest by non-chemical means, it is first necessary to identify the key parasitoids species, which is difficult to achieve by morphological methods. Therefore, the results of this study are important, because they demonstrated that DNA barcode sequencing was successful in identifying *C. partellus* as well as two associated egg parasites, *T. brassicae* and *T. busseolae,* by blasting the barcode sequences using the BOLD identification tool (Table 1).

*Telenomus busseolae* is a solitary egg parasitoid of several species of noctuid stem borers including *C. partellus.* Parasitism by *T. busseolae* can be high in some areas of the Mediterranean basin (Kornoşor et al., 1994; Sétamou & Schulthess, 1995). However, periodic releases of mass-produced parasitoids can be necessary to increase the rate of parasitism in the field (Alexandri & Tsitsipis, 1990; Kornoşor et al., 1994). Egg parasitoids of the genus *Trichogramma,* including *T. brassicae,* have been successfully used to control mainly lepidopteran pests in biological control worldwide (Li et al., 1994)

However, changes in the rate of parasitism may happen when hosts are switched. Therefore, the ability of a parasitoid species to be reared on a non-target host and still keep a high rate of parasitism of the in the target pest after successive rearing is an important determinant of parasitoid quality and must be investigated (Pomari-Fernandes et al., 2015). Consequently, these species could be considered as appropriate candidates for biological control of the spotted stem borer. In future, mass rearing and evaluation of releases to support its naturally-occurring activity in areas of Turkey where the efficacy of the parasitoid is insufficient could be undertaken. The data obtained in this study are expected to contribute to biological management studies of the pest.

Table 1. Accession numbers of sequences submitted to the GenBank database

| Species | Sampling location | BOLD process IDs | Accession numbers |
|---|---|---|---|
| *Chilo partellus* | Hatay-Turkey | GBMIX2732-16 | KU687432 |
| | | GBMIX2733-16 | KU687434 |
| | | GBMIX2734-16 | KU687439 |
| | | GBMIX2735-16 | KU687435 |
| | | GBMIX2736-16 | KU687440 |
| | | GBMIX2737-16 | KU687438 |
| | | GBMIX2738-16 | KU687442 |
| | | GBMIX2739-16 | KU687437 |
| Parasitoids | | | |
| *Telenomus busseolae* | Hatay-Turkey | GBMIX2741-16 | KU687431 |
| *Trichogramma brassicae* | Hatay-Turkey | GBMIX2742-16 | KU687441 |
| | | GBMIX2743-16 | KU687433 |
| | | GBMIX2744-16 | KU687436 |

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## References

Aeschlimann, J. P., 1990. Simultaneous occurrence of thelytoky and bisexuality in hymenopteran species, and its implications for the biological control of pests. Entomophaga 35: 3–5.

Alexandri, M. P. & J. A. Tsitsipis, 1990. Influence of the egg parasitoid *Platytelenomus busseolae* (Hym.; Scelionidae) on the population of *Sesamia nonagrioides* (Lep.; Noctuidae) in Central Greece. Entomophaga, 35: 16-25.

Avcı, M., 2003. Parasitism of egg-batches of the cedar processionary moth, *Traumatocampa ispartaensis* in Turkey. Phytoparasitica 31 (2): 1-6.

Bayram, A. & A. Tonğa, 2015. First report of *Chilo partellus* in Turkey, a new invasive maize pest for Europe. Journal of Applied Entomology, 140 (3): 236-240.

Bonhof, M. J., 2000. The Impact of Predators on Maize Stem Borers in Coastal Kenya . Wageningen University, PhD thesis, The Netherlands,181 pp.

Borror, D. J., D. M. De Long & C. A. Triplehorn, 1981. An Introduction to the Study of Insects. Saunders College Publishing, 827 pp.

Bournier, J. P., 1982. “Behaviour of various strains of *Trichogramma* towards eggs of *Heliothis armigera* Hbn (Lep. Noctuidae) and *Chilo partellus* Swinhoe (Lep. Pyralidae), 85-96” .Les trichogrammes. Ier Symposium International (10-15 November 1986, Guangzhou-Chine).

Chang, S. C, N. T. Hu, C. Y. Hsin & C. N. Sun, 2001. Characterization of differences between two *Trichogramma* wasps by molecular markers. Biological Control 21 (1): 75–78.

Divya, K., K. N. Marulasiddesha, K. Krupanidhi & M. Sankar, 2009. Population dynamics of spotted stem borer, *Chilo partellus* (Swinhoe) and its interaction with natural enemies is sorghum. Indian Journal of Science and Technology, 3 (1): 70-74.

Edgar, R. C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32 (5): 1792-1797.

FAO-STAT, 2014. Food and Agriculture Organization of the United States Statistics, (Web page: http://faostat.fao.org), (Date accessed: January 2016).

Farid, A., M. I. N. Khan, A. Khan, S. U. K. Khattak & A. Sattar, 2007. Studies on maize stem borer, *Chilo partellus* in Peshawar Valley. Pakistan Journal of Zoology, 39 (2): 127-131.

Grabenweger, G., P. Kehrli, I. Zweimüller, S. Augustin, N. Avtzis, S. Bacher, J. Freise, S. Girardoz, S. Guichard, W. Heitland, C. Lethmayer, M. Stolz, R. Tomov, L. Volter & M. Kenis, 2010. Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe. Biological Invasions, 12: 2797–2813.

Hebert, P. D. N., A. Cywinska, S. L. Ball & J. R. Deward, 2003. Biological identifications through DNA barcodes. Proceedings Royal Society London Biological Sciences, 270:313–321.

Hoy, M.A., A. Jeyaprakash, R. Morakote, P. K. C. Lo & R. Nguyen, 2000. Genomic analyses of two populations of *Ageniaspis citricola* (Hymenoptera: Encyrtidae) suggest that a cryptic species may exist. Biological Control 17: 1–10.

Jalali, S. K. & S. P. Singh, 2006. Biological control of *Chilo partellus* using egg parasitoid *Trichogramma chilonis* and *Bacillus thuringiensis*. Indian Journal of Agricultural Research 40 (3): 184-189.

Kfir, R., 1990. Parasites of the spotted stalk borer, *Chilo partellus* (Lep: Pyralidae) in South Africa. Entomophaga 35 (3): 403-410.

Khan, Z. R., P. Chiliswa, K. Ampong-Nyarko, L. E. Smart, A. Polaszek, J. Wandera & M. A. Mulaa, 1997. Utilisation of wild gramineous plants for the management of cereal stemborers in Africa. International Journal of Tropical Insect Science, 17 (1): 143–150.

Kornoşor, S., E. Sertkaya & A. Özpınar, 1994. "Distribution of the egg parasitoid *Platytelenomus busseolae* (Gahan) (Hym., Scelionidae) and its effect on the population of *Sesamia nonagrioides* Lef. (Lep., Noctuidae) in the Mediterranean Region of Turkey, 193-200". 4th. International Symposium on *Trichogramma* and Other Egg Parasites (4-7 October, Cairo, Egypt).

Kumar, H. & J. A. Mihm, 1995. Antibiosis and tolerance to fall armyworm, *Spodoptera frugiperda* (J. E. Smith), southwestern corn borer, *Diatraea grandiosella* Dyar and sugarcane borer, *Diatraea saccharalis* Fabricius in selected maize hybrids and varieties. Maydica. 40: 245-251.

Kumar, H., 2002. Resistance in maize to larger grain borer, *Prosphanus truncates* (Horn) (Coleoptera: Bostrichidae). Journal of Stored Products Research, 38: 267-280.

Landry, B. S., L. Dextraze & G. Boivin, 1993. Random amplified polymorphic DNA markers for DNA fingerprinting and genetic variability assessment of minute parasitic wasp species (Hymenoptera: Mymaridae and Trichogrammatidae) used in biological control programs of phytophagous insects. Genome, 36 (3): 580–587.

LaSalle, J. & I. D. Gauld, 1991. Parasitic Hymenoptera and the biodiversity crisis. Redia, 74 (3): 315–334.

Leray, M., J. Y. Yang., C. P. Meyer, S. C. Mills, N. Agudelo, V. Ranwez, J. T. Boehm & R. J. Machida, 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in Zoology, 10: 34.

Li, S. Y., D. E. Henderson & J. H. Meyer, 1994. Selection of suitable *Trichogramma* species for potential control of the blackheaded fire worm infesting cranberries. Biocontrol, 4 (3): 244-248.

Masner, L. & N. F. Johnson, 1979. A new species of *Telenomus* (Hymenoptera: Scelionidae) an egg parasite of the Ambush bug, *Phymata* sp. (Heteroptera: Phymatidae). Canadian Entomologist 111 (10): 1115-1119.

Midega, C. A. O., C. K. P. O. Ogol & W. A. Overholt, 2004. Effect of agroecosystem diversity on natural enemies of maize stemborers in coastal Kenya. International Journal of Tropical Insect Science, 4 (4): 280-286.

Nasir, M. F., G. Hagedorn, C. Büttner, C. Reichmuth & M. Schöller, 2013. Molecular identification of *Trichogramma* species from Pakistan, using ITS-2 region of rDNA. BioControl 58 (4): 483-491.

Neupane, F. P., H. C. Coppel & R. K. Chapman, 1985. Bionomics of the maize borer, *Chilo partellus* (Swinhoe), in Nepal. International Journal of Tropical Insect Science. 6 (4): 547-553.

Pinto, J. D., R. K. Velten, G. R. Platner & E. R. Oatman, 1989. Phenotypic plasticity and characters in *Trichogramma*. Annals of the Entomological Society America, 85: 413–422.

Pinto, J. D. & R. Stouthamer, 1994. "Systematics of the Trichogrammatidae with Emphasis on *Trichogramma*.1-36". In: Biological Control with Egg Parasitoids (Eds: E. Wajnberg & S. A. Hassan). CAB international, 266 pp.

Pomari- Fernandes, A., A. F. Bueno, A. P. Queiroz & S. A. De Bortoli, 2015. Biological parameters and parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Platygastridae) reared on natural and factitious hosts for successive generations. African Journal of Agricultural Research, 10 (33): 3225-3233.

Poorjavad, N., S. H. Goldansaz, T. Machtelinckx, L. Tirry, R. Stouthamer & T. van Leeuwen, 2012. Iranian *Trichogramma*: ITS2 DNA characterization and natural *Wolbachia* infection. BioControl, 57 (3): 361-374.

Ratnasingham, S. & P. D. Hebert, 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife.org). Molecular Ecology Notes, 7 (3), 355-364.

Rebe, M., J. Van Den Berg & M. A. McGeoch, 2004. Colonization of cultivated and indigenous graminaceous host plants by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) under field conditions. African Entomolology, 12 (2): 187-199.

Rwomushana, I., S. Kyamanywa, A. J. Ngi-Song & C. O. Omwega, 2005. Incidence of larval and pupal parasitoids of Lepidoptera in Uganda with special reference to new associations. International Journal of Tropical Insect Science, 25 (4): 274-280.

Sertkaya, E., V. Akmeşe & E. Atay, 2014. Türkiye'de mısırda yeni bir zararlı, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). Türkiye Entomoloji Bülteni, 4 (3): 197-200.

Seshu Reddy, K. V., 1988. Assessment of on-farm yield losses in sorghum due to insect pests. International Journal of Tropical Insect Science, 9: 679-685.

Sétamou, M. & F. Schulthess, 1995. The influence of egg parasitoids belonging to *Telenomus busseolae* (Hym. Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) population in maize fields in Southern Benin. Biocontrol Science and Technology 5: 69–81.

Smith, S. M. & M. Hubbes, 1986. Strains of the egg parasitoid *Trichogramma minutum* Riley. Journal of Applied Entomology, 101 (1-5) : 223-239.

Somchoudhury, A. K. & N. Dutt, 1988. Influence of hosts and host ages on the bionomics of *Trichogramma perkinsi* Girault and *Trichogramma australicum* Girault. Indian Journal of Entomology, 50 (3): 374-379.

Sorokina, A. P., 1993. Keys to the Species of Genus *Trichogramma* Westw. (Hymenoptera: Trichogrammatidae) of the World Fauna. Kolos Publishing House, Moscow, 77 pp.

Sümer, F., A. S. Tunçbilek, S. Öztemiz, B. Pintureau, P. Rugman-Jones & R. Stouthamer, 2009. A molecular key to the common species of *Trichogramma* of the Mediterranean region. BioControl, 54: 617-624.

Tagne, A., T. P. Feujio & C. Sonna, 2008. "Essential oil and plant extracts as potential substitutes to synthetic fungicides in the control of fungi" ENDURE International Conference, Diversifying crop protection (12-15 October 2008, La Grande-Motte-France), 42. Oral presentation.

Tamura, K., G. Stecher, D. Peterson, A. Filipski & S. Kumar, 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, 30 (12): 2725–2729.

TÜİK, 2014. Türkiye İstatistik Kurumu. (Web page: http://www.tuik.gov.tr/PreTablo.do?alt_id=1001), (Date accessed: January 2016).

Valentini, A., F. Pompanon & P. Taberlet, 2009. Barcoding for ecologist. Trends in Ecology and Evolution, 24: 110–117.

Van den Berg, J., A. F. Nur & A. Polaszek, 1998. "Cultural Control, 333–347" In: Cereal Stem Borers in Africa: Economic Importance, Taxonomy, Natural Enemies and Control (Ed. A. Polaszek). International Institute of Entomology. CAB International, 592 pp.

Van den Berg, J., 2009. "Can Vetiver grass be used to manage insect pests on crops, 254-264". Third International Conference on Vetiver (6-9 October 2003, Guangzhou- China), China Agriculture Press, 572 pp.

Wakeil-El, N., N. Gaafar, A. Sallam & C. Volkmar, 2013. Side effects of insecticides on natural enemies and possibility of their integration in plant protection strategies. Insecticides-development of safer and more effective tecnologies-Chapter 1-January 2013. 56 pp.

Yuliarti, N., P. Hidayat & D. Buchori, 2002. Molecular identification of egg parasitoid, *Telenomus* spp. (Hym.: Scelionidae) from several locations in Java using RAPD-PCR. Biotropia 19: 57-64.

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*Original article (Orijinal araştırma)*

# Parasitoids (Hymenoptera: Encyrtidae) of an invasive mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in Turkey

İstilacı bir unlubit türü *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)'in Türkiye'deki parazitoitleri

**A. Filiz ÇALIŞKAN[1]** **Mohammad HAYAT[2]*** **M. Rifat ULUSOY[1]** **M. Bora KAYDAN[3]**

## Summary

Three parasitoids, a hyperparasitoid and an associate parasitoid were recorded on the invasive mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in Turkey: *Anagyrus aligarhensis* Agarwal & Alam, *Anagyrus* sp. near *dactylopii* (Howard), *Leptomastix epona* (Walker), *Prochiloneurus uyguni* Hayat, sp. n. and *Homalotylus hemipterinus* (De Stefani). *L. epona* and *H. hemipterinus* are new records for the fauna of Encyrtidae of Turkey.

**Keywords:** Associate hyperparasitoid, invasive mealybug parasitoids, Turkey

## Özet

Türkiye'de istilacı unlubit türü *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) üzerinde üç parazitoit, bir hiperparazitoit ve bir bağlantılı parazitoit türü tespit edilmiştir: *Anagyrus aligarhensis* Agarwal & Alam, *Anagyrus* sp. near *dactylopii* (Howard), *Leptomastix epona* (Walker), *Prochiloneurus uyguni* Hayat, sp. n. ve *Homalotylus hemipterinus* (De Stefani). *L. epona* and *H. hemipterinus* Türkiye Encyrtidae faunası için yeni kayıt türlerdir.

**Anahtar sözcükler:** Bağlantılı hipeparazitoit, istilacı unlubit parazitoitleri, Türkiye

[1] Çukurova University, Agriculture Faculty, Plant Protection Department, Adana, Turkey
[2] Department of Zoology, Aligarh Muslim University, Aligarh 202 002, India
[3] Çukurova University, Imamağlu Vocational School, Adana, Turkey
* Corresponding author. e-mail: hayat.mohd44@gmail.com
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## Introduction

An invasive mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), cotton or solenopsis mealybug, has a new world origin (Abbas et al., 2010). This mealybug is reported in the Australasian, Afrotropical, Nearctic, Oriental and Neotropical Regions on 202 host plants from 55 families (Garcia et al., 2015; Fand & Suroshe, 2015; McKenzie, 1967). In the Palaearctic Region it has been recorded in Cyprus, Egypt, France, Iran, Israel, Japan, and recently in Turkey (Garcia et al., 2015; Kaydan et al., 2013). This polyphagous species is regarded as an important pest of cotton (*Gossypium hirsutum* L., Malvaceae) (Fand & Suroshe, 2015) and it has caused 30-60% yield losses in cotton in India and Pakistan in 2005 and 2009 (Fand & Suroshe, 2015). In addition, *P. solenopsis* is also an important pest of ornamental plants, such as *Hibiscus rosa-sinensis* L., *H. syriacus* L. (Malvaceae) and *Lantana camara* L. (Verbenaceae), and some vegetable crops, such as *Solanum esculentum* Lam., *S. melongena* L. and *Capsicum annuum* L. (Solanaceae) (Fand & Suroshe, 2015). This mealybug feeds and develops in the canopy of its host plants and reproduces sexually and through ovoviviparity (Abbas et al. 2010).

*Phenacoccus solenopsis* is parasitized by 21 species of chalcidoids, 19 of these belong to the hymenopteran family Encyrtidae, one each to the Eulophidae and Signiphoridae (Noyes, 2015).

In this study, we recorded three primary parasitoids, one hyperparasitoid, and one primary parasitoid of the larvae of the Coccinellidae (Coleoptera) feeding on *P. solenopsis* in Turkey and one new species is described, namely *Prochiloneurus uyguni* Hayat, sp. n.

## Material and Methods

Mealybug samples including parasitized and unparasitized samples were collected from ornamental plants from Adana, Turkey. Each sample was placed in a plastic bag and taken to the laboratory for examination. Mealybug specimens were prepared for light microscopy using the slide-mounted method of Kosztarab & Kozár (1988) and identified according to key of Williams (2004). Identification of mealybug was made by one of us (Mehmet Bora KAYDAN).

The specimens were reared from the mealybug, and initially preserved in 80% alcohol. These were card-mounted, and at least one specimen of each species (or parts of one specimen) were mounted on slides.

The terminology of Hayat (2006) is followed, except for the use of the terms mesosoma for the thorax plus propodeum, and metasoma for the petiole plus gaster. Only body lengths are given in millimeters; all other measurements are relative, taken with the help of an ocular micrometer with a linear scale of 100 divisions, placed in the eye piece of a stereo zoom binocular microscope (one micrometer division = 0.01234 mm) for card-mounted specimens, and placed in the eye piece of a compound microscope at 100X magnification (one micrometer division = 0.00987 mm) for slide-mounted parts.

The following abbreviations are used in the text:

AOL = minimum distance between a posterior ocellus and the anterior ocellus.

F1, F2, onwards = Funicle segments 1, 2, onwards.

OCL = minimum distance between a posterior ocellus and the occipital margin.

OOL = minimum distance between a posterior ocellus and the corresponding eye margin.

POL = minimum distance between the posterior ocelli.

TI, TII, onwards = Tergites 1, 2 onwards of the gaster.

The following acronym is used for the depository:

ZDAMU = Insect Collections, Department of Zoology, Aligarh Muslim University, Aligarh, India.

## Result and Discussion

### Primary parasitoids

***Anagyrus aligarhensis* Agarwal & Alam** (Figures 1-11)

*Anagyrus diversicornis* Mercet, 1921: 134-135, female. Spain. Preoccupied in *Anagyrus* by *A. diversicornis* (Howard, 1894).

*Anagyrus aligarhensis* (Man Mohan) Agarwal & Alam, 1959: 392, female, male. India, Aligarh. Next available name for *diversicornis* Mercet.

*Anagyrus aligarhensis* Agarwal, 1965: 52-53, female, male. Redescription of *aligarhensis* Agarwal & Alam, 1959, as new species.

*Anagyrus micans* Noyes, 2000: 2, 36. Unnecessary replacement name for *diversicornis* Mercet, not Howard. Synonymy by Hayat, 2003: 189.

For further synonyms and misidentifications, see Noyes & Hayat (1994).

This species is widely distributed in the old world, and introduced into the USA. A redescription of the species and some relevant figures are given here based on the Turkish specimens.

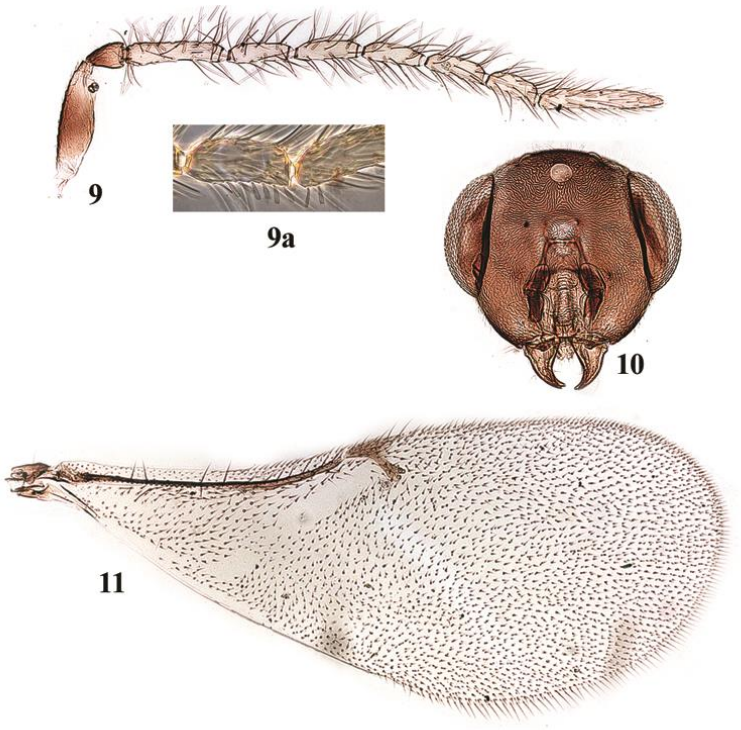
### Redescription

*Female.* Length, 1.15–1.30 mm (n = 4). Body dark brown, except as follows: occiput behind eyes, and frontovertex along eye margins orange yellow (Fig. 1); malar space with a yellow streak from below eye margin; collar of pronotum white, interrupted by a dark brown band in lateral fourth and sides anteriorly brownish yellow; tegula white, apically dark brown (Fig. 2); prepectus white; mesopleuron brown, but anteriorly, dorsally and posteriorly yellow. Antenna (Fig. 5) with radicle, scape except a small basal spot and a subapical curved band, pedicel in about distal third, F2 and F3, white; F1 dark brown; F4–6 pale brown; clava pale brownish yellow. Mandible in about apical half reddish brown. Wings hyaline; fore wing with discal setae pale brown; hind wing with discal setae translucent. Legs, including coxae, largely white, except brown to dark brown as follows: fore leg with coxa in one specimen with a brown streak in basal half on outer margin; femur with brownish streaks on both margins; tibia brownish yellow; tarsus brownish yellow, fifth segment brown; mid leg with coxa in about basal half on ventral surface brown; femur with a brownish streak on outer surface; tibia with a subbasal brownish infuscation; tarsal segments 1–4 yellowish white, fifth segment dark brown; hind leg with coxa largely brown to dark brown; femur largely brownish, becoming yellowish apically; tibia whitish yellow with pale brown infuscation; tarsal segments 1–4 brownish yellow, fifth segment dark brown.

Head. Frontovertex width 0.45X head width; head, in frontal view (Fig. 3), 1.18X as broad as high; antennal torulus with upper margin at most in line with lower margin of eye; eye height 2.63X malar space; frontovertex and face with raised, rugose-reticulate sculpture (Figs 3 and 4); head densely setose, setae silvery white; eye setose, setae hyaline, each seta shorter than a facet diameter. Antenna (Fig. 5) with scape 2.3X as long as broad; pedicel subequal in length to F1; F2–6 each distinctly longer than broad, and each shorter than F1; clava shorter than F4–6 combined.

Mesosoma (Fig. 2). Mesoscutum and scutellum with rugose-reticulate sculpture, that on scutellum not deeper than on mesoscutum; mesothorax densely setose, setae silvery white; propodeum with silvery white setae, a few mesal to spiracle and several setae distal to spiracle. Fore wing 2.6X as long as broad; setae and venation as in Figs 6 and 6a. Hind wing 5X as long as broad. Mid tibia 3.2X as long as mid basitarsus; mid tibial spur shorter (0.8X) than mid basitarsus. *Relative measurements* (from slide): fore wing length (width), 115 (44); hind wing length (width), 85 (17); mid tibia length, 48; mid basitarsus length, 15; mid tibial spur length, 12.


Figures 1-8. *Anagyrus aligarhensis* Agarwal & Alam, female: 1. head, in profile, with antennae; 2. mesosoma dorsal; 3. head, frontal view; 4. sculpture on frons; 5. antenna; 6. fore wing; 6a, distal veins of fore wing; 7. outer plate of ovipositor; 8. ovipositor.




Figures 9-11. *Anagyrus aligarhensis* Agarwal & Alam, male: 9. antenna; 9a. F6 and basal part of clava enlarged showing scale-like sensilla; 10. head, frontal view; 11. fore wing.

Metasoma elongate, longer than head and mesosoma combined, and 1.53X as long as mesosoma; ovipositor as in Fig. 8; outer plate of ovipositor as in Fig. 7; ovipositor with second valvifer 3.55X as long as third valvula. *Relative measurements* (from slide): TVII length, 66; ovipositor length, 82; third valvula length, 18. [Ovipositor 1.7X as long as mid tibia; third valvula longer than both mid basitarsus and mid tibial spur.]

*Male.* Length, 0.74–0.92 mm (n = 3). Similar to female in color and sculpture, but differs in antennal structure, genitalia, and head dimensions.

Head, in frontal view (Fig. 10), 1.3X as broad as high; frontovertex width 0.56X head width; antennal torulus with lower margin in line with lower margin of eye; eye height 1.82X malar space. Antenna as in Fig. 9; F6 and clava basally with scale-like sensilla (Fig. 9a). Fore wing 2.35X as long as broad, venation and discal setation as in Fig. 11. Hind wing 5.95X as long as broad, and 0.73X fore wing length. Gaster as long as mesosoma; phallobase without digiti, and 0.88X mid tibia length.

Material examined. 5 females, 4 males. TURKEY: Adana, 5 females (one on slide, No. EH.1792), 12.ix.2014, Coll. A.F. Çalışkan (No. 31-d); Adana, 2 males (one on slide, No. EH.1798), 4.ix.2014, Coll. A.F. Çalışkan (No. 7-b); 1 male, 12.ix.2014, Coll. A.F. Çalışkan (No. 31-b); 1 male, 12.ix.2014, Coll. A.F. Çalışkan (No. 37). All specimens ex *Phenacoccus solenopsis* on *Hibiscus rosa-sinensis* L. (Malvaceae). (ZDAMU)

Hosts. *Phenacoccus solenopsis* Tinsley, in Turkey (new host record). See Noyes & Hayat (1994) for other host records.

Distribution. England, Spain, western and southern former USSR, Hungary, Portugal, Italy, Bulgaria, former Yugoslavia, Romania, France, Turkey, Iran, India, Nepal, China, Indonesia; USA (Texas, introduced) (Noyes & Hayat, 1994).

***Anagyrus* sp. near *dactylopii* (Howard)** (Figures 12-19)

*Female.* Length, 1.7 mm. Habitus, Fig. 12. Head yellow; occiput and intertorular area brown; mouth margin and malar space dark brown. Antenna with radicle, scape except a small basal spot and a subapical curved white band, dark brown; pedicel in about basal third dark brown, distal two-thirds white; F1 dark brown; F2–6 and clava, white. Mesosoma yellow except as follows: pronotum in about middle half and anterior fourth of mesoscutum, dark brown; sutures dark brown; axilla and scutellum washed with pale brownish yellow; scutellum with a mid-longitudinal brown streak; metanotum laterally brown; propodeum mesal to spiracles and along lateral margins brown. Fore wing hyaline, with discal setae especially distal to linea calva minute, and translucent. Hind wing hyaline. Legs, including fore and mid coxae, white; both margins of fore femur and fore tibia pale brown; upper (= outer) margin of mid femur pale brown; extreme base of mid tibia dark brown, and with a diffuse pale brown infuscation in about basal third; hind femur washed with very pale brown; extreme base of hind tibia with a small dark brown spot, and its outer margin pale brown. Gaster dark brown; ovipositor sheaths (= third valvulae) dark brown.

Head. Frontovertex width 0.32X head width; head, in frontal view (Fig. 14), 1.14X as broad as high; antennal torulus close to mouth margin and with upper margin below lower margin of eye; eye height 2.53X malar space; frontovertex with raised rugose-reticulate sculpture (Fig. 14a); setae on head silvery white; eye setose, setae brown, and each seta at least about as long as a facet diameter. Antenna with scape 2X as long as broad, otherwise as in Fig. 13. *Relative measurements* (from slide): head frontal width, 58; head frontal height, 51; frontovertex width, 18.5; eye height, 38; malar space, 15; antennal scape length (width), 32 (16).

Mesosoma. Sculpture on mesoscutum and scutellum as in Figs 15 and 16; setae silvery white, except two pairs of brown setae subapically on scutellum; propodeum with silvery white setae, 5-6 proximal to each spiracle, and several setae distal to each spiracle. Fore wing 2.28X as long as broad; venation reaching to 0.52X wing length; costal cell with a single line of setae on ventral surface in distal two-thirds, and 3–4 lines in basal third; postmarginal vein virtually absent or very short, both combined 0.56X stigmal vein; linea calva interrupted posteriorly by 6–7 lines of setae, and the basal cutoff portion nearly rounded (Figs 19 and 19a). Hind wing 3.65X as long as broad. Mid tibia 2.94X as long as mid basitarsus; mid tibial spur 0.84X mid basitarsus length. *Relative measurements* (from slide): mesosoma length, 70; mesoscutum length (width), 30 (50); scutellum length (width), 31 (34); fore wing length (width), 132.5 (58); hind wing length (width), 95 (26); mid tibia length, 56; mid basitarsus length, 19; mid tibial spur length, 16.

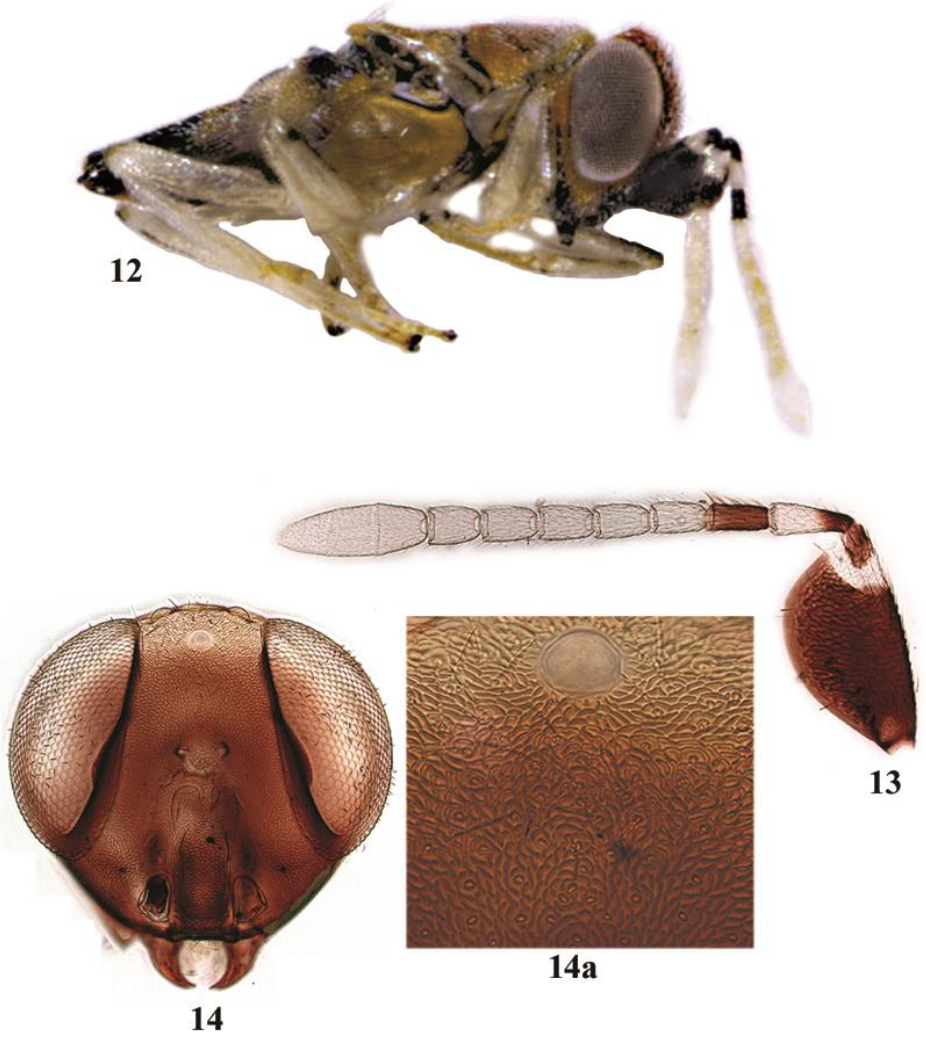
Metasoma. Ovipositor as in Fig. 17; outer plate of ovipositor as in Fig. 18. *Relative measurements* (from slide): TVII length, 65; ovipositor length, 60; third valvula length, 12.5. [Ovipositor slightly longer than mid tibia, 60:56; third valvula distinctly shorter than both mid basitarsus and mid tibial spur, 12.5:19:16.]

*Male.* Unknown.

Material examined. 1, female (on slide under 5 coverslips, slide No. EH.1794). TURKEY: Adana, 12.ix.2014 Coll. A.F. Çalışkan (31-c) and ex *Phenacoccus solenopsis* on *Hibiscus rosa-sinensis* L. (ZDAMU)

Host. *Phenacoccus solenopsis* on *Hibiscus rosa-sinensis* L. (Malvaceae).

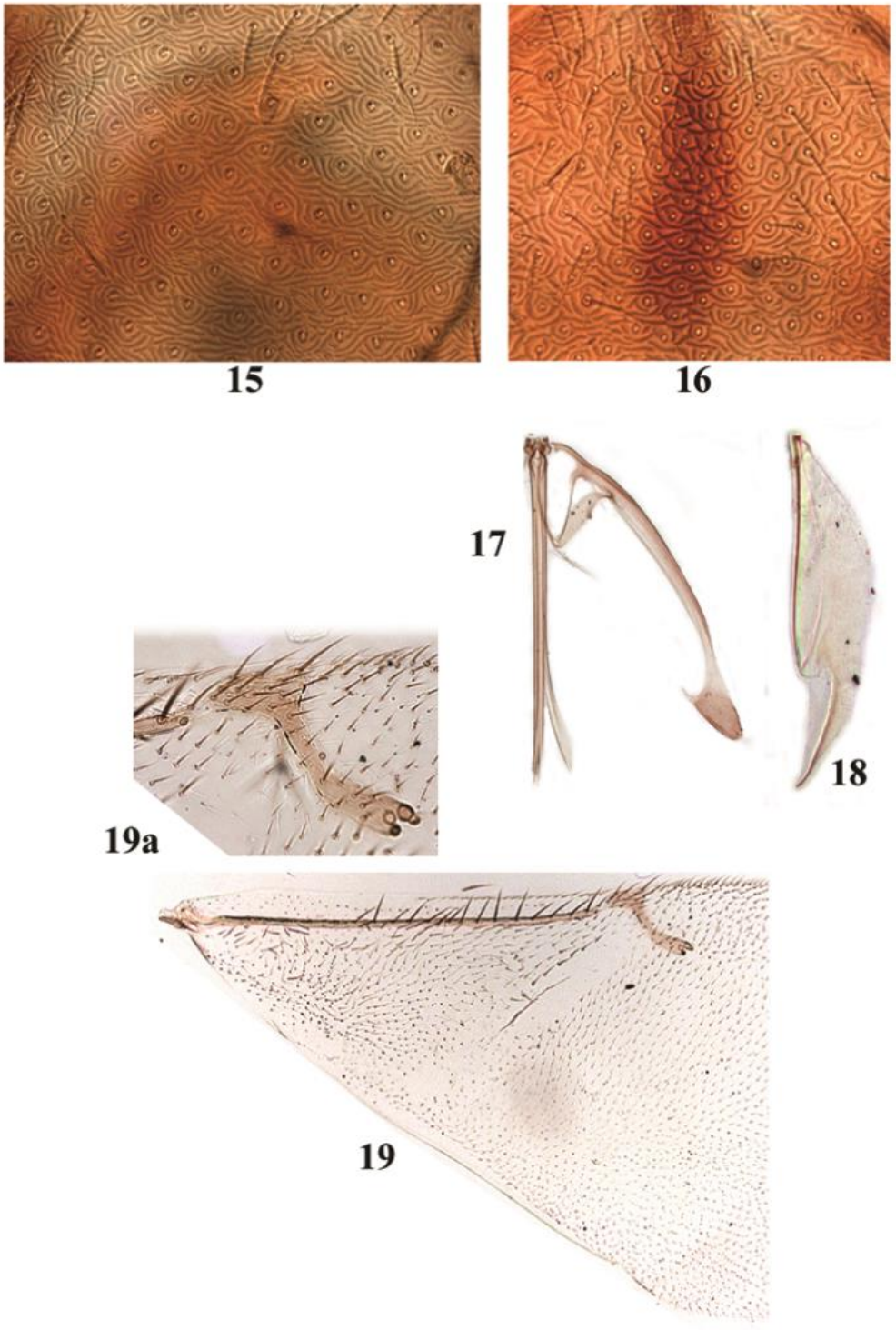
Distribution. Turkey (Adana).




Figures 12-14. *Anagyrus* sp. near *dactylopii* (Howard), female: 12. body, lateral view; 13. antenna; 14. head, frontal view; 14a. sculpture on frons.

Comments. This *Anagyrus* sp. is apparently similar to *A. dactylopii* (Howard) based on the description and key characters given by Noyes & Hayat (1994) (See also the paper on *A. pseudococci* (Girault) and related species or forms, by Triapitsyn et al., 2007). However, it differs from *A. dactylopii*, *A. pseudococci* and *Anagyrus chrysos* Noyes & Hayat (1994), by the following combination of characters: frontovertex width nearly one-third head width; antennal scape 2X as long as broad; fore wing with postmarginal vein very short or absent; costal cell with a single line of setae on ventral surface in distal two-thirds which become 3-4 lines in proximal third; ovipositor slightly longer than mid tibia; third valvula (fused with second valvifer, a generic character) short, 0.26X second valvifer length, and distinctly shorter than mid basitarsus or mid tibial spur.

We initially considered this species from Turkey as a new species, but following the opinion of one of the reviewer, eventually decided to regard it as a species near *A. dactylopii*. However, we are describing this form or strain reared from *P. solenopsis* in Turkey because it may eventually prove to be of help to those dealing with the identities of the *pseudococci*-complex based on the morphological characters.




Figures 15-19. *Anagyrus* sp. near *dactylopii* (Howard), female: 15. sculpture on mesoscutum; 16. sculpture on scutellum; 17. ovipositor; 18. outer plate of ovipositor; 19. fore wing, basal part; 19a. distal veins of fore wing.

***Leptomastix epona* (Walker)** (Figures 20-31)

*Encyrtus epona* Walker, 1844: 184, male.

*Leptomastix epona* (Walker): Graham, 1969: 217.

*Leptomastix histrio* Mayr: Mercet, 1921: 123, female, male. Misidentification of *L. epona* according to Graham, 1969: 217.

**Redescription**

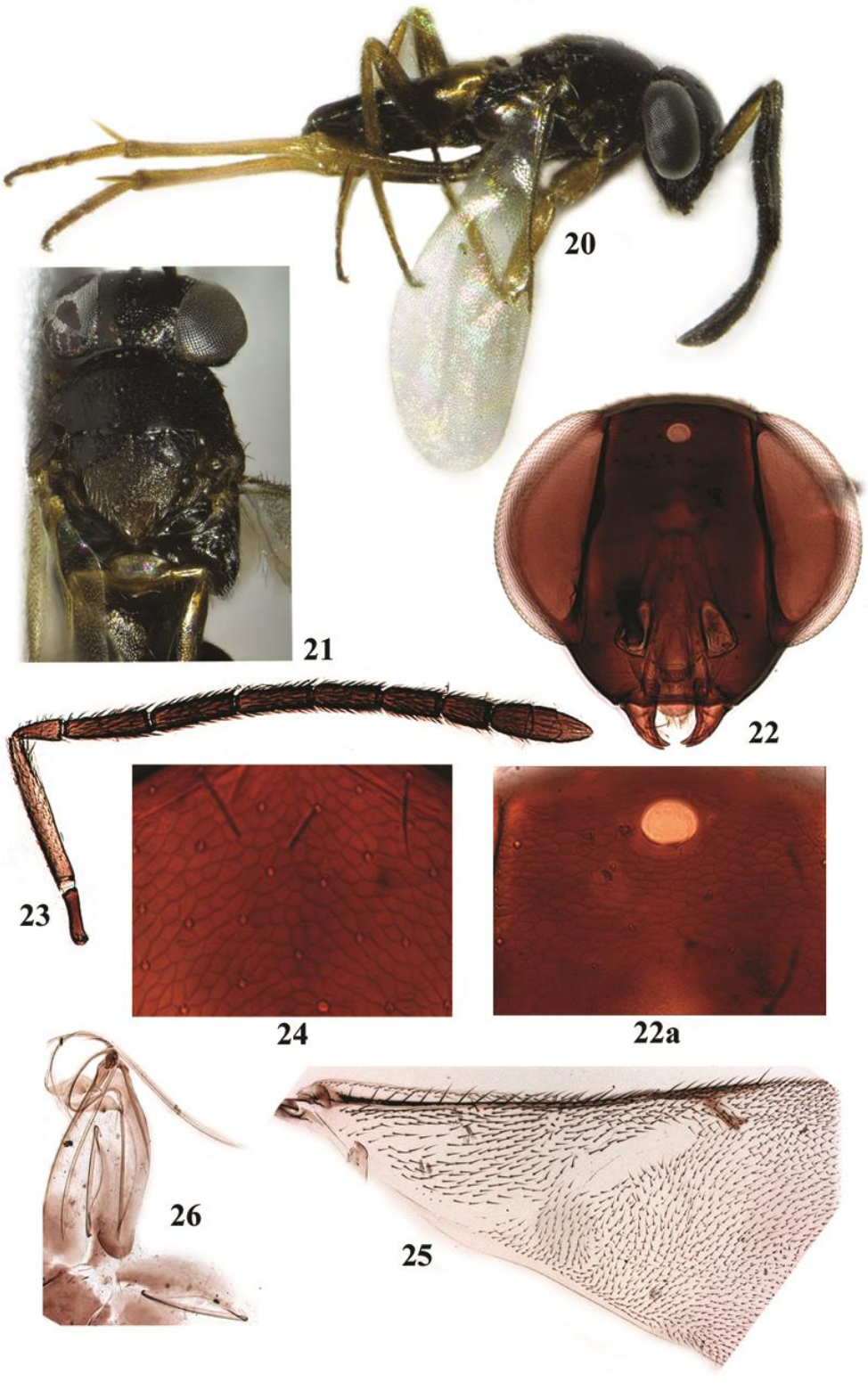
Female. Length, 1.49 mm, 1.75 mm. Body completely dark brown to nearly black (Fig. 20). Head in one specimen with a fine yellow line along each eye margin (Fig. 22). Antenna dark brown; scape ventrally yellow. Scutellum dark brown to yellowish brown, but in one specimen nearly black. Wings hyaline. Legs, including fore coxa, yellow to brownish yellow, except as follows: mid and hind coxae dark brown; mid femur in about basal two-thirds, hind femur except apex, dark brown; fore tarsus brown; mid tarsus with segments 1-4 each apically brown, fifth segment dark brown; hind tarsus with basitarsus brownish yellow, second segment onwards gradually becoming brown to dark brown. In one specimen, fore coxa dark brown; fore femur brown in about basal half; mid femur except apex, dark brown. Gaster with TI across base and postero laterally yellow, with a black spot on each side; in one specimen TI completely dark brown; ovipositor sheaths dark brown.

Head (Fig. 21). Occipital margin sharp; head, in dorsal view, 2.45X as broad as frontovertex or frontovertex width 0.4X head width; ocellar triangle with apical angle a right angle; POL, OOL, OCL, ratios, 9:3:4; head, in frontal view, 1.08X as broad as high, but on slide 1.18X as broad as high (Fig. 22); scrobes inverted U-shaped, margins rounded; intertorular area convexly elongate with a mid-longitudinal ridge; head, in profile, with eye height 3.27X malar space; frontovertex with slightly raised polygonal reticulate sculpture, the cells small (Fig. 22a), and with sparse, silvery white setae; the sculpture fine on facial region, and elongate reticulate on malar space; eye bare. Antenna (Fig. 23) with scape about 7X as long as broad; F1 nearly 2X as long as pedicel; clava slightly shorter than F5 and F6 combined. Relative measurements (from card): head dorsal width, 54; frontovertex width, 22; head frontal height, 50; eye height, 36; malar space, 11.

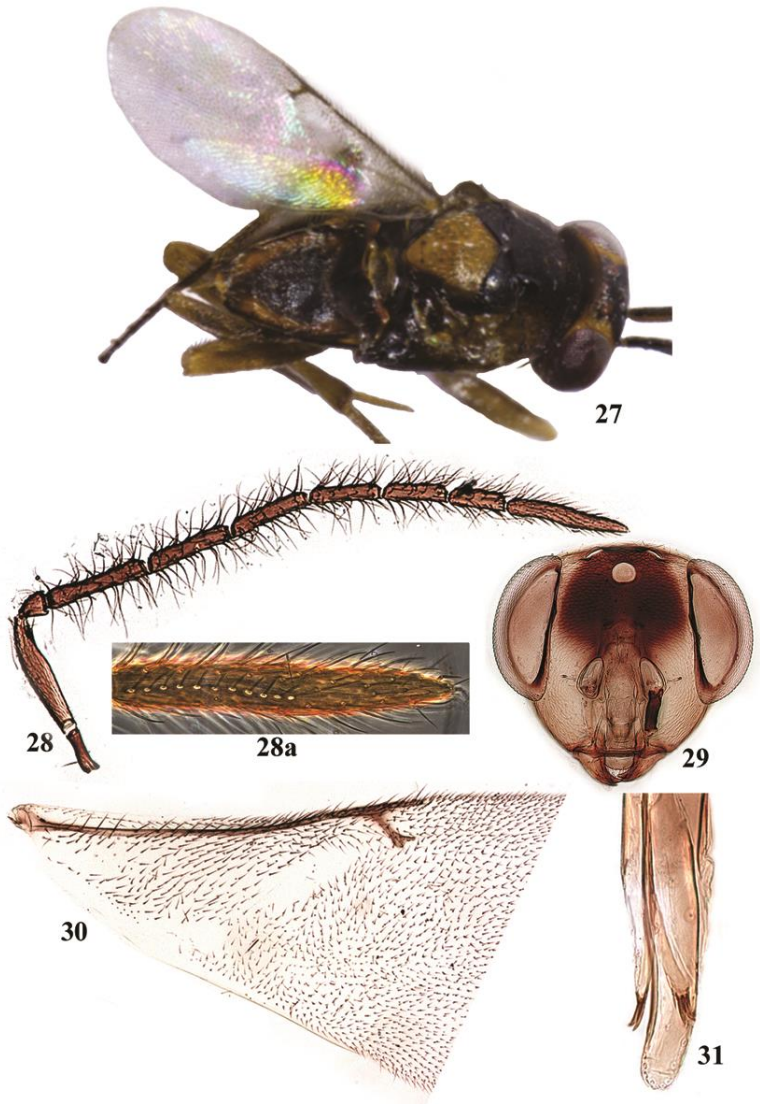
Mesosoma (Fig. 21). Pronotum with transversely elongate reticulate sculpture; mesoscutum with fine polygonal reticulate sculpture; axilla with slightly raised, reticulate sculpture; scutellum with irregular, polygonal reticulations, only slightly deeper than on mesoscutum; pronotal collar with brown setae; mesoscutum with off-white to pallid setae, except brown setae along posterior margin; scutellum with off-white to pallid setae, except 4 brown subapical setae; propodeum on both sides of spiracle with white setae. Fore wing 2.77X as long as broad; marginal vein at least 1.3X as long as postmarginal vein which is subequal in length to stigmal vein; otherwise, wing venation and discal setation as in Fig. 25. Mid tibia 3X as long as mid basitarsus; mid basitarsus slightly longer than mid tibial spur. *Relative measurements* (from slide): fore wing length (width), 172 (62); mid tibia length, 84; mid basitarsus length, 28; mid tibial spur length, 26.

Metasoma. Ovipositor and outer plate as in Fig. 26. *Relative measurements* (from slide): TVII length, 80; ovipositor length, 49; third valvula length, 7. [Ovipositor 0.58X mid tibia length; third valvula 0.25X mid basitarsus length, and 0.27X mid tibial spur length.]

Male. Length, 1.05–1.46 mm (n = 6). Color, especially of the head, and mesoscutum and scutellum variable. Head varies from dark brown with narrowly yellow along eye margins, and brownish yellow between toruli to mouth margin; the bands along eye margins may be broader, and facial region and malar space completely yellow to pale brownish yellow (Fig. 29). Antenna dark brown, with radicle nearly black, and ventral half of scape yellow to brownish yellow. Mesosoma completely dark brown, but mesoscutum on sides from narrowly brownish yellow to one-fourth to one-third of sides yellow to brownish yellow; scutellum dark brown to yellowish brown with brown to completely yellow apically (Fig. 27). Leg color about as in female, but fore femur except apex brown to completely yellow; mid femur brownish yellow, and hind femur dark brown except pale apex to light brown; fore tarsus brown; mid tarsus with segments 1–3 segments apically dark brown, segments fourth and fifth dark brown; hind tarsal segment 1 or segments 1 and 2 yellowish, segments 2–5 or 3–5 dark brown.




Figures 20-26. *Leptomastix epona* (Walker), female: 20. body, lateral view; 21. head, mesosoma and TI of gaster, dorsal view; 22. head, frontal view; 22a. sculpture on frons; 23. antenna; 24. sculpture on scutellum; 25. fore wing, basal half; 26. ovipositor and outer plate.




Figures 27-31. *Leptomastix epona* (Walker), male: 27. body, dorsolateral view; 28. antenna; 28a. clava enlarged showing scale-like sensilla; 29. head, frontal view; 30. fore wing, basal half; 31. genitalia, distal part.

Head (Fig. 29) with frontovertex width about 0.5X head width; torulus with lower margin in line with lower margin of eye. Antenna as in Fig. 28; only clava with a line of scale-like sensilla (Fig. 28a). Fore wing venation and setation as in Fig. 30. Genitalia (Fig. 31) with phallobase (20) slightly shorter than mid tibial spur (22.5).

Material examined. 3 females, 8 males. TURKEY: Adana, 3 females (one on slide, No. EH.1795), 12.ix.2014, Coll. A.F. Çalışkan (No. 31-b); Adana, 6 males (two on slides, Nos. EH.1796 and EH.1876), 12.ix.2014, Coll. A.F. Çalışkan (No. 31-c); Adana, 1 male, 4.ix.2014, Coll. A.F. Çalışkan (No. 12-b); Adana, 1 male, 4.ix.2014, Coll. A.F. Çalışkan (No. 18). All specimens ex *Phenacoccus solenopsis* on *Hibiscus rosa-sinensis* L. (Malvaceae). (ZDAMU)

Hosts. *Phenacoccus solenopsis* Tinsley in Turkey (new host record). For other hosts see Noyes (2015).

Distribution. Turkey (new record). Recorded from several countries in the Palearctic Region, and the USA (California) (See Noyes, 2015).

Comments. The problem with the identity of *Leptomastix epona* (Walker, 1844) is a little complicated and dealt with by Graham (1969). In short, it may be stated that the genus *Leptomastix* Foerster (1856) was erected without any included species, and it was Mayr (1876) who included his (new) species, *histrio* which thus became the type species by subsequent reference. Foerster in the same paper (1856) described *Sterrhocoma* and included one species, *histrio* Foerster. Graham (1969: 216–218) synonymized *Sterrhocoma* with *Leptomastix*, and has shown that *L. histrio* Mayr of Mercet (1921: 121–123) is a misidentification for *L. epona* (Walker). It is, however, not clear how *L. histrio* (Foerster) differs from *L. histrio* Mayr or *L. epona* (Walker). As *L. histrio* (Foerster, 1856) is a senior homonym of *L. histrio* Mayr (1876), Özdikmen (2011: 804) proposed the replacement name *L. mayri* Özdikmen for the junior homonym.

The specimens identified as *L. epona* may be confused with *L. algirica* Trjapitzin (1989). However, Anga & Noyes (1999) note that *L. algirica* may be a black form of *L. epona* since they indicated that it was not possible to find any consistent morphological characters to separate the two species.

It is apparent that the problem of identity of *L. histrio* (Foerster) remains unsolved. Our identification of the Turkish *Leptomastix* as *L. epona* is largely based on the description of the male by Walker (1844), and of both sexes of *epona* misidentified by Mercet (1921) as *histrio* Mayr. This is also supported by the presence of pale setae on the mesoscutum and scutellum of *epona* as given in the key by Graham (1969: 213). We have, therefore, redescribed *L. epona* based on the Turkish specimens obtained from *Phenacoccus solenopsis.*

**Hyperparasitoid**

***Prochiloneurus uyguni* Hayat, sp. n.** (Figures 32-34)

*Female.* Holotype (Fig. 32). Length, 1.2 mm. Head dark brown; face in scrobal impression brownish yellow; occiput brownish yellow with dark brown in upper part; frontovertex with purple shine. Antenna (Figs 32 and 33) with scape pale yellow, with base and both margins dark brown, except apex yellow; pedicel, F1and F2 brown, F3–5 white, and F6 dark brown; clava dark brown to nearly black. Mesosoma with pronotal collar white, otherwise pronotum brownish yellow and medially dark brown; mesoscutum dark brown; axilla yellowish brown; scutellum dark brown; tegula brownish yellow, apically brown; metanotum pale brown; propodeum brown; prepectus yellow; mesopleuron anteriorly yellow, becoming in large part pale brownish yellow. Fore wing (Fig. 34) hyaline in basal two-sevenths and apically, rest infuscate, the infuscation distally convex, with the width of the hyaline apex one-sixth of width of infuscated area. Hind wing hyaline. Legs with all coxae and trochanters white; fore leg with femur white, dark brown in distal fourth or so; tibia yellow with a faint brown streak on outer surface; tarsus yellow to brownish yellow; mid leg with femur white, brownish along outer surface in distal half, except white apex; tibia brownish from base to near apex, the apex nearly white; tarsus and spur white; hind leg with femur yellowish white with a brownish streak on inner surface in about distal half; tibia dark brown with base and a streak at apical fifth on outer surface, white; tarsus with segments 1–4 white, fifth brownish yellow. Gaster dark brown with violet and greenish blue shine; exserted part of ovipositor yellow.

Head. Occipital margin sharp, concave between eyes; frontovertex width one-sixth (0.16X) head width; eye posteriorly separated from occipital margin by about two facet diameters; ocellar triangle with apical angle acute; posterior ocellus touching eye margin; OOL 2X POL, and 0.5X AOL; head, in frontal view, 1.36X as high as broad; upper margin of facial impression straight, transverse and reaching only to one-fourth head height; head, in profile, with eye height 1.68X malar space; vertex with raised, polygonal reticulate sculpture, the cells very small; frons with hardly raised sculpture; frontovertex with sparse, short, white setae; eye apparently bare. Antenna (Fig. 33) with scape 6X as long as broad; pedicel as long as F1 and F2 combined; F1 slightly longer than broad, F 2–5 quadrate to slightly broader than long; F6 nearly 1.5X as broad as long; clava large, strongly truncate, and as long as funicle. *Relative measurements* (from card): head dorsal width, 30; frontovertex width, 5; head frontal height, 41; eye height, 27; malar space, 16; distance from mouth margin to upper margin of facial impression, 10.


Figures 32-34. *Prochiloneurus uyguni* Hayat, sp. n., holotype, female: 32. body, lateral view; 33. antenna; 34. fore wing.

Mesosoma (Fig. 32) 1.25X as long as metasoma; mesoscutum in about anterior half with slightly raised polygonal reticulate sculpture and in about posterior half finely reticulate; scutellum with raised, polygonal reticulate sculpture, cells very small; pronotal collar with silvery white setae; mesoscutum in about anterior half with golden brown setae, in about posterior half with silvery white setae; axilla (strongly elevated above surface of scutellum) with largely brown setae; scutellum with golden brown setae; scutellar brush of black setae (14 long setae followed by several shorter setae, about two-thirds the length of the longer setae); with a subapical pair of white, recurved setae; propodeum with sides, proximal as well as distal to spiracles, with silvery white setae. Fore wing (Fig. 34) 2.76X as long as broad; disc in basal hyaline area with a few translucent setae adjacent to proximal margin of infuscation; apical hyaline area with brown setae. Hind wing 3.56X as long as broad. *Relative measurements* (from card): mesosoma length, 44; mesoscutum length (width), 20 (32); scutellum length (width), 19 (21); (from slide, at 100X): fore wing length (width), 108 (39); hind wing length (width), 89 (25).

Metasoma (Fig. 32) with exserted part of ovipositor 0.62X gaster length.

*Male.* Unknown.

Material examined. Holotype (ZDAMU; registration No. HYM.CH.735), female (on card, with one antenna and both fore wings and left hind wing, on slide, No. EH.1797), labeled “TURKEY: Adana, 12.ix.2014 Coll. A.F. Çalışkan (41-b)” and “*Phenacoccus solenopsis* on *Lantana camara* L.”

Host. Hyperparasitoid on encyrtid primary parasitoid of *Phenacoccus solenopsis* on *Lantana camara* L. (Verbenaceae).

Distribution. Turkey (Adana).

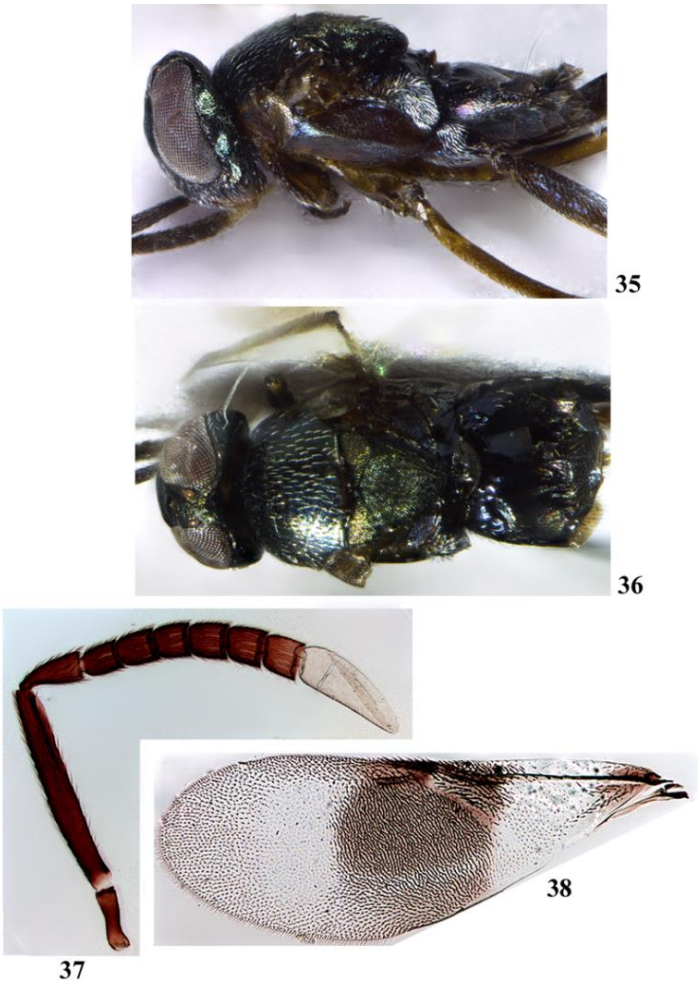
Etymology. This species is named after one of the most well-known entomologists who worked on the Coccinellidae (Coleoptera) in Turkey, Prof. Dr. Nedim Uygun (Çukurova University, Agriculture Faculty, Plant Protection Department, Adana, Turkey).

Comments. This new species was initially confused with *P. dactylopii* (Howard) [Synonym: *Achrysopophagus argentinensis* De Santis, 1964], but differs in several characters: head and mesosoma, except axilla, dark brown; head, in frontal view, 1.36X as high as broad; frontovertex width one-sixth head width; posterior ocellus touching eye margin; eye height 1.68X malar space; antennal scape 6X as long as broad; and width of hyaline apex of fore wing one-sixth width of infuscate area. In *P. dactylopii*, based on the description given by De Santis (1964): head and mesosoma orange yellow, with posterior third of mesoscutum dark brown; head, in frontal view, as broad as high; frontovertex width one-fourth head width; posterior ocellus separated from eye margin by one ocellus diameter; eye height 1.45X malar space; antennal scape 4.63X as long as broad; width of hyaline apex of fore wing 0.4X width of infuscate area.

**Associate parasitoid of predator**

***Homalotylus hemipterinus* (De Stefani)** (Figures 35-38)

*Homalotylus hemipterinus* (De Stefani): Noyes, 2010: 146-148, female, male, figures, synonymy, diagnosis, material listed from several countries in the Palaearctic, Oriental, Afrotropical and Neotropical Regions.




Figures 35-38. *Homalotylus hemipterinus* (De Stefani), female: 35. body, lateral view; 36. body, dorsal view; 37. antenna; 38. fore wing.

Material examined. 2 females (one antenna and one fore wing of one female on slide, No. EH.1880), 1 male. TURKEY: Adana, 4.ix.2014, Coll. A.F. Çalışkan (No. 11-b) and [with] *Phenacoccus solenopsis*. (ZDAMU)

Host. The real host of this species is some coccinellid beetle (Coleoptera: Coccinellidae) whose larvae feed on the mealybug, *Phenacoccus solenopsis* on *Lantana camara.*

Distribution. Turkey (new record). See Noyes (2015) for world distribution.

Comments. This is a well-known species with a wide distribution, and was recently considered by Noyes (2010). Therefore, we are only providing some relevant figures which may be of help in identifying this species in Turkey.

## Acknowledgments

The authors (MRU, MBK, AFC) would like to thank Ç.Ü. Scientific Research Foundation (Project No. ZF2013BAP8) for their financial support. One of us (MH) particularly thanks the Indian Council of Agricultural Research, New Delhi and the Director, Dr. A. Verghese, National Bureau of Agricultural Insect Resources, Bengaluru and Coordinator of the Network Project on Insect Biosystematics, for financial help; the Aligarh Muslim University, Aligarh, and the Chairman, Department of Zoology, for providing research facilities; and Dr. J.S. Noyes, Natural History Museum, London, for his comments on the status of *Leptomastix* species dealt with in this paper which proved to be helpful in identifying the Turkish specimens as *L. epona*.

## References

Abbas, G., M. J. Arif, M. Ashfaq, M. Aslam & S. Saeed, 2010. The impact of some environmental factors on the fecundity of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae): a serious pest of cotton and other crops. Pakistan Journal of Agricultural Sciences, 47(4): 321–325.

Agarwal, M. M. & S. M. Alam, 1959. "New species of the genus *Anagyrus* Howard (Chalcidoidea: Encyrtidae) recorded from Aligarh" In: Proceedings of the 4th Indian Science Congress, Part III: 392 pp.

Agarwal, M. M. 1965. Taxonomy of encyrtid parasites (Hymenoptera: Chalcidoidea) of Indian Coccoidea. Acta Hymenopterologica, Fukuoka, 2: 37–97.

Anga, J-M. & J. S. Noyes, 1999. A revision of the African and Malagasy species of the genus *Leptomastix* (Hymenoptera, Encyrtidae), parasitoids of mealybugs (Homoptera: Pseudococcidae). Bulletin of the Natural History Museum London (Entomology), 68 (2): 93–128.

De Santis, L., 1964. Encirtidos de la Republica Argentina (Hymenoptera: Chalcidoidea). Anales de la Comisión de Investigación Cientifica Provincia de Buenos Aires Gobernación, 4: 9–422.

Fand, B. B. & S. S. Suroshe, 2015. The invasive mealybug *Phenacoccus solenopsis* Tinsley, a threat to tropical and subtropical agricultural and horticultural production systems. Crop Protection, 69: 34–43.

Foerster, A., 1856. Hymenopterologische Studien. II Heft. Chalcidiae und Proctotrupii. 152 pp. Aachen.

García M., B. Denno, D. R. Miller, G. L. Miller, Y. Ben-Dov & N. B. Hardy, 2015. ScaleNet: A Literature-based model of scale insect biology and systematics. (Web page: http://scalenet.info).

Graham, M. W. R. de V., 1969. Synonymic and descriptive notes on European Encyrtidae (Hym., Chalcidoidea). Polskie Pismo Entomologiczne, 39 (2): 211–319.

Hayat, M., 2003. Records and descriptions of Indian Encyrtidae (Hymenoptera: Chalcidoidea). Oriental Insects, 37: 187–259.

Hayat, M., 2006. Indian Encyrtidae (Hymenoptera: Chalcidoidea).Published by M. Hayat, Department of Zoology, Aligarh Muslim University, India viii + 496 pp.

Howard, L. O. 1894. Report upon the parasitic Hymenoptera in the island of St. Vincent. Journal of the Linnean Society (Zoology), 25: 56–254.

Kaydan, M. B., Çalışkan, A. F. & M. R. Ulusoy, (2013). New record of invasive mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in Turkey. EPPO Bulletin, 43: 169–171.

Kosztarab M. & F. Kozár, 1988. Scale Insects of Central Europe. Akademiai Kiado, Budapest, Hungary, and Dr W. Junk Publishers, Dordrecht, The Netherlands, 456 pp.

Mayr, G., 1876. Die europäischen Encyrtiden. Verhandlungen der Zoologisch-Botanischen Gessellschaft in Wien, 25, 675–778.

McKenzie, H. L., 1967. Mealybugs of California with taxonomy, biology, and control of North American species (Homoptera: Coccoidea: Pseudococcidae). California: University of California Press.

Mercet, R. G., 1921. Fauna Ibérica Himenópteros Fam. Encírtidos. Museo Nacional de Ciencias Naturales, Madrid, Spain, 727 pp.

Noyes, J. S. & M. Hayat, 1994. Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae).CAB International, Wallingford, Oxon, UK, viii + 554 pp.

Noyes, J. S., 2000. Encyrtidae of Costa Rica (Hymenoptera: Chalcidoidea), 1. Memoirs of the American Entomological Institute*,* 62: 1–355.

Noyes, J. S., 2010. Encyrtidae of Costa Rica (Hymenoptera: Chalcidoidea), 3. Memoirs of the American Entomological Institute*,* 84: 1–848.

Noyes, J. S., 2015. Universal Chalcidoidea Database. (Web page: http://www.nhm.ac.uk/research-curation/projects/chalcidoids) (Date accessed: 30 December 2015).

Özdikmen, H., 2011. New names for some preoccupied specific epithets in Chalcidoidea I: Families Agaonidae, Aphelinidae, Chalcididae, Encyrtidae, Eulophidae (Hymenoptera: Parasitica). Munis Entomology & Zoology*,* 6 (2): 796–814.

Triapitsyn, S. V., D. González, D. B. Vickerman, J. S. Noyes & E. B. White, 2007. Morphological, biological, and molecular comparisons among the different geographical populations of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), parasitoids of *Planococcus* spp. (Hemiptera: Pseudococcidae), with notes on *Anagyrus dactylopii*. Biological Control, 41: 14–24.

Trjapitzin, V. A., 1989. Parasitic Hymenoptera of the fam. Encyrtidae of Palaearctics. Opredeleteli po Faune SSSR, Izdavavaemiye Zoologicheskim Institutom Akademii Nauk SSSR, 158: 1–489.

Walker, F., 1844. Descriptions of some British chalcidites. Annals and Magazine of Natural History*,* 14: 181–185.

Williams, D. J., 2004. Mealybugs of Southern Asia. The Natural History Museum, London, UK and Southdene SDN. BHD., Kuala Lumpur, Malaysia, 896 pp.

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*Original article (Orijinal araştırma)*

# The lepidopteran pests of sweet potato: First record of *Helcystogramma triannulella* (Herrich-Schäffer) (Lepidoptera: Gelechiidae) with population development and natural enemies in Turkey[1]

Tatlı patatesteki zararlı lepidopterler: Türkiye'de *Helcystogramma triannulella* (Herrich-Schäffer) (Lepidoptera: Gelechiidae)'nın ilk kaydı, popülasyon gelişmesi ve doğal düşmanları

**Kamuran KAYA[2]*** **Feza CAN CENGIZ[3]**

**Mehmet Emin ÇALIŞKAN[4]** **Sevgi ÇALIŞKAN[4]**

## Summary

The study was conducted in Hatay Province, east Mediterranean Region, Turkey in 2012 and 2013 to determine major lepidopteran pests of sweet potato and to document the population development of the newly recorded species *Helcystogramma triannulella* (Herrich-Schäffer) (Lepidoptera: Gelechiidae) in 2013. Four lepidopteran species, *Aedia leucomelas* (L.) (Noctuidae), *Agrius convolvuli* (Linnaeus) (Sphingidae), *Hydriris ornatalis* (Duponchel) (Crambidae) and *H. triannulella* were found. This was the first detection of *H. triannulella* in Turkey, so its population development was studied in the second year. The larval population of *H. triannulella* began to increase towards the end of July and reached its peak in mid-August. During the study, predators, *Hippodamia variegata* (Goeze), *Oenopia conglobata* (L.), *Scymnus interruptus* (Goeze), *Scymnus mediterraneus* Khnzorian, *Stethorus gilvifrons* (Mulsant) (Coleoptera; Coccinellidae); *Nabis viridulus* Spinola (Hemiptera: Nabidae) were found in the folded parts of the leaves, and parasitoids, *Apanteles* sp., *Chelonus* sp. (Hymenoptera: Braconidae) and *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) were obtained in the laboratory from larvae of *H. triannulella*.

**Keywords:** *Helcystogramma triannulella, Ipomoea batatas*, first record, sweet potato

## Özet

Bu çalışma Türkiye'nin, Doğu Akdeniz Bölgesi'nde bulunan Hatay ilinde tatlı patates bitkisinin başlıca lepidopter zararlılarını ortaya çıkarmak amacıyla 2012-2013 yıllarında yürütülmüş ve Türkiye için yeni kayıt tür olan *Helcystogramma triannulella* (Herrich-Schäffer) (Lepidoptera: Gelechiidae) 'nın popülasyon gelişimi 2013 yılında belirlenmiştir. Tarla gözlemleri sonucunda dört lepidopter tür, *Aedia leucomelas* (L.) (Noctuidae), *Agrius convolvuli* (Linnaeus) (Sphingidae), *Hydriris ornatalis* (Duponchel) (Crambidae) ve *H. triannulella* belirlenmiştir. Bu çalışmada *H. triannulella* Türkiye'de ilk kez kaydedilmiştir. İkinci yıl bu türün popülasyon gelişmesi araştırılmıştır. Deneme alanında *H. triannulella*'nın larva popülasyonu temmuz sonunda artmaya başlamış ve ağustos ortasında tepe noktasına ulaşmıştır. Çalışma boyunca predatörler, *Hippodamia variegata* (Goeze), *Oenopia conglobata* (L.), *Scymnus interruptus* (Goeze), *Scymnus mediterraneus* Khnzorian, *Stethorus gilvifrons* (Mulsant) (Coleoptera; Coccinellidae); *Nabis viridulus* Spinola (Hemiptera: Nabidae) yaprakların katlanmış kısımlarından ve parazitoidler *Apanteles* sp., *Chelonus* sp. (Hymenoptera: Braconidae) ve *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) laboratuvarda *H. triannulella* larvalarından elde edilmişlerdir.

**Anahtar sözcükler:** *Helcystogramma triannulella, Ipomoea batatas*, yeni kayıt, tatlı patates

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[2] Mustafa Kemal University, Agricultural Research and Application Center 31040 Hatay, Turkey
[3] Mustafa Kemal University, Agricultural Faculty, Plant Protection Deparment, Entomology Section 31040 Hatay, Turkey
[4] Nigde University, Faculty of Agricultural Sciences and Technology, 51240 Niğde, Turkey
* Corresponding author (Sorumlu yazar) e-mail: kayakamuran@gmail.com
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## Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam., Solanaceae], is native to South America, and it is now grown in more than 100 countries with a combined annual production of over 100 Mt (FAO, 2016). Its storage roots and green parts are used as food and animal nutrition (Bovell-Benjamin, 2007; Kaya & Çalışkan, 2010) as well as natural colorant in layer egg yolk (Kaya & Yıldırım, 2011). Dry matter content of storage roots is around 20-30%, while the starch, sugar and protein account for about 30,10 and 5% of total dry matter, respectively (Woolfe, 1992). Storage roots are also quite rich in provitamin A (β-carotene), vitamin C (ascorbic acid) and vitamin B complex (Edmond & Ammerman, 1971; Woolfe, 1992).

As an important cash crop for small farms, sweet potato is grown in some villages of Hatay Province, in the east Mediterranean Region of Turkey. Çalışkan et al. (2007) indicated that sweet potato could be adapted to both the Mediterranean and southeastern Anatolia region of Turkey, and that high yields could be achieved in these areas.

There are several biotic and abiotic factors that limit growth and yield of sweet potato crops around the world. Insects are one of major the biotic constraints to crop growth, yield and quality given that both sweet potato storage roots and haulms are damaged by a diversity of insects (Jansson & Raman, 1991; Schalk et al., 1991; Ames et al., 1997). Clearwing moths *Synanthedon* spp. (Lepidoptera: Sesiidae) are reported as storage root feeders and stemborers; sweet potato stemborer *Omphisia anastomasalis* (Lepidoptera: Pyralidae) is reported as stemborer; sweet potato butterfly *Acraea acerata* Hew. (Lepidoptera: Nymphalidae), sweet potato hornworm *Agrius convolvuli* L. (Lepidoptera: Sphingidae), armyworms *Spodoptera eridania* (Stoll)*, Spodoptera exigua* (Hübner) and *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), leaf folders *Helcystogramma convolvuli* (Walsingham) (Lepidoptera: Gelechiidae) and *Herpetogramma hipponalis* (Walker) (Lepidoptera: Pyralidae) are reported as foliage feeders of sweet potato by Ames et al. (1997). Also, leaf folders are considered important defoliators of sweet potato. *H. convolvuli* is known to be a common leaf folder of sweet potato. However, the leaf folder, *Helcystogramma triannulella* (Herrich-Schäffer) (Lepidoptera: Gelechiidae) had not been reported to occur in Turkey. *Helcystogramma triannulella* had been recorded in China, Europe, India, Kazakhstan, Korea, Japan and Russia (Ponomarenko, 1997; Li & Zhen, 2011). Across this distribution*, Ipomoea aquatica* Forsk, *Ipomoea batatas* (L.), *Calonyction aculeatum* (L.), *Calystegia sepium* var. *japonica* (Choisy), *Convolvulus aroensis*, *Convolvulus arvensis* L. and *Pharbitis nil* (L.) from Convolvulaceae, and *Hibiscus syriacus* L. from Malvaceae are recorded as host plants of *H. triannulella*. Insecticides are usually applied to suppress populations of this pest, but alternative control methods such as biological control and using resistant cultivars have also been applied recently in different regions (Jones et al., 1987; Chalfant et al., 1990; Jansson & Raman, 1991; Schalk et al., 1993; Thompson et al., 1999).

Choosing the most appropriate insect management program mainly depends on insect species, feeding types, damage level, as well as the socioeconomic status of growers. Hence, determination of major insect pests of sweet potato crops, as well as their effects on crop yield and quality in a certain environments is essential for ecological and economic pest control program. Unfortunately, there is currently limited information available about sweet potato insect diversity in the Mediterranean Basin. Initially this study was conducted to determine major lepidopteran pests of sweet potato crops in a Mediterranean-type environment in Turkey, then subsequently to investigate the population development and natural enemies of *H. triannulella*, which was detected for the first time in Turkey during the first year of the study.

## Materials and Methods

### Determination of sweet potato lepidopteran pests

The study was conducted in Reyhanlı, Hatay Province (36° 50” N, 36° 25” E, 128 m) in the Mediterranean Region of Turkey. Larval samples of Lepidoptera were collected by irregular sweet potato field surveys during 2012 and 2013. Larvae of each species were placed into different plastic containers with part of sweet potato foliage and taken to the laboratory. The collected larval samples were incubated

in a plant growth chamber at 26±1°C, 70±5% RH and 16L:8D photoperiod, until pupal formation or adult hatching, or emergence of any possible natural enemies. Emerged adults of lepidopteran pests were identified based on male genitalia. All specimens of the pest were deposited in the Museum of Mustafa Kemal University, Hatay, Turkey.

**Population development and natural enemies of *Helcystogramma triannulella***

In late July 2012, some folded leaves of sweet potato were noticed during field observations. When the folded leaves were examined, a larva was seen in each folded part of leaf (Figure 1). This was also found on *Convolvulus* sp. in these fields. Larvae of pest species were cultured to obtain adults in plastic containers in the laboratory. Four of the six larvae collected developed to adults within 10 d, while the other two died. The adult specimens were identified as *H. triannulella* by Ole Karsholt (Zoological Museum, Natural History Museum of Denmark, Universitetsparken 15, DK-2100 Copenhagen, Denmark). This was a new record for Turkey.

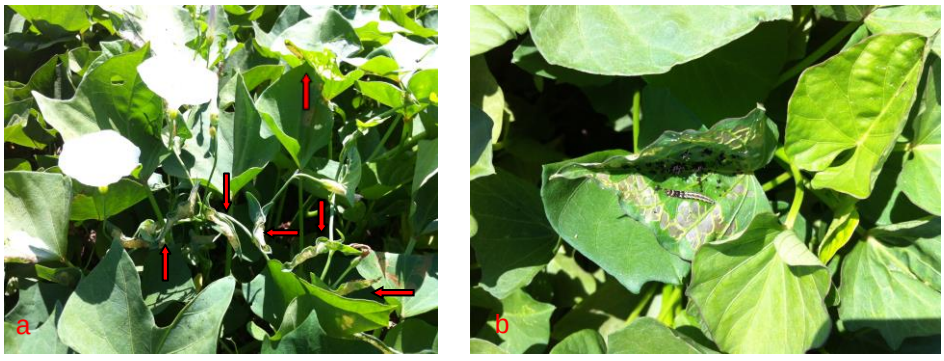


Figure 1. (a) Folded leaves of sweet potato, and (b) larva of *Helcystogramma triannulella.*

Consequently, in 2013, the population development of *H. triannulella,* was studied. For this purpose, sweet potato seedlings were planted on 11 June 2013 and 40 randomly selected plants were examined weekly to record the number of folded leaves and larvae within these folds. At the same time, folded leaves were also examined for predators; individual predators were collected for identification. Additionally several larvae (5-7) of the *H. triannulella* were collected every week and cultured to obtain potential parasitoids. Predator species (Nabidae and Coccinellidae) and tachinid parasitoids were mounted on insect pins, and braconid parasitoids were placed in 70% alcohol for identification.

## Results and Discussion

Four lepidopteran species, *Aedia leucomelas* (L.) (Noctuidae), *Agrius convolvuli* (L.) (Sphingidae), *H. triannulella* and *Hydriris ornatalis* (Duponchel) (Crambidae) were found in sweet potato fields. The lepidopteran species obtained were as follows:

***Aedia leucomelas* (L.) (Noctuidae)**

Material examined: 1♂, Reyhanlı, Hatay Province (36° 50” N, 36° 25” E, 128 m) 14.IX.2012, leg. K. Kaya.

Host plants: Asteraceae: *Chondrilla juncea* L.; Convolvulaceae: *Convolvulus arvensis* L., *Convolvulus erubescens* Sims, *Ipomoea batatas* (L.), *Ipomoea pes-caprae* **(**L**.)** R**.** Br**.,** (Anonymous, 2016a).

Distribution in Turkey: In the warm coastal areas close to the Black Sea and the Mediterranean Sea (Hacker, 1989; also Hacker, 1990): Adana, Amasya, Antalya, Bilecik, Bursa, Giresun, İstanbul, Ordu, Samsun, Tekirdağ (Koçak & Kemal, 2009) and Muğla (Baron, 2014).

***Agrius convolvuli* (L.) (Sphingidae)**

Material examined: 1♀, Reyhanlı, Hatay province (36° 50” N, 36° 25” E, 128 m) 23.VIII.2012; 1♂**,** 09.IX.2013, leg. K. Kaya.

Host plants: Convolvulaceae: *Calystegia* spp., *Convolvulus* spp., *Ipomoea batatas (L.)*; Zygophyllaceae: *Zygophyllum dumosum Boiss.* (Akın, 2012; Ames et al., 1997).

Distribution in Turkey: Amasya, Ankara, Balıkesir, Bursa, Konya, Malatya and Tokat (Koçak & Kemal, 2009).

***Hydriris ornatalis* (Duponchel) (Crambidae)**

Material examined: 1♂, 1♀, Reyhanlı, Hatay Province (36° 50" N, 36° 25" E, 128 m) 13.VIII.2013; 2♀♀, 18.VIII.2013, leg. K. Kaya.

Host plants: Convolvulaceae: *Ipomoea batatas (L.)*; Mimosaceae: *Acacia monticola* J.M. Black; Polygonaceae: *Emex spinosa* (L.); Rosaceae: *Malus pumila* Mill. (Anonymous, 2016b).

Distribution in Turkey: Adana and Antalya (Koçak & Kemal, 2009).

***Helcystogramma triannulella*** (Herrich-Schäffer)

Material examined: 2♂♂, 1♀, Reyhanlı, Hatay Province (36° 50" N, 36° 25" E, 128 m) 9.VIII.2012; 2♂♂, 10.VIII.2013; 3♀♀, 11.VIII.2013; 4♂♂, 2♀♀, 18.VIII.2013; 1♂, 1♀, 24.VIII.2013, leg.K. Kaya.

New record for Turkish fauna.

Diagnosis: Wingspan 13.0-17.0 mm. Head smooth; ocellus absent; antenna ciliate; forewings with circular spots at middle and near end of cell, with oval dark brown spot at middle of upper side (Figure 2a). Valva elongate, exceeding apex of uncus in the male genitalia (Figure 2b); aedeagus stout (Figure 2c).

Host plants: Convolvulaceae: *Calonyction aculeatum* (L.), *Calystegia sepium* var. *japonica* (Choisy) *Convolvulus arvensis* L., *Ipomoea aquatica* Forsk, *Ipomoea batatas* (L.), *Pharbitis nil* (L.); Malvaceae: *Hibiscus syriacus* L. (Li, 2002; Lee & Byun, 2015).

Distribution: China, Europe, Kazakhstan, Korea, India, Japan, Russia, (Ponomarenko, 1997; Li & Zhen, 2011; Lee and Byun, 2015), Central Asia, Taiwan, Transcaucasian region Caucasus, (Ponomarenko, 1997; Lee & Byun, 2015) and Europe (Karsholt & Razowski, 1996; Ponomarenko, 1997; Lee & Byun, 2015).

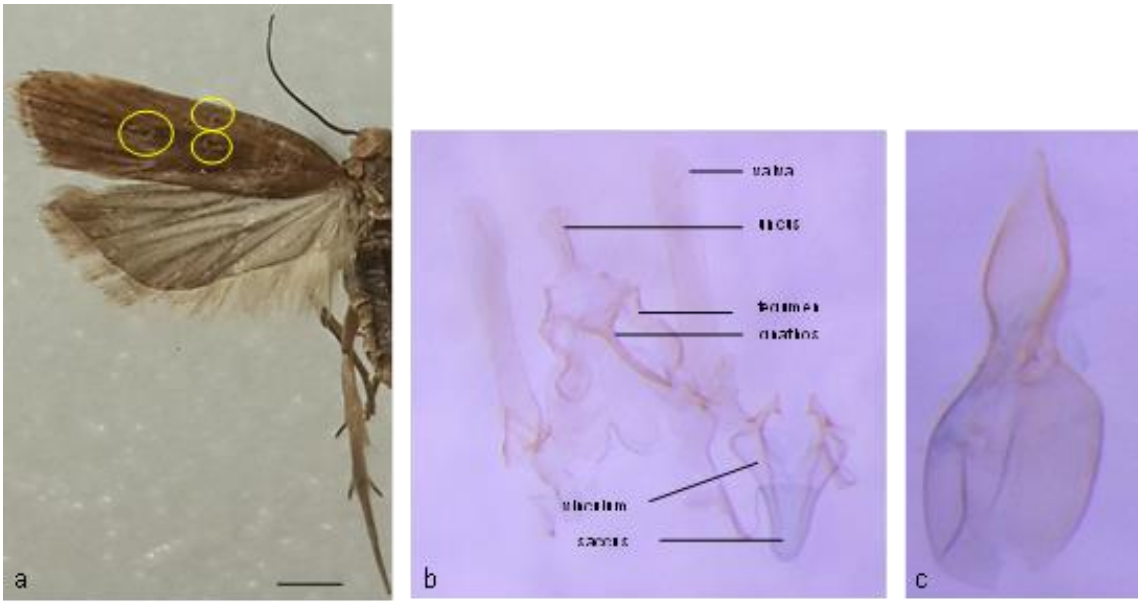


Figure 2. (a) Adult of *Helcystogramma triannulella,* (b) male genitalia, and (c) aedeagus. Scale bar = 1 mm.

Population development of *H. triannulella* is shown in Figure 3. First larvae of *H. triannulella* were seen on 7 July. The larval population of the pest in the experimental plot began to increase in late July and reached its peak on 10 August. In August, 10 larvae per plant were observed on some plants. After the August peak, density of the pest larvae declined gradually until14 September, and finally disappeared (Figure 3). Over this period several natural enemies were detected in the field or from cultured larvae *H. triannulella*. During the sampling period, the pest was also observed feeding on *Convolvulus* sp. in the same field.

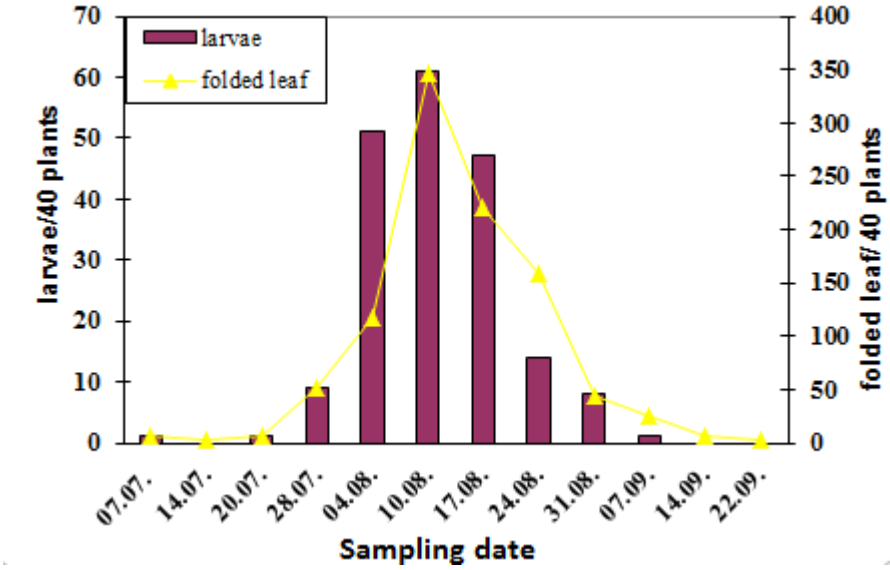


Figure 3. Number of folded leaves and larval population of *Helcystogramma triannulella* in sweet potato in 2013.

During the sampling, predator species belonging to Nabidae (Hemiptera) and Coccinellidae (Coleoptera) families were found between some folded leaves although no larvae of *H. triannulella* were detected. The parasitoid species from Braconidae (Hymenoptera) and Tachinidae (Diptera) families emerged when the sampled larvae were incubated in the laboratory. Especially the species belonging to Coccinellidae were abundant between empty folds, with up to 12 larvae per folded leaf (Figure 4).

Figure 4. Coccinellid individuals found between empty folds of sweet potato leaves.

In total, six predators and three larval parasitoid species were found in the field. Predators obtained were *Hippodamia variegata* (Goeze), *Oenopia conglobata* (L.), *Scymnus interruptus* (Goeze), *Scymnus mediterraneus* Khnzorian, *Stethorus gilvifrons* (Mulsant) (Coleoptera: Coccinellidae) and *Nabis viridulus* Spinola (Hemiptera: Nabidae). Franzman (2002) reported that *H. variegata* is the most important natural enemy of aphids, whiteflies, mealybugs, lepidopteran and coleopteran insects in many countries. It is known that this species feeds on eggs and first instars of *Ostrinia nubilalis* (Kayapınar & Kornoşor, 1993), *Pieris rapae* L. (Schmaedıck & Shelton, 2000) and *S. exigua* (Atlıhan et al., 2003). Likewise, Yanık (2011) used the eggs of the pyralid, *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae), for mass production of *O. conglobata*. This work revealed that it is possible to produce up to 20 generations per year of *O. conglobata* on *E. kuehniella.* However, it was reported that *E. kuehniella* eggs were a superior diet for the development rather than the reproduction of *O. conglobata* (Mirhosseini et al., 2015). Also, Atlıhan et al. (2003) reported with reference to Summy et al. (1997) that some *Scymnus* species feed on eggs and larvae of *S. exigua*. Several researchers have also reported that *Nabis* spp. feed on other small and soft insects such as aphids, cicadellids, small larvae of lepidopteran species, nymphs of Heteroptera (Lodos, 1986; Kayapınar & Kornoşor, 1993).

The parasitoids obtained from the larvae were determined as *Apanteles* sp., *Chelonus* sp. (Hymenoptera: Braconidae) and *Compsilura concinnata* (Meigen) (Diptera: Tachinidae). Thirteen species from eight lepidopteran families were reported as hosts for *C. concinnata* (Kara & Tschorsnig, 2003). The braconid species are an important parasitoid group preying on pest lepidopteran populations. Balevski (1999) found that many species from the families Gelechiidae, Gracillariidae, Pyralidae, and Tortricidae are hosts for *Apanteles* species in Bulgaria, and *Apanteles anarsiae* Faure & Alabouvette (Hymenoptera: Braconidae) is also a parasitoid of *Anarsia lineatella* Zell. (Gelechiidae) and *H. triannulella*. Balevski (1999) also reported that different species belong to the genus *Chelonus* are parasitoids of *Helicoverpa armigera* (Hübner) (Noctuidae), *Etiella zinckenella* (Treitschke), *Ostrinia nubilalis* (Hübner) (Pyralidae), *Synanthedon myopaeformis* (Borkhausen) (Sesiidae) and *Cydia pomonella* (L.) (Tortricidae). *Apanteles* sp. and *Chelonus* spp. were also detected on different lepidopteran hosts in Turkey, i.e. *Apanteles* sp. from *Hellula undalis* Fabricius, *Mythimna loreyi* Duponchel, *O. nubilalis*, *Sesamia cretica* Led., *Sesamia nonagrioides* (Lef.), *Spodoptera littoralis* Boisd. and *Chelonus* sp. from *H. undalis*, *M. loreyi* and *S. exigua* (Kaya, 2008; Gözüaçık et al., 2009).

Four harmful lepidopteran species from sweet potato crops, an important cash crop for small farms in Hatay Province, were found in this study. This includes also the first detection of *H. triannulella* in Turkey. These four lepidopteran species can be considered to be potentially harmful insects for sweet potato in the Mediterranean-type environments. Several natural enemies of these species were also found in the sampling area. Therefore, further studies should be conducted to evaluate the effects of these species on yield and quality deterioration in sweet potato crops to determine the economic threshold for chemical treatments or other management practices.

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## References

Akın, K., 2012. Mutki (Bitlis) Lepidoptera Faunasına Katkılar I. Sphingidae. BEÜ Fen Bilimleri Dergisi, 1 (1): 45-49.

Ames, T., N. E. J. M. Smit, A. R. Braun, J. N. O'Sullivan & L. G. Skoglund, 1997. Sweet potato: Major Pests, Diseases and Nutritional Disorders. International Potato Center, Lima, Peru, 153 pp.

Anonymous 2016a. Coffs harbour, Butterfly house, maze and tearooms (Web page: http://lepidoptera.butterflyhouse.com.au/acro/leucom.html) (Date accessed: April 2016).

Anonymous 2016b. Coffs harbour, Butterfly house, maze and tearooms (Web page: http://lepidoptera.butterflyhouse.com.au/spil/ornatalis.html) (Date accessed: April 2016).

Atlıhan R., E. N. Yardım, M. S. Özgökçe & M. B. Kaydan, 2003. *Spodoptera exigua* (Hübner) (Noctuidae: Lepidoptera)'nın Van ili patates alanlarındaki popülasyon gelişmesi ve doğal düşmanları. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi [Journal of Agricultural Science], 13 (1): 39-43.

Balevski, N. A., 1999. Catalogue of the Braconid Parasitoids (Hym.; Braconidae) Isolated From Various Phytophagus Insect Hosts in Bulgaria. Pensoft Publishers, Sofia, 126 pp.

Baron, T., 2014. Collection records of Noctuoidea and Bombycoidea (Lepidoptera) from a location near Marmaris in South-Western Anatolia, Turkey. Esperiana Band, 19: 263-296.

Bovell-Benjamin, A. C., 2007. Sweet potato: A review of its past, present and future role in human nutrition. Advances in Food and Nutrition Research, 52: 1-59.

Chalfant, R. B., R. K. Jansson, D. R. Seal & J. M. Schalk, 1990. Ecology and management of sweet potato insects. Annual Review of Entomology, 35: 157-180.

Çalışkan, M. E., T. Söğüt, E. Boydak, E. Ertürk & H. Arıoğlu, 2007. Growth, yield, and quality of sweet potato (Ipomoea batatas (L.) Lam.) cultivars in the Southeastern Anatolian and East Mediterranean Regions of Turkey. Turkish Journal of Agriculture and Forestry, 31: 213-227.

Edmond, J. B. & G. R. Ammermann, 1971. Sweet potatoes: Production, Processing, Marketing. Major Feed and Food Crops in Agriculture and Food Series, The AVI Publishing Company, Inc., Connecticut, USA, 334 pp.

FAO, 2016. Statistical Database. (Web page: http://faostat3.fao.org/home/E) (Accessed on 30.01.2016)

Franzman, A. B. 2002. *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), A predacious ladybird new in Australia. Australian Journal of Entomology, 41: 375-377.

Gözüaçık, C., C. Mart & K. Kara, 2009. Parasitoids of several lepidopterous pests in maize plantations in the Southeast Anatolian Region of Turkey. Turkish Journal of Zoology, 33: 475-477.

Hacker, H., 1989. Die Noctuidae Griechenlands. Herbipoliana, 2: 1-589.

Hacker, H., 1990. Die Noctuidae Vorderasiens (Lepidoptera) - Neue Entomologische Nachrichten 27: 1-707.

Jansson, R. K. & K. V. Raman, 1991. Sweet Potato Pest Management. A Global Perspective. Westview Press, Inc., Boulder, USA, 458 pp.

Jones, A., J. M. Schalk & P. D. Dukes, 1987. Control of soil insect injury by resistance in sweet potato. Journal of American Society Horticultural Science, 112: 195-197.

Kara, K. & H. P. Tschorsnig, 2003. Host catalogue for the Turkish Tachinidae (Diptera). Journal of Applied Entomology, 127 (8): 465-476.

Karsholt, O. & J. Razowski, 1996. The Lepidoptera of Europe. Apollo Books, Stenstrup, Denmark, 380 pp.

Kaya, K., 2008. Hatay İlinde Önemli Yazlık ve Kışlık Sebze Alanlarında Bulunan Zararlı Lepidopter Türleri, Popülasyon Yoğunlukları ve Parazitoitleri Üzerinde Araştırmalar. Çukurova Üniversitesi Fen Bilimleri, Doktora Tezi, Adana, 86 pp.

Kaya, Ş. & M. E. Çalışkan, 2010. Effects of molasses and ground wheat additions on the quality of groundnut, sweet potato, and Jerusalem artichoke tops silages. African Journal of Agricultural Research, 5 (9): 829-833.

Kaya, Ş. & H. Yıldırım, 2011. The effect of dried sweet potato (*Ipomoea batatas*) vines on egg yolk color and some egg yield parameters. International Journal of Agriculture and Biology, 13 (5):766-770.

Kayapınar, A. & S. Kornoşor, 1993. *Ostrinia nubilalis* Hübner (Lep.; Pyralidae)'in larva dönemleri üzerine avcı böceklerin etkisinin araştırılması. Türkiye Entomoloji Dergisi- Turkish Journal of Entomology, 17 (2): 69-76.

Koçak, A.Ö., & M. Kemal, 2009. Revised of the Lepidoptera of Turkey. Priamus, Serial Publication of the Centre for Entomological Studies, Ankara, 17, 253 pp.

Lee, Sangmi & B. Byun, 2015. New faunistik data of Gelechioidea (Lepidoptera) from North Korea, based on the material deposited in Hungarian Natural History Museum. Journal of Asia-Pacific Biodiversity, 8: 227-229.

Li, H.H. & H. Zhen, 2011. Review of the genus Helcystogramma Zeller (Lepidoptera: Gelechiidae: Dichomeridinae) from China. Journal of Natural History, 45 (17-18): 1035-1087.

Li H.H. 2002. The Gelechiidae of China (I) (Lepidoptera: Gelechioidea). Nankai University Press, Tianjin, China; 538 pp. [in Chinese].

Lodos, N., 1986. Türkiye Entomolojisi II: Genel, Uygulamalı ve Faunistik. Ege Üniversitesi Ziraat Fakültesi Yayınları, No: 429, İzmir, 580 pp.

Mirhosseini, M. A., M. R. Hosseini & M. A. Jalali, 2015. Effects of diet on development and reproductive fitness of two predatory coccinellids (Coleoptera: Coccinellidae). European Journal of Entomology, 112 (3): 446-452.

Ponomarenko, M.G., 1997. Catalogue of the subfamily Dichomeridinae (Lepidoptera: Gelechiidae) of the Asia. Far Eastern Entomologist, 50: 1-67.

Schalk, J. M., J. R. Bohac, P. D. Dukes & W. R. Martin, 1993. Potential of non-chemical control strategies for reduction of soil insect damage in sweet potato. Journal of American Society Horticultural Science. 118: 605-608.

Schalk, J.M., P.D. Dukes, A. Jones & R.L. Jarret, 1991. Evaluation of sweet potato clones for soil insect damage. Horticultural Science, 26: 1548-1549.

Schmaedick, M. A. & A. M. Shelton, 2000. Arthropod predators in cabbage (Cruciferae) and their potential as naturally occurring biological control agents for *Pieris rapae* (Lepidoptera: Pieridae). The Canadian Entomologist, 132**:** 655-675.

Summy, K. R., J. R. Raulaton, D. W. Spurgeon & A. W. Scott, 1997. Population trends of beet armyworm on cotton in the lower Rio Grande Valley. Proceedings Beltwide Cotton Conferences, (January 6-10, 1997). New Orleans, LA, USA, Volume 2, 1035-1039.

Thompson, P. G., J. C. Schneider, B. Graves & R. C. J Sloan, 1999. Insect resistance in sweet potato plant introductions. Horticultural Science, 34: 711-714.

Woolfe, J. A., 1992. Sweet Potato: An Untapped Food Resource. Cambridge University Press, Cambridge, UK, 634 pp.

Yanık, E., 2011. Farklı sıcaklıkların *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae) yumurtalarında beslenen *Oenopia conglobata* (L.) (Coleoptera: Coccinellidae)'nın biyolojik özelliklerine etkisi. Harran Üniversitesi Ziraat Fakültesi Dergisi, 15 (1): 21-28.

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# Six new records for the spider fauna of Turkey (Araneae: Salticidae)

Türkiye örümcek faunası için altı yeni kayıt (Araneae: Salticidae)

**İlhan COŞAR[1]*** **Muhammed İsmail VAROL[2]**

## Summary

Six Salticidae spider species, *Evarcha laetabunda* (C. L. Koch, 1846), *Heliophanus verus* Wesolowska, 1986, *Pellenes seriatus* (Thorell, 1875), *Plexippoides flavescens* (O. Pickard-Cambridge, 1872), *Pseudicius palaestinensis* Strand, 1915 and *Saitis barbipes* (Simon, 1868) are new records for the spider fauna of Turkey. Their morphology is briefly described and illustrated.

**Keywords:** Araneae, Salticidae, fauna, new record, Turkey

## Özet

*Evarcha laetabunda* (C. L. Koch, 1846), *Heliophanus verus* Wesolowska, 1986, *Pellenes seriatus* (Thorell, 1875), *Plexippoides flavescens* (O. Pickard-Cambridge, 1872), *Pseudicius palaestinensis* Strand, 1915 ve *Saitis barbipes* (Simon, 1868)'in yer aldığı altı örümcek türü Türkiye örümcek faunası için yeni kayıttır. Bu türlerin morfolojileri kısaca tanımlanmış ve tasvir edilmiştir.

**Anahtar sözcükler:** Araneae, Salticidae, fauna, yeni kayıt, Türkiye

[1] Department of Biology, Faculty of Arts and Sciences, Kırıkkale University, 71451, Yahşihan, Kırıkkale, Turkey
[2] Faculty of Medicine, Department of Medical Biology, Kırıkkale University, Kırıkkale, Turkey
* Corresponding author (Sorumlu yazar) e-mail: ilhancsr88@gmail.com
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## Introduction

The Salticidae Blackwall, 1841 is the largest family in Araneae and currently represented by 5862 species belonging to 595 genera worldwide (World Spider Catalog, 2016). There are 106 species in 40 salticid genera listed for Turkey (Bayram et al., 2016). With latest records, the number of salticid species in Turkey has since increased to 128 in the same 40 genera. Logunov (2015) recorded; *Ballus rufipes* (Simon, 1868), *Evarcha armeniaca* Logunov, 1999, *Habrocestum egaeum M*etzner, 1999, *Habrocestum shulovi* Prószyński, 2000, *Heliophanus dunini* Rakov et Logunov, 1997, *Marpissa pomatia* (Walckenaer, 1802), *Neon rayi* (Simon, 1875), *Pellenes ostrinus* (Simon, 1868), *Phlegra cinereofasciata* (Simon, 1868), *Salticus ressli* Logunow, 2015, *Salticus tricinctus* (C. L. Koch, 1846), *Sitticus atricapillus* (Simon, 1882), *Sitticus inexpectus* Logunov et Kronestedt, 1997, *Sitticus saltator* (O. Pickard-Cambridge, 1868), *Sitticus zimmermanni* (Simon, 1877), *Talavera aperta* (Miller, 1971) and *Yllenus univittatus* (Simon, 1871). Coşar (2015) recorded; *Euophrys sulphurea* (L. Koch, 1867), *Neon levis* (Simon, 1871), *Pellenes brevis* (Simon, 1868) and *Sibianor aurocinctus* (Ohlert, 1865). Azarkina & Komnenov (2015) recorded; *Aelurillus alboclypeus* Azarkina & Komnenov, 2015.

In this paper, six additional species of the spider fauna of Turkey are recorded. These are *Evarcha laetabunda* (C. L. Koch, 1846), *Heliophanus verus* Wesolowska, 1986, *Pellenes seriatus* (Thorell, 1875), *Plexippoides flavescens* (O. Pickard-Cambridge, 1872), *Pseudicius palaestinensis* Strand, 1915 and *Saitis barbipes* (Simon, 1868). These records bring the total number of salticid recorded in Turkey to 134 species.

## Material and Methods

This study of specimens collected from different regions of the Turkish provinces of Kırıkkale, Manisa and Sinop. Specimens were collected either by sweep net from herbaceous plants or by hand aspirator from plants or under stones. Identifications were made using a Leica S8APO stereo microscope. The identification keys of Heimer & Nentwig (1991), Metzner (1999) and Prószyński (2003) were used to identify species. Specimens were preserved in 70% ethanol. All measurements are given in millimeters (± 0.01). Abbreviations used in the text are as follows; ALE, anterior lateral eyes and ME, anterior median eyes. Pictures were taken using a Leica DC 160 camera mounted on the stereo microscope. Specimens have been deposited in the collection of the Arachnological Museum of Kırıkkale University (KUAM).

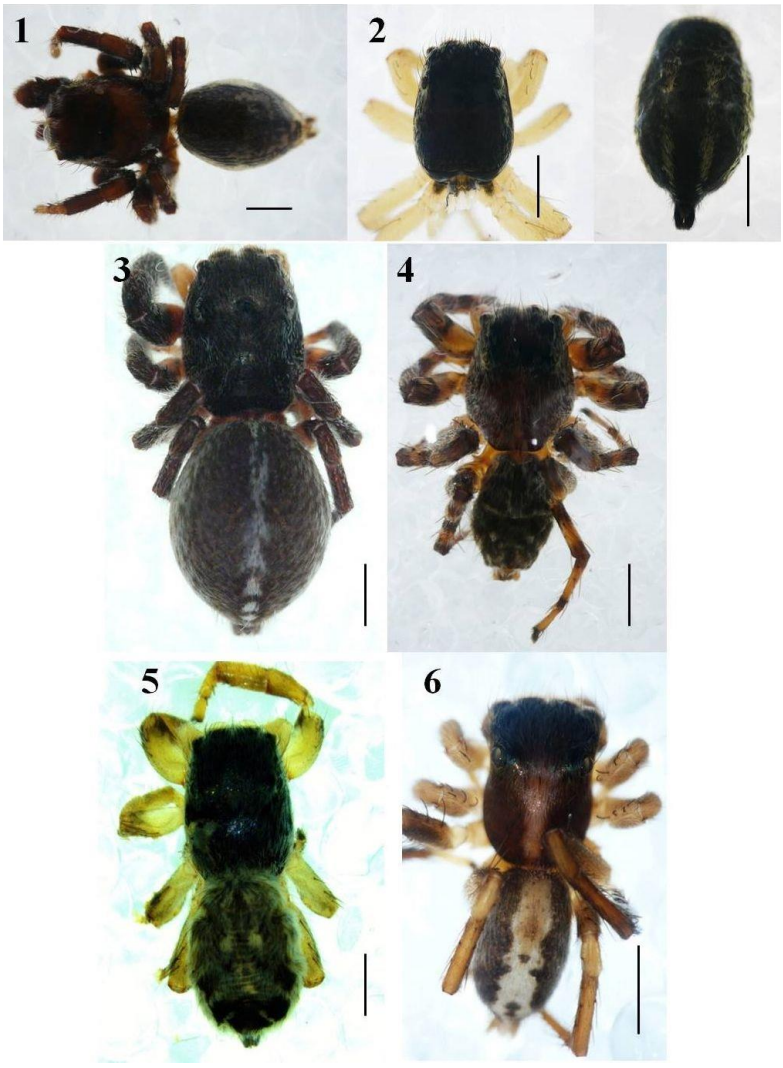
## Results

### *Evarcha laetabunda* (C. L. Koch, 1846)

Material examined : Manisa Province, Turgutlu District, pine forest (38˚22’49" N, 27˚52’12" E), 01.VII.2003, 3♂.

Distribution: Palearctic (World Spider Catalog, 2016).

Comments : Total length 4.60, prosoma length 2.20, prosoma width 1.70, abdomen length 2.40, abdomen width 1.80. Prosoma dark brown with vivid and light black color laterally (Figure 1). AME and ALE surrounded by dense white hairs. Chelicerae narrow, with light brown and white haired dorsally (Figure 7). Abdomen grayish. First legs black and thicker than other legs (Figure 1). Tibial apophysis apex forked. Embolus sclerotized, and sperm ducts not clearly distinguishable. Terminal and median apophysis absent. Tegulum bulging laterally and distal tegular projection light colored (Figure 13-14). Spinnerets dark brown. Male palp as in Figures 13-14.



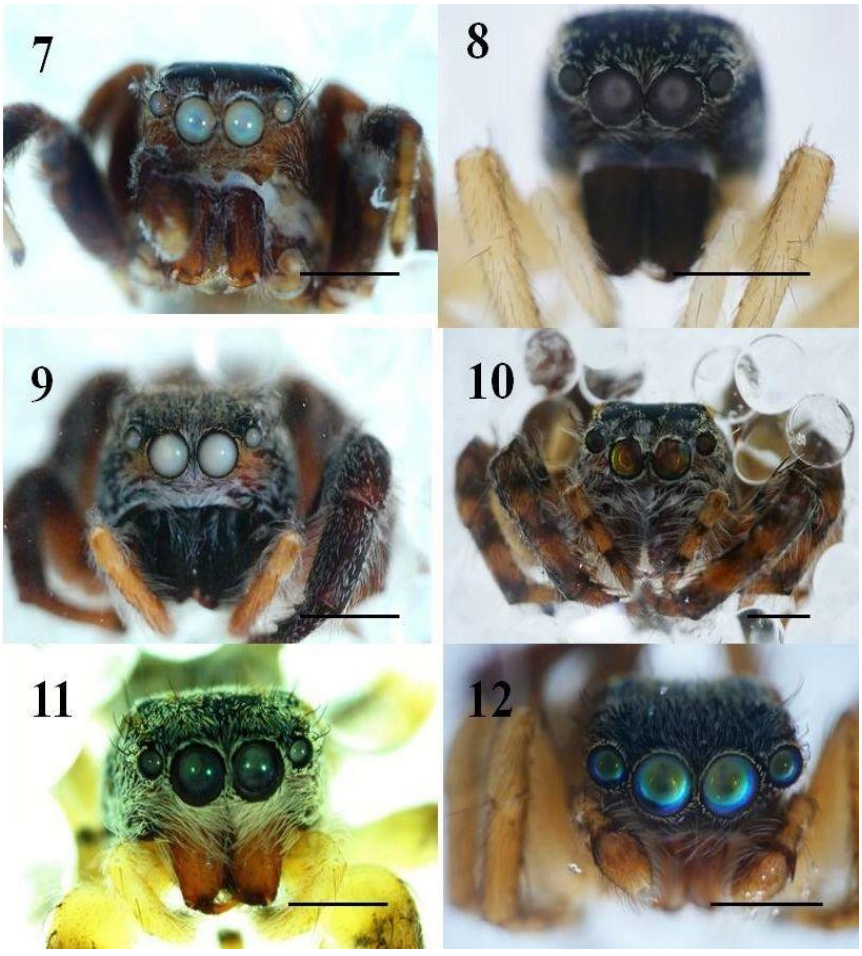

Figures 1-6. Dorsal views; 1: *Evarcha laetabunda* (♂), 2: *Heliophanus verus* (♀), 3: *Pellenes seriatus* (♀), 4: *Plexippoides flavescens* (♀), 5: *Pseudicius palaestinensis* (♀), 6: *Saitis barbipes* (♂) (Scale: 1.0 mm).

***Heliophanus verus* Wesolowska, 1986**

Material examined : Kırıkkale Province, Karacalı Village (39˚53'01" N, 33˚32'25" E), 11.V.2014, 2♀.

Distribution : Azerbaijan, Iran (World Spider Catalog, 2016).

Comments : Total length 4.50, prosoma length 2.0, prosoma width 1.15, abdomen length 2.50, abdomen width 2.0. Prosoma black, bright, with few white hairs and long dark colored hairs on anterior part. White hairs located on lateral side of prosoma (Figure 2). AME and ALE covered by white hairs. Chelicerae dark brown. Clypeus narrow, dark colored, and has intense white hairs (Figure 8). Abdomen with black and white hairs on lateral part and middle section. White hairs central forming two striped patterns. Legs light yellow and covered with dark colored hairs. Spinnerets black and hairy (Figure 2). Female epigyne as in Figures 18-19.



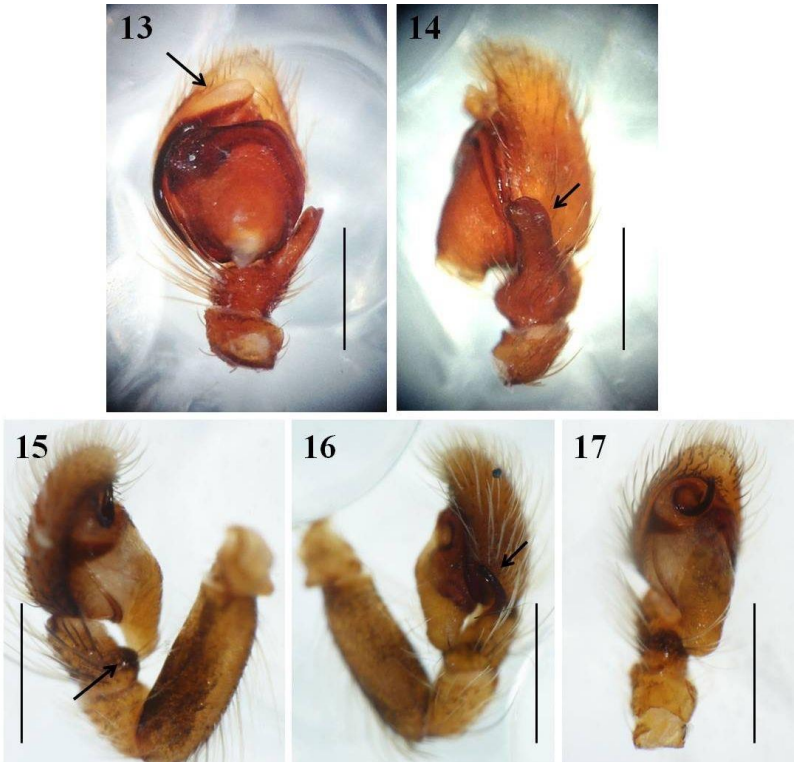

Figures 7-12. Ocular area; 7: *Evarcha laetabunda* (♂), 8: *Heliophanus verus* (♀), 9: *Pellenes seriatus* (♀), 10: *Plexippoides flavescens* (♀), 11: *Pseudicius palaestinensis* (♀), 12: *Saitis barbipes* (♂) (Scale: 1.0 mm).

***Pellenes seriatus* (Thorell, 1875)**

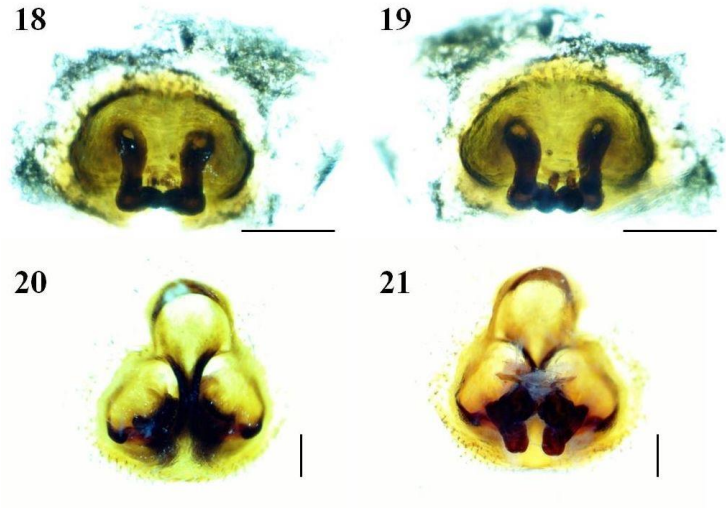
Material examined : Sinop Province, Boyabat District, Drannaz Mountain (41˚41'40" N, 34˚52'50" E), 07.VI.2013, 3♀.

Distribution : Bulgaria, Central Asia, Greece, Macedonia, Romania, Russia (World Spider Catalog, 2016).

Comments : Total length 7.84, prosoma length 3.28, prosoma width 2.56, abdomen length 4.56, abdomen width 3.52. Prosoma black, sparsely covered with white hairs. Clypeus reddish, densely covered with white hairs (Figure 3). AME surrounded by dense white hairs. Chelicerae black and white haired dorsally (Figure 9). Abdomen dorsum dark brown, with a white longitudinal line, ventral yellowish gray and covered with white hairs. Legs dark brown, only femur and tarsus yellowish brown. Spinnerets light brown (Figure 3). Female epigyne as in Figures 20-21.




Figures 13-17. Palps, *Evarcha laetabunda* (♂), 13: ventral view, 14: retrolateral view, *Saitis barbipes* (♂), 15-16: retrolateral view, 17: ventral view (Scale: 0.5 mm).




Figures 18-21. *Heliophanus verus* (♀), 18: ventral view of epigyne , 19: dorsal view of vulva, *Pellenes seriatus* (♀), 20: ventral view of epigyne , 21: dorsal view of vulva (Scale: 0.2 mm).

***Plexippoides flavescens* (O. Pickard-Cambridge, 1872)**

Material examined : Kırıkkale Province, Ahılı Village (39˚47'41" N, 33˚32'42" E), 15.V.2014, 1♀.

Distribution : Greece to Central Asia, Pakistan, Sudan (World Spider Catalog, 2016).

Comments : Total length 3.40, prosoma length 1.80, prosoma width 1.40, abdomen length 1.60, abdomen width 1.10. Prosoma dark brown, eye area blackish brown and intensively covered with grayish hairs (Figure 4). AME and ALE covered with white hairs. Chelicerae dark brown with white hairs located in the dorsal direction. Clypeus dark brown, narrow, intensely covered with long white hairs (Figure 10). Abdomen blackish brown with patterns formed by grayish hairs localized on dorsal part. Coxa and trochanter parts of the legs yellow, remaining parts brown and covered with white hairs. Spinnerets light brown (Figure 4). Female epigyne as in Figures 22-23.

***Pseudicius palaestinensis* Strand, 1915**

Material examined : Kırıkkale Province, Karacalı Village (39˚53'50"N, 33˚32'30"E), 25.VI.2015, 1♀.

Distribution : Iran, Israel (World Spider Catalog, 2016).

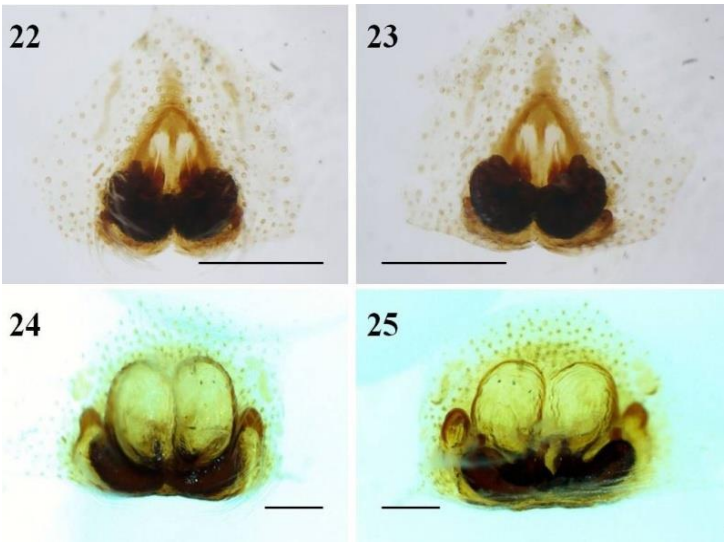
Comments : Total length 4.25, prosoma length 2.10, prosoma width 1.35, abdomen length 2.15, abdomen width 1.50. Prosoma black, covered with white hairs. Frontal eyes surrounded by white hairs (Figure 5). Clypeus brown, densely covered with white hairs. Chelicerae light brown and dorsum covered dark colored hairs (Figure 11). Sternum dark brown. Abdomen gray and covered with shiny grayish hairs. Locations close to spinnerets, intensely covered with black hairs. Spinnerets light brown (Figure 5). Legs light yellow. Female epigyne as in Figures 24-25.

***Saitis barbipes* (Simon, 1868)**

Material examined : Sinop Province, Boyabat District (41˚50’32" N, 35˚09’44" E), 09.VI.2013, 3♂. Sinop Province (42˚02’10" N, 35˚11’24" E), 18.IV.2010, 1♂.

Distribution : Central Europe, Mediterranean (World Spider Catalog, 2016).

Comments : Total length 3.90, prosoma length 1.90, prosoma width 1.30, abdomen length 2.0, abdomen width 1.10. Posterior of prosoma dark brown with white striped pattern centrally. Anterior of prosoma blackish brown and intensely covered with dark hairs (Figure 6). AME and ALE covered with white hairs. Clypeus narrow, dark brown with long white hairs. Chelicerae light brown (Figure 12). Abdomen dark brown with white striped pattern centrally. Brown pattern in the middle of stripe and close to spinnerets. Spinnerets light brown (Figure 6). Male palp as in Figures 15-17.




Figures 22-25. *Plexippoides flavescens* (♀), 22: ventral view of epigyne , 23: dorsal view of vulva, *Pseudicius palaestinensis* (♀), 24: ventral view of epigyne , 25: dorsal view of vulva (Scale: 0.5).

## References

Azarkina, G. N. & M. Komnenov, 2015. Descriptions of two new species of *Aelurillus* Simon, 1884 (Araneae, Salticidae) from the Mediterranean, with the synonymization of *A. steliosi* Dobroruka, 2002. Zookeys 516: 109-122.

Bayram, A., K. B. Kunt & T. Danışman. 2016. The Checklist of the Spiders of Turkey. Version 2016. (Web page: http://www.spidersofturkey.info) (Date accessed: 15 March 2016)

Coşar, İ., 2015. Four new records for the spider fauna of Turkey (Araneae: Salticidae). Turkish Journal of Zoology 39: 368-371.

Heimer, S. & W. Nentwig, 1991. Spinnen Mitteleuropas. Verlag Paul Parey, Berlin, 543 pp.

Logunov, D. V., 2015. Taxonomic-faunistic notes on the jumping spiders of the Mediterranean (Aranei: Salticidae). Arthropoda Selecta 24 (1): 33-85.

Metzner, H., 1999. Die Springspinnen (Araneae, Salticidae) Griechenlands. Andrias 14: 1-279.

World Spider Catalog, 2016. World Spider Catalog. Natural History Museum Bern. Version 17.0. (Web page: http://wsc.nmbe.ch) (Date accessed: 15 March 2016)

Prószyński, J., 2003. Salticidae (Araneae) of the Levant. Annales Zoologici (Warszawa) 53: 1-180.

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# Constituents and termiticide potential of some wood extracts against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae)

Bazı odun ekstraktlarının bileşenleri ve *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae)'ye karşı termitisit potansiyeli

**Khalid Zamir RASIB**[1 *] **Ayesha AlHETASHAM**[2]

## Summary

Wood extractives are one of the main reasons for the resistance of wood to termite attack. A study was carried out to determine the chemical constituents of wood extractives from *Pinus roxburghii* Sargent*, Morus alba* L. and *Eucalyptus camaldulensis* Dehnh against *Coptotermes heimi* (Wasmann) under laboratory conditions in Forman Christian College University (Lahor, Pakistan) in June 2015. Gas chromatography-mass spectrometry of wood extractives of *P. roxburghii* detected 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and octadecanoic acid (methyl ester). Hexadecanoic acid (methyl ester) and 1-methyl-3-(1-methylethyl)-benzene were present in *M. alba* in addition to the compounds present in *P. roxburghii* and in *E. camaldulensis* 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and β-phellandrene were also present. Based on the feeding activity, wood extracts were arranged in descending order of preference; *P. roxburghii* > *M. alba* > *E. camaldulensis*. Extracts of *P. roxburghii, M. alba* and *E. camaldulensis* proved repellent at higher concentrations, with tunneling activity almost fully inhibited. So these could prove useful in developing a soil barrier to block termite activity and serve as a replacement to synthetic chemicals.

**Keywords:** Barrier*, Eucalyptus camaldulensis,* extractives*, Morus alba, Pinus roxburghii,* wood

## Özet

Odun özütleri, termit saldırılarına karşı odun dayanıklılığının ana nedenlerinden birisidir. Bu çalışma 2015 yılı haziran ayında, Forman Christian College Üniversitesi (Lahor, Pakistan)'nde laboratuvar koşullarında *Coptotermes heimi* (Wasmann)'ye karşı *Pinus roxburghii* Sargent*, Morus alba* L. ve *Eucalyptus camaldulensis* Dehnh'in odun özütlerinin kimyasal bileşenlerini belirlemek amacıyla yürütülmüştür. *P.* roxburghi'nin odun özütlerinin gaz kromatografisi-kütle spektrometresi sonucunda, 1,2-benzendikarboksilik asit, mono (2-etilheksil) ester ve oktadekanoik asit (metil ester) tespit edilmiştir. *M. alba*'da *P. roxburghii*'de mevcut bileşiklere ilave olarak heksadekanoik asit (metil ester) ve 1-metil-3-(1-metil-etil)-benzen bulunurken, *E. camaldulensis*'te 1,2-benzendikarboksilik asit, mono (2-etilheksil) ester ve β-fellandren de bulunmuştur. Odun özütleri beslenme aktivitesi tercih sırasına göre, *P. roxburghii* > *M. alba* > *E. camaldulensis* şeklinde olmuştur. *P. roxburghii*, *M. alba* ve *E. camaldulensis* özütlerinin daha yüksek konsantrasyonlarda itici olduğu tespit edilmiş, tünel aktivitesi ise neredeyse tamamen engellenmiştir. Böylece, bu özütler termit aktivitesini engellemek için bir toprak bariyer geliştirilmesinde yararlı olabilir ve sentetik kimyasallara bir alternatif olarak hizmet edebilir.

**Anahtar sözcükler:** Bariyer*, Eucalyptus camaldulensis,* özütler*, Morus alba, Pinus roxburghii,* odun

[1] Department of Biological Science, Forman Christian College University, Lahore, Pakistan
[2] Department of Zoology, Punjab University. New Campus, Lahore, Pakistan
* Corresponding author (Sorumlu yazar) e-mail: khalidrasib786@gmail.com
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## Introduction

Termites remain the most dreaded insect pest of human dwellings and other structures because of their ability to destroy wood and wood products (Pedigo, 1986). Wood being a biological material is readily degraded by bacteria, fungi and termites (Walker, 1993; Schultz & Nicholas, 2002). Termites are responsible for much of the degradation of wood and other cellulose materials in the terrestrial environment mainly in the tropics and subtropics (Peralta et al., 2003, 2004). The injudicious use of pesticides for the control of termites has generated a number of biological and environmental hazards in air, water, soil and food. More than 1000 species of plants have been reported to have chemicals in leaves, stems, flowers, seeds and roots which have insecticidal properties, but only a few of them have been used for practical insect control on a commercial scale. The chemical poisons of plants are mostly alkaloids (Shahid, 2003). Termites are members of the insect order, Isoptera, and more than 2600 species of termites are found around the world (Kambhampati & Eggleton, 2000; Eggleton, 2001, Ohkuma et al., 2004). Ecologically, termites are classified under three main groups based on their feeding and nesting behavior: damp wood termites, dry wood termites and subterranean termites (Chung & Lee, 1999). Termites have economic importance because they damage a wide variety and quantity of wood work in buildings, crops, local plantation and forests. For the prevention of heavy timber damage, we need extensive knowledge of natural resistance of native plants to termites and their feeding preference (Rasib, 2008). The fauna of Pakistan includes a range of termites species having diverse feeding preferences in various ecological zones (Akhtar, 1974).

In the past, the control of termites was totally based on the synthetic insecticides especially the persistent organochlorine (Khan & Singh, 1985; Anonymous, 2000, Ahmed et al., 2006). Their maximum residual effects, as well as the development of resistance in target pests, are considered their main drawbacks. Also, adverse effects on human health and concerns about environmental deterioration (Potter & Hillery, 2001; Verkerk & Bravery, 2001) have resulted in the replacement of synthetic insecticides with biorational ones, and is now universally accepted and practiced worldwide. The pool of plants possessing insecticidal substances is enormous, with plant chemicals from roots, stems, leaves, flowers and seeds of more than 1000 species having been used for insect control. Like alkaloids, plant flavonoids are naturally occurring substances and play an important role in insect control. These compounds function as constitutive or inducible anti-insect compounds (Dixon, 1999), so they may serve as natural pesticides. Many of them are being tested for repellent and deterrent effects against the insects (Simmonds, 2003). Flavonoids can also modulate the feeding behavior of the insects (Nawrot et al., 1986).

***Eucalyptus camaldulensis* Dehn.**

*Eucalyptus* species have promising essential oils with repellent and pesticide effects (Nerio *et al.*, 2010). From an environmental and toxicological point of view, the use of eucalyptus essential oil as a natural pesticide has a better effect than irregular use of other pesticides, and thereby the problem of pest resistance can be overcome (Batish et al., 2008). Durable and termites-resistant *Eucalyptus camaldulensis* Dehn. wood is extensively used in some industrial processes like pulp, paper, chipboard and plywood manufacturing (Abbas et al., 2010).

***Morus alba* L.**

White mulberry heartwood (*Morus alba* L.) is the main timber used for crafting three traditional long necked lutes in Iran. The chemical structure of wood is composed not only of its primary constituents, but also of a relatively small fraction of extractable compounds. Extractives can include different categories like phenols, tannins and oils. The primary role of these compounds is to protect the tree and timber against decay agents; therefore it is expected that these would have termiticidal, fungicidal and even toxic properties (Hillis, 1962; Highley & Scheffer, 1970; Tsunoda, 1990; Schultz & Nicolas, 2000; Taylor et al., 2002).

***Pinus roxburghii* Sargent**

*Pinus roxburghii* Sargent is a large evergreen tree, which is sometimes deciduous in dry locations or dry seasons (Troup, 1921). *Pinus roxburghii* oil has been traditionally used to treat cuts, wounds, boils, and blisters (Gewali, 2008). *Pinus roxburghii* is known for its natural resistance to termites (Rasib, 2008).

This study was specifically designed to assess the effectiveness of wood extractives against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae) by determining different compounds present in these three tree species using Soxhlet extraction and gas chromatography-mass spectrometry (GC-MS) to understand their efficacy against termites and then suggest their application for termite control as an alternative to synthetic insecticides.

## Materials and Methods

### Selection of plant species

Commercial timber species were selected for this study. Wood of *E. camaldulensis, M. alba* and *P. roxburghii* was cut from standing trees in the, Forman Christian College Botanical Garden, Lahore, Pakistan in June 2015

### Extraction method

For crude extracts of the wood, a non-polar and polar solvents, n-hexane and methanol, were used by incubating sawdust of *E. camaldulensis, M. alba* and *P. roxburghii* at 70$^{o}$C for 72 h in order to remove moisture. The samples were then preserved in zip lock bags for future use and to avoid any contamination. Two hundred g of each species with solvent were placed separately in a Soxhlet extractor and extracted with 150 ml of hexane and methanol according to ASTM (2003) standards. After extraction, each extract was kept separately under refrigeration at 4°C and subsequently the bioactivity of the extracted compounds was checked against termites.

### Gas chromatography-mass spectrometry

For the identification of components extracts from the Soxhlet extractor were processed through hydro distillation, for GC-MS analysis. Samples were distilled below 200°C and filtered through 0.20 µm pore size filter paper. The gas chromatography temperature ranged of 50 to 250 °C with 4°C/min, with a solvent delay of 5 min. The temperature of the injector was 250°C. The inert gas was helium at a flow rate of 1.0 mL/min, and 2 µL of sample was injected sample in the splitless mode. The percent composition of the samples was calculated. The quantitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with those of NIST 98 (Mass Spectral Library, National Institute of Standards and Technology, MD, USA) as modified by Jianu et al., 2013.

### Collection of *Coptotermes heimi*

*Coptotermes heimi* were collected from different areas of the Lahore District including visits to Changa Manga Forest, Jallo Park, Wagha Border, Jinnah Garden, but mainly from Forman Christian College. Some were also collected through the baiting. Collection were made feasible using artificial baiting methods such as bucket traps, wetted toilet rolls, and cardboard in of plastic bottles with small holes in the base and sides to permit the entry of termites. The baits were buried in soil and termites were collected as and when required.

### Feeding bioassays

### Choice test and repellency bioassay

Repellent responses of wood extracts from *E. camaldulensis, M. alba* and *P. roxburghii* were observed against *C. heimi* by cutting Whatman filter paper No. 1 into two halves according to size of Petri dish (70 x 10 mm) and placed into the dish in such a way that there was space between two halves to

allow separate treatment. One half was treated with specific amount of plant extracts 2, 5, 10, 20 or 30% by pipette while the other half was treated with water to serve as a control. Then filter papers were air dried for few minutes. Three replicates of each extract including control were prepared. Ten mature workers of *C. heimi* were released separately into each dish between treated and untreated zone and observations were made at 15-min intervals. After introduction of termites into the dishes, the number of termites oriented towards the control half were counted as repelled. A treatment concentration was considered repellent when 21 (sum of three replicates) of 30 termites were present on untreated area against respective percent concentration.

**No choice test**

Filter paper for each concentration *E. camaldulensis, M. alba* and *P. roxburghii* was treated separately as mentioned above to evaluate the mortality and feeding of *C. heimi.* Termite mortality was recorded periodically during the bioassays up to 7 days. Percentage mortality was calculated using the following formula:

$$Mc = (Mo - Me) / (100 - Me) \times 100$$

Where, Mo = mortality rate of treated termite (%); Me = mortality rate of control (%); Mc =corrected mortality rate (%).

**Tunneling test**

**Tunneling apparatus**

Tunneling apparatus consists of a transparent tube with two chambers i.e. nest/feeding chamber and treated chamber attached with a rectangular plate. Termites were released into the nest chamber which was treated with the distilled water. The chamber was cylindrical (53 mm high by 55 mm wide) with holes in the lid to provide a source of oxygen to the termites. The nest chamber was connected with treated chamber through a tube (71 mm long, 8 mm diameter). Termites could move from this tube to reach the treated chamber. The treated chamber was rectangular (253 mm long x 75 mm wide x 14 mm high) and treated with the different concentrations of the wood extracts.

**Tunneling method**

Tunneling apparatus was filled with soil in both chambers i.e. nest/feeding chamber and treated chamber. In the nest chamber, a layer of 10 g of soil was evenly spread and moistened with distilled water. In the treated chamber, 48.9 g of soil was evenly spread with filter paper in the center to provide a food source for the termites. This compartment was treated with five different concentrations (2, 5, 10, 20 and 30%) of each wood extract. A group of 60 workers were added to each nest container, with three replicates for each wood extract. Test set ups were examined at 24-h intervals for one week after establishment. At each examination, tunnel length was measured.

**Statistical analysis**

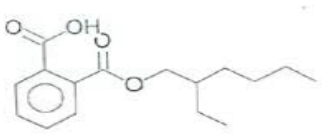
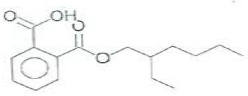
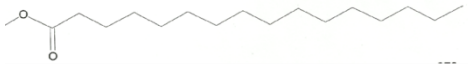
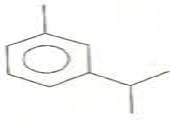
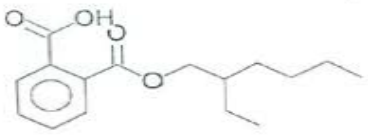
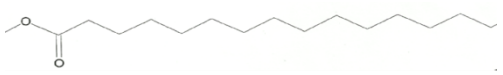
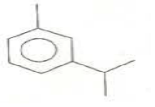
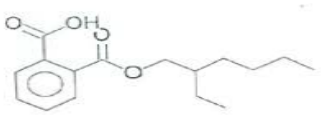
Data were analyzed statistically by using paired t-test and standard deviation to evaluate the consumption, mortality and the tunneling activity on the wooden extracts against *C. heimi* in MINITAB 15 software (Minitab Inc., State College, PA, USA). Results were statistically significant in all cases ($P < 0.05$).

## Results

**Compounds identified in extracts of *Eucalyptus cameldulensis, Morus alba* and *Pinus roxburghii***

Compounds identified in gas chromatography using methanol and hexane as extraction solvent are given in Table 1.

Table 1. Phytocompounds identified in chromatogram of *Eucalyptus cameldulensis, Morus alba* and *Pinus roxburghii* using hexane and methanol as extraction solvent

| Tree species | Retention time (minutes) | Phytocompounds | Extraction solvent | Structural formulae |
|---|---|---|---|---|
| *Eucaluyptus camaldulensis* | 21.264 and 21.695 | 1,2-Benzenedicarboxylic acid, Mono (2-ethylhexyl) ester | hexane |  |
| *Morus alba* | 18.080 | Octadedanoic acid, methyl ester | methanol |  |
| | 21.633 | 1,2 Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | |  |
| | 16.185 | Hexadedanoic acid, methyl ester | |  |
| | 5.119 | Benzene, 1- methyl-3-(1-methylethyl)-methanol | |  |
| | 18.080 | Octadedanoic acid, methyl ester | |  |
| | 21.633 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | |  |
| | 16.185 | Hexadedanoic acid, methyl ester | |  |
| | 5.119 | Benzene, 1- methyl-3-(1-methylethyl)- | |  |
| *Pinus roxburghii* | 18.074 | Octadedanoic acid, Methyl ester | hexane |  |
| | 21.639 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | |  |

### Effect of wood extracts on *Coptotermes heimi* under laboratory conditions

#### No choice laboratory trials

Table 2 shows workers of *C. heimi* exposed to different *E. camaldulensis* extract concentrations. The highest mortality rate of 97.5% was observed at an extract concentration of 30% and the lowest mortality rate of 30.1% at an extract concentration of 2%. The table also shows the consumption of filter paper by *C. heimi* when treated with different concentrations of *E. camaldulensis* extract. The maximum consumption of 0.41 g was observed at an extract concentration of 2% and minimum consumption was at an extract concentration of 30%. There were statistically significant concentration effects on mortality ($t = 7.22$; $P = 0.002$; $P > 0.05$) and feeding ($t = 2.53$; $P = 0.065$; $P > 0.05$).

Table 2. Mortality of and filter paper consumption by *Coptotermes heimi* exposed for 7 days to varying concentrations of wood extracts of *Eucalyptus cameldulensis, Morus alba* and *Pinus roxburghii* using hexane as the solvent

| Tree species | Extract concentration (%) | Mean mortality (%) | Filter paper consumed (g) | Solvent Used |
|---|---|---|---|---|
| *Eucalyptus camaldulensis* | Control | 0.00 | 0.50 | hexane |
| | 2 | 30.1 | 0.41 | |
| | 5 | 50.2 | 0.35 | |
| | 10 | 70.8 | 0.24 | |
| | 20 | 84.7 | 0.18 | |
| | 30 | 97.5 | 0.13 | |
| *Morus alba* | Control | 0.00 | 0.49 | methanol |
| | 2 | 20.8 | 0.41 | |
| | 5 | 40.2 | 0.39 | |
| | 10 | 40.8 | 0.37 | |
| | 20 | 50.7 | 0.31 | |
| | 30 | 70.6 | 0.24 | |
| *Pinus roxburghii* | Control | 0.00 | 0.47 | hexane |
| | 2 | 20.5 | 0.42 | |
| | 5 | 30.8 | 0.38 | |
| | 10 | 70.9 | 0.25 | |
| | 20 | 80.2 | 0.18 | |
| | 30 | 90.6 | 0.14 | |

N = 100 (sum of three replicates).

Table 2 shows workers of *C. heimi* exposed to different concentrations of *M. alba* extract. The highest mortality rate of 70.6% was observed at an extract concentration of 30% and the mortality rate of 20.8% was at an extract concentration of 2%. The table also shows the consumption of filter paper by *C. heimi* when treated with different concentrations of *M. alba* extract. Maximum consumption of 0.41 g was observed at an extract concentration of 2% and minimum consumption of 0.24 g was at an extract concentration of 30%. There statistically significant concentration effects on mortality ($t = 8.68$; $P = 0.001$; $P > 0.05$) and feeding ($t = 2.52$; $P = 0.065$; $P > 0.05$).

When workers of *C. heimi* were exposed to the different concentrations of *P. roxburghii* extract, the highest mortality rate of 90.6% was observed at an extract concentration of 30% and the lowest mortality rate of 20.5% was at an extract concentration of 2% (Table 2). The table also shows the consumption of filter paper by *C. heimi* when treated with different percentage concentrations of *P. roxburghii*. The maximum consumption of 0.42 g was observed at an extract concentration of 2% and minimum consumption of 0.14 g was at an extract concentration of 30%. There were statistically significant concentration effects on mortality ($t = 4.77$; $P = 0.009$; $P > 0.05$) and feeding ($t = 2.52$; $P = 0.065$; $P > 0.05$).

**Choice test and repellency bioassay**

Table 3 shows the results when workers of *C. heimi* were exposed to *E. camaldulensis* extracts to detect the repellent and attractive effects on treated and untreated filter paper in Petri dishes. There were repellent effects on termites, which moved from treated to untreated; at 2%, 18 termites were observed on untreated filter paper as the concentrations increased fewer termites were observed on treated filter paper. However, at 10-30%, concentration most of the termites were present on untreated filter paper indicating repellency.

Table 3. Repellency effects of wood extracts on *Coptotermes heimi* when exposed to filter paper with five different concentrations of *Eucalyptus camaldulensis, Morus alba* and *Pinus roxburghii* extracts for 60 mins

| Tree species | Extract concentration (%) | Termites* on treated filter paper | Termites* on untreated filter paper |
|---|---|---|---|
| *Eucalyptus camaldulensis* | 2 | 12 | 18 |
| | 5 | 15 | 15 |
| | 10 | 9 | **21** |
| | 20 | 6 | **24** |
| | 30 | 2 | **28** |
| *Morus alba* | 2 | 18 | 12 |
| | 5 | 12 | 18 |
| | 10 | 6 | **24** |
| | 20 | 6 | **24** |
| | 30 | 0 | **30** |
| *Pinus roxburghii* | 2 | 12 | 18 |
| | 5 | 15 | 15 |
| | 10 | 9 | **21** |
| | 20 | 6 | **24** |
| | 30 | 2 | **28** |

*N = 30 (sum of three replicates). Values in bold in columns indicate repellency.

Table 3 shows the results when workers of *C. heimi* were exposed to *M. alba* extracts to detect the repellent and attractive effects on treated and untreated filter paper in Petri dishes. There were repellent effects on termites, which moved from treated to untreated; at 2%, 12 termites were observed on untreated filter paper as the concentrations increased fewer termites were observed on treated filter paper. However at 10-30% concentration, most of the termites were present on untreated filter paper indicating repellency.

Table 3 shows the results when workers of *C. heimi* were exposed to *P. roxburghii* extracts at different concentrations to detect the repellent and attractive effects on treated and untreated filter paper in Petri dishes. There were repellent effects on the termites, which moved from treated to untreated; at 2%, 18 termites were observed on untreated filter paper as the concentrations increased fewer termites were observed on treated filter paper. However at 10-30% concentration, most of the termites were present on untreated filter paper indicating repellency.

**Effect of wood extracts on tunneling activity of *Coptotermes heimi***

The tunnel length formed by *C. heimi* at 2, 5, 10, 20 and 30% concentrations of wood extracts of *E. camaldulensis*, *M. alba* and *P. roxburghii* in different solvents is given in Table 4.

Table 4. Effect of different wood extracts on tunneling activity of *Coptotermes heimi* at varying concentrations of wood extracts of *Eucalyptus camaldulensis, Morus alba* and *Pinus roxburghii* after 7 days exposure

| Extract concentration (%) | Tunneling distance (mm, mean ± SD) | | |
|---|---|---|---|
| | *Eucalyptus camaldulensis* | *Pinus roxburghii* | *Morus alba* |
| Control | 294.2 ± 1.53 | 298.3 ± 1.32 | 295.4 ± 1.68 |
| 2 | 60.1 ± 0.98 | 72.0 ± 1.42 | 70.1 ± 1.95 |
| 5 | 46.1 ± 1.05 | 56.2 ± 1.47 | 55.1 ± 1.58 |
| 10 | 24.2 ± 0.95 | 34.2 ± 1.20 | 33.2 ± 1.205 |
| 20 | 20.4 ± 1.21 | 25.3 ± 0.86 | 24.3 ± 0.80 |
| 30 | 10.9 ± 1.00 | 12.7 ± 1.05 | 11.6 ± 1.2 |

## Discussion

Compounds present in the extracts of *M. alba* wood have been previously reported (Venkataraman, 1972; Rowe & Conner, 1979; Se Golpayegani, 2007; Sadeghifar et al., 2011). Higher hydrocarbons, fatty acids, sterols and phenols constitute the majority of the compounds found in white mulberry. Extracts of *M. alba* were protective against decay caused by fungi and termites (Se Golpayegani et al., 2014). Siramon et al. (2007), investigated the essential oils from *E. camaldulensis* leaves and found both fumigants and contact toxicants against *Coptotermes formosanus*. The antitermite functions were dependent on the chemical composition of the oils. The various chemical constituents isolated from the bark of *P. roxburghii* include 3,4-dihydroxybenzoic acid, terpenoids, flavonoids, tannins, xanthones and some other compounds and all showed antitermite activity (Kaushik et al., 2013). In the present study, similar results were found using *E. camaldulensis, M. alba* and *P. roxburghii.* The preference of termites for a particular wood species could be altered by the wood combination offered (Smythe & Carter, 1970a; Morales-Ramos & Rojas, 2001). The choice feeding test was a more appropriate method for determining termite wood preference than no choice test (forced feeding), which is why it was adopted here because in the later test method, termites were forced to feed on whatever resource was available to survivor (Smythe & Carter, 1970b). The results of present study were consistent with above findings, as the workers of *C. heimi* more easily identified the palatable food source than the distasteful one when offered these in choice feeding experiments. In the laboratory trials more termites were observed feeding on palatable food source. *Eucalyptus camaldulensis, M. alba* and *P. roxburghii* sawdust with ethanol, methanol and hexane in the bioassay of *C. heimi* exhibited mortality at different concentrations of the extracts. In the feeding bioassays on *C. heimi*, the impact of different wooden extracts used against this termite indicated the following descending order of feeding preference: *P. roxburghii* > *M. alba* > *E, camaldulensis*. Resistance to these species to termites after preservation could be due to the repellent

characteristics of the extractives. The repellent characteristics could be due to the toxic chemical composition of the various wood extractives and the durability of the heartwood of the tree species from which they were extracted.

**Conclusions and recommendations**

1) In the present study, the chemical constituents of wood extracts from *E. camaldulensis, M. alba* and *P. roxburghii* was studied using gas chromatographic analysis. Several compounds were identified. Fatty acids and waxes were the major groups of compounds detected and identified.

2) The main compounds detected in *P. roxburghii* were 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and octadecanoic acid, methyl ester. The main compounds detected in *M. alba* were 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, octadecanoic acid (methyl ester), hexadecanoic acid (methyl ester) and 1-methyl-3-(1-methylethyl)-benzene. The main compounds detected in *E. camaldulensis* were 1, 2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester. Bioactivity bioassay revealed antitermite activity was common and were different as far as their feeding was concerned.

## References

Abbas, S. Q., T. Iftikhar, M. Niaz & N. Sadaf, 2010. An addition to fungal flora of *Eucalyptus* sp. Pakistan Journal of Botany , 42 (5): 3317-3321.

Ahmed, S. & M. Farhan, 2006. Laboratory evaluation of chlorpyrifos, bifenthrin, imidacloprid, thiamethoxam and flufenoxuron against *Microtermes obesi* (Isoptera: Termitidae). Pakistan Entomologist, 28: 45-50.

Ahmed, S., M. A. Riaz & M. Shahid, 2006. Response of *Microtermes obesi* (Isoptera: Termitidae) and its gut bacteria towards some plant extracts. Journal of Food Agriculture and Environment, 4 (1): 317-320.

Akhtar, M.S., 1974. Zoogeography of the termites of Pakistan. Pakistan Journal of Zoology, 6: 85-104.

Anonymous, 2000. Finding Alternatives to Persistent Organic Pollutants (POPS) for Termite Management. Global IPM Facility Expert Group Termite Biol. Manag., Stockholm Conven., FAO, Rome, Italy. pp. 118-168.

ASTM, American Standard Test Methods, 2003. Standard Test Method for Wood Preservatives by Laboratory Soil-Block Cultures. D1413-99. ASTM International, West Conshohocken, PA.

Batish, D. R., H. P. Singh, R. K. Kohli & S. Kaur, 2008. Eucalyptus essential oil as a natural pesticide. Forest Ecology and Management, 256: 2166-2174.

Chung, K. M. & C. Y. Lee, 1999. Termites, in Urban Pest Control, a Malaysian Perspective University Sains Malaysia, Penang, pp: 107-114.

Dixon, R. A.,1999. Isoflavonoids: Biochemistry, Molecular Biology and Biological Functions. Elsevier, New York. pp. 773-823.

Eggleton, P., 2001. "Global Patterns of Termite Diversity, 25-51". In: Termites: Evolution, Sociality, Symbioses, Ecology. (Eds: T. Abe, D. E. Bignell & M. Higashi), Kluwer Academic Publishing, Dordrecht, 466 pp.

Gewali, M. B., 2008. Institute of Natural Medicine. Japan: University of Toyama; Aspects of Traditional Medicine in Nepal; pp. 19–20.

Highley, T. L. & T. C. Scheffer, 1970. Natural Decay Resistance of 30 Peruvian Woods, Research Paper FPL 143. USDA Forest Service, Forest Products Laboratory, Madison WI. 4 pp.

Hillis, W. E, 1962. Wood Extractives and their Significance to the Pulp and Paper Industry. Academic press, New York.

Kambhampati, S. & P. Eggleton, 2000. "Phylogenetics and Taxonomy, 1-23". In: Termites: Evolution, Sociality, Symbioses, Ecology. (Eds: T. Abe, D. E. Bignell & M. Higashi), Kluwer Academic Publishing, Dordrecht, 466 pp.

Kaushik, P., D. Kaushik & S. L. Khokra, 2013. Ethnobotany and phytopharmacology of *Pinus roxburghii* Sargent: a plant review. Journal of integrated Medicine, 11 (6): 371-376.

Khan, R. M. & A. P. Singh, 1985. Control of termites in wheat crop with insecticides applied through irrigation. Indian Journal of Entomology, 3:197-201.

Morales-Ramos, J. A. & M. G. Rojas, 2001. Bait matrix for delivery of chitin synthesis inhibitors to the Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology, 94: 506- 510.

Nawort, J., E. Bloszyk & J. Harmatha, 1986. Action of antifeedants of plant origin on beetles infesting stored products. Acta Entomologica Bohemoslovaca, 83: 327-335.

Nerio, L. S., J. Olivero-Verbel & E. Stashenko, 2010. Repellent activity of essential oils: A review. Bioresource Technology, 101: 372-378.

Ohkuma, M., H. Yuzawa, W. Amornsak, Y. Sornnuwat, Y. Takematsu, A. Yamada, C. Vongkaluang, O. Sarnthoy, N. Kirtibutr, N. Noparatnaraporn, T. Kudo & T. Inoue, 2004. Molecular phylogeny of Asian termites (Isoptera) of the families Termitidae and Rhinotermitidae based on mitochondrial COII sequences. Molecular phylogenetics and Evolution, Vol: 31, pp: 701-710.

Pedigo, L. P., 1986. Entomology and Pest Management. 2nd ed. Upper Saddle River (NJ): Prentice Hall. 655 pp.

Peralta, R. C. G., E. B. Menezes, A. G. Carvalho, E. L. A. Menezes, 2003. Feeding preferences of subterranean termites for forest species associated or not to wood decaying fungi. Floresta e Ambiente, 10 (2):58-63.

Peralta, R. C. G., E. B. Menezes, A. G. Carvalho, E. L. A. Menezes, 2004. Wood consumption rates of forest species by subterranean termites (Isoptera) under field conditions. Revista Arvore, 28 (2): 283-289.

Potter, M. F. & A. E. Hillery, 2001. Exterior-targeted liquid termiticides: An alternative approach to managing subterranean termites (Isoptera: Rhinotermitidae) in buildings. Sociobiol, 39 (3): 373-405.

Rasib, K. Z., 2008. Feeding preferences of Microcerotermes champoni (Snyder) on different timbers dried at different temperatures under choice and no choice trials. Nature Preceedings. (Web page: http://hdl.handle.net/10101/npre.2008.20481), (Accessed date: Jan 2016).

Rowe, J. W. & A. Conner, 1979. Extractives in Eastern Hardwoods-A Review. Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, Madison, Wisconsin, USA, 72 pp.

Sadeghifar, H., A. Sheikh, M. A. Khalilzadeh & A. G. H. Ebadi, 2011. Heartwood extractives of Iranian *Morus alba* wood. Journal of Chemistry Pakistan, 33 (1): 104-106.

Schultz, T. P. & D. D. Nicholas, 2000. Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. Phytochemistry, 54: 47–52.

Schultz, T. P., & Nicholas, D. D., 2002. Development of environmentally-benignwood preservatives based on the combination of organic biocides with antioxidants and metal chelators. Phytochemistry, 61: 555–560.

Se Golpayegani, A., 2007. Primary evaluation of mulberry's wood (*Morus alba* L.). Master thesis (in Farsi), University of Tehran, Iran, 150 pp.

Se Golpayegani, A., 2014. Toxicity potential in the extraneous compounds of White Mulberry wood (*Morus alba*). Maderas. Ciencia y tecnología, 16 (2): 227-238.

Shahid, M., 2003. Principles of insect pest management. Pub. No.19 (233)/Mono/HEC/96. 183 pp.

Simmonds, M. S. J., 2003. Flavonoid-insect interactions: Recent advances in our knowledge. Phytochemistry, 64: 21-30.

Siramon, P. & Y. Ohtani, 2007. Antioxidative and antiradical activities of *Eucalyptus camaldulensis* leaf oils from Thailand. Journal of Wood Science, 53: 498–504.

Smythe, R. V. & F. L. Carter, 1970a. Feeding responses to sound wood by *Coptotermes formosanus*, *Reticulitermes flavipes* and *R. vivginicus* (Isoptera: Rhinotermitidae). Annals of Entomological Society of America, 63: 841-846.

Smythe, R. V. & F. L. Carter, 1970b. Survival and behavior of three subterranean termite species in sawdust of eleven wood species. Annals of Entomological Society of America, 63: 847-850.

Taylor, A. M., B. L. Gartner & J. J. Morrell, 2002. Heartwood formation and natural durability -A review. Wood and Fiber Science, 34 (4): 587-611.

Troup, R. S., 1921. The Silviculture of Indian Trees, Volume I, II, III. Oxford at the Clarendon Press.

Tsunoda, K., 1990. The natural resistance of tropical woods against biodeterioration. Wood Research, 77: 18–27.

Venkataraman, K., 1972. Wood phenolics in the chemotaxonomy of the moraceae. Phytochemistry, 11: 1571-1586.

Verkerk, R. H. J. & A. F. Bravery, 2001. The UK termite eradication programme: Justification and implementation. Sociobiology, 37: 351-360.

Walker, J. C. F., 1993. Primary Wood Processing. Principles and Practice.1st Edition. Chapman and Hall. 285 pp.

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# Determination of the efficacy of some entomopathogenic nematodes against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions[1]

Bazı entomopatojen nematodların *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'ya karşı etkilerinin laboratuvar koşullarında belirlenmesi

**Sevgi TÜRKÖZ[2]** **Galip KAŞKAVALCI[2]***

## Summary

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which was first detected in Izmir Province, Turkey in 2009 has spread quickly and has become the major pest in tomato producing areas. The efficacy of three different entomopathogenic nematode (EPN) species isolates (from e-nema® GmbH Schwentinental, Germany), *Heterorhabditis bacteriophora* (Poinar), *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) was investigated against *T. absoluta* during 2013-2014 under laboratory conditions. The EPNs were applied with different inoculation rates (1, 2, 5, 10, 15, 20, 25 and 40 infective juveniles per larva) for each species to the third instar larvae of *T. absoluta* outside the leaves. The mortality rates for *H. bacteriophora, S. carpocapsae* and *S. feltiae* were found between 21.2 - 74.2%, 28.8 - 99.4% and 17.5 - 95.2%, respectively. According to the results, *S. carpocapsae* and *S. feltiae* caused similar mortalities at the given inoculation rates while *H. bacteriophora* had lower efficacy compared to those two species. The values of $LD_{50}$ for *H. bacteriophora, S. carpocapsae* and *S. feltiae* were 21.67, 7.13 and 6.25 infective juveniles per larva, respectively. Based on these data, *S. feltiae* was the most efficient nematode species, and was then applied against the larva of *T. absoluta* inside the mines and pupae. However, *S. feltiae* only caused low mortality of larvae both inside mines (19%) and the pupae (7%). These results revealed that EPN have good potential for the control of *T. absoluta* larvae outside the leaves and should be studied further.

**Key words:** Efficacy, *Heterorhabditis, Steinernema,* tomato, *Tuta absoluta*

## Özet

Türkiye'de ilk defa 2009 yılında İzmir ilinde tespit edilen domates güvesi, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) hızlı bir şekilde yayılarak domates üretim alanlarında ana zararlı konumuna gelmiştir. Üç farklı entomopatojen nematod (EPN) türü olan, *Heterorhabditis bacteriophora* (Poinar), *Steinernema carpocapsae* (Weiser) ve *Steinernema feltiae* (Filipjev) izolatları(e-nema® GmbH Schwentinental, Almanya)'nın *Tuta absoluta*'ya karşı olan etkileri 2013-2014 yılları arasında Ege Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nematoloji Laboratuvarı'nda laboratuvar koşulları altında araştırılmıştır. EPN'lerin her bir türü, yaprak dışında bulunan üçüncü dönem *T. absoluta* larvalarına farklı inokulasyon oranlarında (1, 2, 5, 10, 15, 20, 25, 40 IJ/larva) uygulanmıştır. Ölüm oranları *Heterorhabditis bacteriophora, S. carpocapsae* ve *S. feltiae* için sırasıyla %21.2-74.2, %28.8-99.4 ve %17.5-95.2 olarak bulunmuştur. Elde edilen sonuçlara göre, *S. carpocapsae* ve *S. feltiae* belirlenen inokulasyon oranlarında benzer ölüm oranlarına sebep olurken, *H.* bacteriophora' nın bu iki türe kıyasla daha düşük düzeyde etkiye sahip olduğu görülmüştür. *H. bacteriophora, S. carpocapsae* ve *S. feltiae* için $LD_{50}$ değerleri sırasıyla 21.67, 7.13 ve 6.25' dir. Bu değerlendirmelere göre en yüksek etkiye sahip nematod türü olarak saptanan *S. feltiae*' nin $LD_{50}$ dozu yaprak içerisinde bulunan *T. absoluta* larvalarına ve pupa dönemlerine uygulanmıştır. Ancak *S. feltiae* yaprak içerisindeki larvalarda (% 19) ve pupalarda (% 7) düşük miktarda ölüme yol açmıştır. Bu sonuçlar, EPN'ların yaprak dışında bulunan *T. absoluta* larvalarının mücadelesinde iyi bir potansiyel olabileceğini ve ileride daha detaylı bir şekilde araştırılması gerektiğini ortaya koymuştur.

**Anahtar sözcükler:** Etkinlik, *Heterorhabditis*, *Steinernema*, domates, *Tuta absoluta*

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[2] Ege University, Agricultural Faculty, Plant Protection Department, 35100, Bornova, İzmir-Türkiye
* Corresponding author (Sorumlu yazar) e-mail: galip.kaskavalci@ege.edu.tr

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## Introduction

In Turkey, about 46.7 Mt of fresh vegetables and fruit are produced each year of which 28.5 Mt is vegetables. Tomato is one of the most widely grown vegetable with the production capacity of 11.8 Mt (Anonymous, 2016). There are many diseases and pest that can economically affect tomatoes. Among these, the tomato leaf miner [*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)] is one of the most economically harmful. This pest originates from South America and was first seen in Europe in Spain in 2006 (Urbaneja et al., 2007), later on in France, Italy, Malta, the Netherlands, England, Hungary, Bulgaria and in the North African countries, Algeria, Morocco and Tunisia (Potting, 2009). It was then detected in West Africa in 2010 (USDA-APHIS, 2011), Sudan and Ethiopia in 2011 (Anonymous, 2013) and crossed the Sahara Dessert in West Africa and reached to Senegal in 2013 (Pfeiffer et al., 2013; Brévault et al., 2014). In Turkey, it was first detected in Urla District, İzmir Province, in 2009 (Kılıc, 2010). It has since spread quickly in Mediterranean Basin as well as the other regions. In Mediterranean Region, it has been caught in pheromone traps of a producer in Kumluca District, Antalya Province (Erler et al., 2010).

*Tuta absoluta* has high potential for reproduction and with 12 generations per year. Although its primary host is tomato, it can also feed on secondary host from the Solanaceae. One female can lay approximately 120 to 260 eggs. The pest generally lays its eggs under leaves, on buds or on immature fruit. The larvae are able to feed on the entire plant above the soil, leaves, stalks, stems and fruit. It feeds between the epidermis of the leaves by tunnelling irregular galleries. These galleries may become a brownish color and the whole plants may die (Desneux et al., 2010).

The control of tomato leaf miner is difficult because the pest feeds inside the mines, develop resistance to insecticides and reproduce quickly. Since chemical control is not sufficient alone, the damage (if it is not controlled) may increase to 80 to 100% in open field and greenhouse tomato production. There is a pressing need to develop sustainable control methods against this pest. In this context, biological control has a significant role because it is safe for the environment and non-target organisms, decreases residue problems in food, protects natural enemies and increases biological diversity in ecosystems. Thus, the entomopathogenic nematodes (EPN) are efficient biological control agents for many pests that are economically important (Grewal et al., 2005).

EPNs, *Steinernema* and *Heterorhabditis,* have infective juvenile (IJ) stages like the other orders of Rhabditida. The IJs are adapted to long term survival and contains between 0-250 symbionts in the anterior region of their intestine (Spiridonov et al., 1991; Glazer, 1996). The EPNs belonging to the family of Steinernematidae and Heterorhabditidae are obligate parasites of many pathogens. They can kill pests with their symbiotic bacteria (*Photorhabdus* sp. and *Xenorhabdus* sp.), which live in their intestine. Non-feeding third stage IJs penetrate through natural openings (mouth, anus and stigma), or in some species (*Heterorhabditis* spp.) through the cuticle, and enter the hemocoel of the host releasing their symbiotic bacteria into the haemolymph of their host. These bacteria propagate and produce toxins (Dowds & Peters, 2002) and other metabolites (Webster et al., 2002) to suppress the defense mechanisms of the host, which usually dies within 48 h of invasion by the nematode. The cadaver provides food for the nematode for up to three generations (Poinar, 1990; Kaya & Gaugler, 1993). When the nematodes leave the insect cadaver, they seek a new host.

EPNs are safe biological control agents and have been successfully used against soilborne insects in ornamental plants, turf, mushrooms and strawberries (Ehlers, 1996; Kaya & Gaugler, 1993), and also against pest with cryptic habitats where the pests are highly protected inside galleries of plants (Begley, 1990; Klein, 1990; Williams & Walters, 1999; Tomalak et al., 2005).

There have been some studies conducted to examine the efficacy of EPN on *T. absoluta.* Batalla-Carrera et al. (2010) recorded high mortality (78.6 - 100%) of *T. absoluta* larvae when EPN were applied under laboratory conditions. In addition, they have observed 87 - 95% decrease in the infestation of tomato leaves in a pot experiment. Garcia-del Pino et al. (2013) also found high mortality of *T. absoluta* larvae that fall down to pupate in the soil. The mortality rate of *T. absoluta* larvae caused by *Heterorhabditis bacteriophora* (Poinar)*, Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) was 97, 100 and 52%, respectively. They have also reported the mortality of emerging adults as to be 79% for *S. carpocapsae* and 0.5% for *S. feltiae*. In another study conducted in Turkey by Gözel et al. (2014), the efficacy of EPNs both in the laboratory and under natural conditions was evaluated at different temperatures. Different results were observed in the two contexts. In another study done in the same region by Gözel & Kasap (2015), *S. feltiae* was found to be the most efficient nematode in an open field application over two growing seasons.

EPNs are seen as suitable candidates for sustainable agriculture and integrated pest management owing to their behaviour. They have many advantages such as, wide host range, rapid host death, actively seek and invade their hosts, easily cultured *in vivo* and *in vitro*, suitable for standard application equipment, safe for the environment, and exempted from registration in many countries (Shapiro-Ilan et al., 2012).

The aim of this work was to evaluate the use of EPNs against *Tuta absoluta,* which has recently caused serious losses in tomato production and has become a major tomato pest.

## Materials and Methods

### Host plant culture

Tomato seedlings (cv. Şimşek) were planted with sterilized peat mixture into the pots every 2 months in a greenhouse at the Plant Protection Department, Ege University. Fertilizer was applied regularly from 15 d after planting.

### *Tuta absoluta* culture

The initial population of *T. absoluta* was obtained as culture from Akdeniz University, Plant Protection Department. To culture *T. absoluta*, fresh tomato branches were cut and put in a jar filled with water and placed in 0.5 x 1 x 0.5 m cages. Adults of tomato leaf miner were then put into these cages to deposit eggs. The eggs hatched after 5 days and started feeding within galleries. To maintain the culture, the plants were checked and replaced with fresh branches once per week.

### *Galleria mellonella* L. (Lepidoptera: Pyralidae) culture

*In vivo* EPN cultures were established using the final instar larvae of wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Galleria mellonella* larvae were reared on artificial medium (22% corn groats, 22% wheat flour, 11% honey, 11% glycerol, 5.5% yeast powder and 17.5% beeswax) inside a glass jar at 35°C under laboratory conditions. The adults and larvae of *G. mellonella* were kept separately. Tissue paper is placed on top of the jar for adult to lay eggs. The eggs were collected and placed in fresh medium to maintain the culture.

### Entomopathogenic nematode culture

The EPNs (*H. bacteriophora, S. carpocapsae and S. feltiae*) used in this study were obtained from e-nema® GmbH (Schwentinental, Germany). They were propagated *in vivo* using the final instar larvae of *G. mellonella* as described by Kaya & Stock (1997). Different batches of *G. mellonella* larvae were inoculated with 80 to 100 IJ/larva and kept in the dark at 25°C. Three to 4 d after inoculation, dead cadavers of *G. mellonella* larvae were transferred to White traps in order to allow the emergence of IJs. Freshly emerged IJs were collected from the White traps and stored in Ringer's solution (9.0 g NaCl, 0.42 g KCl, 0.37 g $CaCl_2.2H_2O$ and 0.2 g $NaHCO_3$, in 1 l distilled water) until used.

**Application of entomopathogenic nematodes**

The EPNs were applied to third instar larvae of *T. absoluta* outside the leaves, final instar larvae inside the mines and to pupae at different inoculation rates. The experiment was included 20 replications at three application times (totally 60 larvae) for each species of nematodes. For each group 20 larvae were used as a control group.

For the application of nematodes to the third instar larvae of *T. absoluta* outside the leaves, moistened filter paper and the tomato leaf disks were placed into 24-well plates, then each larvae was transferred individually into a well. Nematodes were checked for viability before use. Nematodes were applied at 1, 2, 5, 10, 15, 20, 25 and 40 IJ per tomato leaf miner larvae for each species. Water was applied to the control larvae. Plates were wrapped with Parafilm and stored at 25°C.

For the application of nematodes to *T. absoluta* inside the mines, leaf disks with one larva were placed in each well of 24-well plates with tissue paper on the bottom. The $LD_{50}$ inoculation rate for *S. feltiae*, the most efficient nematode strain from the previous experiment, was used for the larvae inside the mines. The plates were covered and stored at 25°C.

For the pupa experiment, *T. absoluta* pupae were placed into the 24-well plates and each well filled with 10% moistened sterilized sand. The $LD_{50}$ inoculation rate for *S. feltiae* was applied to each well. The plates were wrapped with Parafilm and stored in dark.

**Statistical analysis**

All data was evaluated using SPSS (Version 15.00; SPSS, Chicago, IL, USA) statistical software. The mean mortality was compared with ANOVA and groups were determined with Duncan's test. The data obtained from the efficacy of EPN on *T. absoluta* outside the leaves was corrected with Abbots formula (Abbott, 1925) and the data was square root transformed before analysis. To evaluate the $LD_{50}$ probit analyses was performed with BioStat 2009 (AnalystSoft Inc., Vancouver, Canada).

## Results and Discussions

This study demonstrated the efficacy of three different species of EPNs (*H. bacteriophora*, *S. carpocapsae* and *S. feltiae*) with 8 different inoculation rates ranging between 1 and 40 IJ/larva on *T. absoluta* larvae outside the leaves. The data obtained from this experiment were evaluated in two ways; the efficacy of each EPN species and a comparison of the efficacy of three different species at the same inoculation rates. Afterwards, $LD_{50}$ values of three different nematode species were calculated and the most effective species, *S. feltiae,* was applied to *T. absoluta* larvae inside mines and to pupae to assess the efficacy this nematode.

**The efficacy of entomopathogenic nematodes on *Tuta absoluta* outside the leaves for each nematode species**

For *S. feltiae,* the mortality was ranged between 17.5 and 95.2%. The highest mortality was obtained at 40 IJ/larva. Significant differences were recorded between the inoculation rates of 1, 2 and 5 IJ/larva ($F = 36.0$; $df = 7$; $P \leq 0.05$) (Table 1). However there were no significant differences at inoculation rates of 15, 20, 25 and 40 IJ/larva.

For *S. carpocapsae,* the mortality was ranged between 28.8 and 99.4%.The highest mortality was obtained at 40 IJ/larva. However, there were no significant differences in the mortality at 40 IJ/larva between the inoculation rates of 20 and 25 IJ/larva. Significant differences were observed at 5 and 10 IJ/larva ($F = 34.5$; $df = 7$; $P \leq 0.05$) (Table 1).

For *H. bacteriophora,* the mortality was ranged between 21.2 and 74.2%. The highest mortality was reached at 40 IJ/larva. However this was not significantly different from 20 and 25 IJ/larva. Also, no significant differences was observed at 1, 2 and 5 IJ/larva (F = 12.0; df= 7; P ≤ 0.05) (Table 1).

Table 1. Mortality of *Tuta* absoluta (Meyrick) (Lepidoptera: Gelechiidae) larvae outside the leaves caused by three different entomopathogenic nematode species [(mean±SD) (min, max)], (n=60)

| Inoculation rates | *Heterorhabditis bacteriophora* | | *Steinernema carpocapsae* | | *Steinernema feltiae* | |
|---|---|---|---|---|---|---|
| | Mortality (%) | | Mortality (%) | | Mortality (%) | |
| 1 | 21.21±1.52 (18.18-22.73) | a* | 28.79±4.01 (22.73-36.36) | a | 17.46±1.59 (14.29-19.05) | a* |
| 2 | 25.76±1.52 (22.73-27.27) | ab | 36.36±2.62 (31.82-40.91) | ab | 30.16±3.17 (23.81-33.33) | b |
| 5 | 31.82±5.25 (22.73-40.91) | abc | 45.45±7.87 (31.82-59.09) | b | 69.84±8.84 (52.38-80.95) | c |
| 10 | 39.39±8.02 (27.27-54.55) | bc | 69.70±6.06 (63.64-81.82) | c | 62.11±8.07 (48.24-76.19) | cd |
| 15 | 42.42±1.51 (40.91-45.45) | cd | 83.33±8.02 (68.18-95.45) | cd | 77.78±1.59 (76.19-80.95) | de |
| 20 | 59.09±9.46 (45.45-77.27) | de | 93.94±1.51 (90.91-95.45) | d | 82.54±1.59 (80.95-85.71) | de |
| 25 | 65.15±1.51 (63.64-68.18) | e | 95.45±0.00 (95.45-95.45) | d | 90.48±2.75 (85.71-95.24) | e |
| 40 | 74.24±7.58 (59.09-81.82) | e | 99.39±0.61 (98.18-100.00) | d | 95.24±2.75 (90.48-100.00) | e |

*The mortalities include the same letter are not statistically different from each other using Duncan's test (P≤0.05).

In a study conducted by Gözel & Kasap (2015), the susceptibility of EPN to *T. absoluta* larvae was also recorded as high for all EPN species used. However the level of susceptibility differed depending on the nematode species. They conducted their experiments in an open field during two successive years. In both years, *S. feltiae* (isolate 879) was recorded as the most efficient with 90.7 and 94.3% mortality rate in the first and second year, respectively. The least efficient species was *Steinernema affine* (Bovien) (isolate 46) in both years with 39.3 and 43.7% mortality. In another study conducted by Gözel et al. (2014), the efficacy of EPNs both in laboratory and natural conditions was evaluated at different temperatures. They have observed 0 to 87.5% mortality for *H. bacteriophora*, 8.3 to 83.3% for *S. affine*, 12.5 to 87.5% for *S. carpocapsae* and 8.3 to 91.6% for *S. feltiae* in laboratory assays. Whereas, under natural conditions they have found 0 to 85.5% mortality for *H. bacteriophora*, 0 to 41.2% for *S. affine*, 0 to 47.4% for *S. carpocapsae* and 0 to 95.6% for *S. feltiae.*

**The efficacy of entomopathogenic nematodes on *Tuta absoluta* outside the leaves at different inoculation rates**

The mortality of *T. absoluta* for each inoculation rate were evaluated separately to compare the differences for each nematode species.

At 1 IJ/larva, the highest mortality was 28.8% for *S. carpocapsae.* The mortality rate difference between *S. feltiae* and *S. carpocapsae* was significant. However, no significant differences were found for *S. carpocapsae* (28.8%) and *H. bacteriophora* (21.2%) (F = 4.78; df = 2; P ≤ 0.05) (Table 2).

At 2 IJ/larva, *S. carpocapsae* caused the highest mortality of 36.4%, while the *H. bacteriophora* caused the lowest mortality (25.8%) and the difference between these two species was significant. No difference was found between the *Steinernema* spp. ($F = 4.43$; $df = 2$; $P \leq 0.05$) (Table 2).

At 5 IJ/larva, *S. feltiae* was the most efficient nematode with a mortality rate of 69.8%. The least efficient nematode was found as *H. bacteriophora* with 31.8% mortality and the differences between *S. feltiae* and *H. bacteriophora* was found significant ($F = 6.64$; $df = 2$; $P \leq 0.05$) (Table 2).

Table 2. Mortality of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae outside the leaves at each inoculation rates [(mean ±SD), (min, max)], (n=60)

| Inoculation rates | Nematode species | Mortality (%) | | |
|---|---|---|---|---|
| 1 | *Heterorhabditis bacteriophora* | 21.21±1.52 | (18.18-22.73) | ab* |
| | *Steinernema carpocapsae* | 28.79±4.00 | (22.73-36.36) | b |
| | *Steinernema feltiae* | 17.46±1.59 | (14.29-19.05) | a |
| 2 | *Heterorhabditis bacteriophora* | 25.76±1.51 | (22.73-27.27) | a |
| | *Steinernema carpocapsae* | 36.36±2.63 | (31.82-40.91) | b |
| | *Steinernema feltiae* | 30.16±3.17 | (23.81-33.33) | ab |
| 5 | *Heterorhabditis bacteriophora* | 31.82±5.25 | (22.73-40.91) | a |
| | *Steinernema carpocapsae* | 45.45±7.87 | (31.82-59.09) | ab |
| | *Steinernema feltiae* | 69.84±8.84 | (52.38-80.95) | b |
| 10 | *Heterorhabditis bacteriophora* | 39.39±8.02 | (27.27-54.55) | a |
| | *Steinernema carpocapsae* | 69.70±6.06 | (63.64-81.82) | b |
| | *Steinernema feltiae* | 62.11±8.07 | (48.24-76.19) | ab |
| 15 | *Heterorhabditis bacteriophora* | 42.42±1.51 | (40.91-45.45) | a |
| | *Steinernema carpocapsae* | 83.33±8.02 | (68.18-95.45) | b |
| | *Steinernema feltiae* | 77.78±1.59 | (76.19-80.95) | b |
| 20 | *Heterorhabditis bacteriophora* | 59.09±9.46 | (45.45-77.27) | a |
| | *Steinernema carpocapsae* | 93.94±1.51 | (90.91-95.45) | b |
| | *Steinernema feltiae* | 82.54±1.59 | (80.95-85.71) | b |
| 25 | *Heterorhabditis bacteriophora* | 65.15±1.51 | (63.64-68.18) | a |
| | *Steinernema carpocapsae* | 95.45±0.00 | (95.45-95.45) | b |
| | *Steinernema feltiae* | 90.48±2.75 | (85.71-95.24) | b |
| 40 | *Heterorhabditis bacteriophora* | 74.24±7.58 | (59.09-81.82) | a |
| | *Steinernema carpocapsae* | 99.39±0.61 | (98.18-100.0) | b |
| | *Steinernema feltiae* | 95.24±2.75 | (90.48-100.0) | b |

*The mortalities include the same letter are not statistically different from each other using Duncan's test ($P \leq 0.05$).

At 10 IJ/larva the highest mortality was seen in *S. carpocapsae* with 69.7% mortality. These results were in accordance with the result of Lacey & Unruh (1998). They studied the effect of three different nematode species (*S. carpocapsae, Steinernema riobrave* Cabanillas, Poinar and Raulston and *H. bacteriophora*) at different temperatures against *Cydia pomonella* (L.) (Lepidoptera : Tortricidae) larvae. In their study, *S. carpocapsae* was also found to be the most efficient nematode at 10 IJ/cm$^2$ inoculation rate with the mortality ranging from 66 to 90%.

When the three different species of EPN (*H. bacteriophora, S. carpocapsae and S. feltiae*) were compared for the remaining inoculation rates, *Steinernema* spp. was found to be more efficient than *H. bacteriophora*. The mortality rates for both *Steinernema* spp. showed differences but generally these were not siginificant. Our results were similar to those of Gözel & Güneş (2013), who investigated

different Turkish isolates of the EPNs against *Sesamia cretica* Lederer (Lepidoptera: Noctuidae) and found that *S. carpocapsae* and *S. feltiae* gave the similar results at the given temperatures.

At 25 IJ/larva in our study, the mortalities were 65.2% for *H. bacteriophora,* 95.5% for *S. carpocapsae* and 90.5% for *S. feltiae. A*t 40 IJ/larva, the mortalties were 74.24% for *H. bacteriophora,* 99.4% for *S. carpocapsae* and 95.2% for *S. feltiae*. Our results were similar to those reported by Batalla-Carrera et al. (2010). They observed the efficacy of three EPN species against *T. absoluta* and found the mortalities at 25 IJ/cm$^2$ 78.6% for *H. bacteriophora* 85.7% *S. carpocapsae* and 100% for *S. feltiae*, and at 50 IJ/cm$^2$, 100% for *H. bacteriophora* 86.6% for *S. carpocapsae* and 100% for *S. feltiae*.

**$LD_{50}$ of nematodes**

The most efficient species for *T. absoluta* was *S. feltiae* ($LD_{50}$ = 6.2 IJ/larva) followed by *S. carpocapsae* ($LD_{50}$ = 7.1 IJ/larva). The least efficient species was *H. bacteriophora* ($LD_{50}$ = 21.7 IJ/larva). In this study *S. carpocapsae* was more efficient than *H. bacteriophora.* Similar results were reported by Salari et al. (2014). They reported that *S. carpocapsae* ($LD_{50}$ = 6.4 IJ/larva) was more effective than *H. bacteriophora* ($LD_{50}$ = 8.4 IJ/larva) against the larvae of *Zeuzera pyrina* (L.) (Lepidoptera: Cossidae). Lacey & Unruh (1998) also concluded that *S. carpocapsae* ($LD_{50}$ = 4.7 IJ/larva) was more efficient than *H. bacteriophora* ($LD_{50}$ = 6 IJ/larva).

**The efficacy of entomopathogenic nematodes on *T. absoluta* larvae inside the mines and pupae**

The most efficient nematode strain, *S. feltiae,* was applied with $LD_{50}$ inoculation rates to the larvae of *T. absoluta* inside the mines and pupae stages. The nematodes caused 19% mortality for the larvae inside the mines. Although previous studies had shown that EPNs can be used successfully for soil dwelling Coleopteran species, there are limited studies for Lepidopteran species that are above ground pest (Klein, 1990). In our study, the mortality of tomato leaf miner larvae was quite low. Arthurs et al. (2004) showed that application on leaves for above ground pest had low efficacy. The major reason for the limited the success of the application on leaves is likely to be desiccation of IJs. Addition of an anti-desiccant may help to increase the efficacy on leaves.

The most efficient nematode strain *S. feltiae* was applied at the $LD_{50}$ inoculation rate to the pupae of *T. absoluta*. This resulted in 7% mortality. The mortality rate for pupae was lower than for to larval stages outside the mines. This result is in agreement with Batalla-Carrera et al. (2010), who also observed lower than 10% mortality for pupae of *T. absoluta*. Similar results were obtained by Garcia-del Pino et al. (2013), who observed no mortality in pupal stages of tomato leaf miner.

The results of this study demonstrated that EPNs could be a good candidates for the control of *T. absoluta* larval stages outside the mines, and further study of their efficacy under natural conditions is warranted.

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## References

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

Anonymous, 2013. *Tuta absoluta* Information Network. (Web page:http://www.tutaabsoluta.com), (Date accessed: December 2013).

Anonymous, 2016. 'Agricultural Land by Provinces. (Web page: http://www.turkstat.gov.tr/PreTablo.do?alt_id=1001), (Date accessed: March 2016).

Arthurs, S., K. M. Heinz & J. R. Prasifka, 2004. An analysis of using entomopathogenic nematodes against above-ground pests. Bulletin of Entomological Research, 94: 297–306.

Batalla-Carrera, L., A. Morton & F. Garcia-del-Pino, 2010. Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. BioControl, 55: 523–530.

Begley, J. W., 1990. ''Efficacy Against Insects in Habitats Others than Soil,215-231''. In: Entomopathogenic Nematodes in Biological Control (Eds. R. Gaugler & H. K. Kaya). CRC Press, Boca Raton, FL.

Brévault, T., S. Sylla, M. Diatte, G. Bernadas & K. Diarra, 2014. *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae): A New Threat to Tomato Production in Sub-Saharan Africa. African Entomology, 22 (2): 441-444.

Desneux, N., E. Wajnberg, K. A. G. Wyckhuys, G. Burgio, S. Arpaia, C. A. Narvaez-Vasquez, J. Gonzalez-Cabrera, D.C. Ruescas, E. Tabone, J. Frandon, J. Pizzol, C. Poncet, T. Cabello & A. Urbaneja, 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. Journal of Pest Science, 83: 197-215.

Dowds, B. C. & A. Peters, 2002. ''Virulence mechanisms, 79–98''. In: Entomopathogenic Nematology (Ed. R. Gaugler). CABI Publishing, Oxon, UK.

Ehlers, R.U., 1996. Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. Biocontrol Science and Technology, 6: 303-316.

Erler, F., M. Can, M. Erdogan, A. O. Ates, & T. Pradier, 2010. New record of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on greenhouse-grown 46 tomato in southwestern Turkey (Antalya). Journal of Entomological Science, 45: 392-393.

Garcia-del-Pino, F., X. Alabern & A. Morton, 2013. Efficacy of soil treatments of entomopathogenic nematodes against the larvae, pupae and adults of *Tuta absoluta* and their interaction with the insecticides used against this insect. BioControl, 58: 723–731.

Glazer, I., 1996. Survival mechanisms of entomopathogenic nematodes. Biocontrol Science and Technology, 6: 373-378.

Gözel, U. & Ç. Güneş, 2013. Effect of entomopathogenic nematode species on the corn stalk borer (*Sesamia cretica* Led. Lepidoptera: Noctuidae) at different temperatures. Türkiye Entomoloji Dergisi-Turkish Journal of Entomology, 37 (1): 65-72.

Gözel, Ç., U. Gözel & İ. Kasap, 2014. "Domates güvesi *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)'nin mücadelesinde entomopatojen nematodların kullanım olanakları, 319". Türkiye V. Bitki Koruma Kongresi (3-5 Şubat 2014, Antalya) Bildirileri, XMAT Matbaa, 417 s.

Gözel, Ç. & İ. Kasap, 2015. Efficacy of entomopathogenic nematodes against the Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato field. Türkiye Entomoloji Dergisi-Turkish Journal of Entomology, 39 (3): 229-237.

Grewal, P. S., R. U. Ehlers & D. I. Shapiro-Ilan, 2005. Nematodes as Biocontrol Agents. CABI Publisher, Wallingford, UK, 505pp.

Kaya, H. K. & R. Gaugler, 1993. Entomopathogenic nematodes. Annual Review of Entomology, 38: 181-206.

Kaya, H. K. & S. P. Stock, 1997. "Techniques in Insect Nematology, 281–324". In: Manual of Techniques in Insect Pathology, (Ed. L. A. Lacey). Academic Press, San Diego.

Kılıc, T., 2010. First record of *Tuta absoluta* in Turkey. Phytoparasitica, 38: 243–244.

Klein, M. G., 1990. "Efficacy against Soil-Inhabiting Insects Pest, 195–214". In: Entomopathogenic Nematodes Biological Control, (Eds. R. Gaugler & H. K. Kaya). CRC Press, Boca Raton, FL.

Lacey, L. A. & T. R. Unruh, 1998. Entomopathogenic nematodes for control of codling moth: Effect of nematode species, dosage, temperature, and humidity under laboratory and simulated field conditions. Biological Control, 13:190–197.

Pfeiffer, D. G., R. Muniappan, D. Sall, P. Diatta, A. Diongue & E. O. Dieng, 2013. First record of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Senegal. Florida Entomologist, 96: 661–662.

Poinar, G. O. Jr., 1990. “Biology and taxonomy of Steinernematidae and Heterorhabditidae, 23-61”. In: Entomopathogenic Nematodes in Biological Control (Eds: R., Gaugler & H. K. Kaya). CRC Press, Boca Raton, FL.

Potting, R., 2009. Pest risk analysis. *Tuta absoluta*, tomato leaf miner moth. Plant Protection Service of the Netherlands, 24.

Salari, E., Karimi, J., Sadeghi-Nameghi, H. & Hosseini, M., 2014. Efficacy of two entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* for control of the leopard moth borer *Zeuzera pyrina* (Lepidoptera: Cossidae) larvae under laboratory conditions. Biocontrol Science and Technology, 25 (3): 260-275.

Shapiro-Ilan, D. I., R. Han & C. Dolinksi, 2012. Entomopathogenic nematode production and application technology. Journal Nematology, 44 (2): 206–217.

Spiridonov, S., E. Akhmedov & F. Belostotskaya, 1991. Proliferation of symbiotic bacteria in the intestinal vesicles of invasive larvae of *Neoaplectana* spp.(Nematoda, Steinernematidae). Helminthologia, 28: 141-142.

Tomalak, M., S. Piggott & G. B. Jagdale, 2005. “Glasshouse Applications, 147–166”. In: Nematodes as Biocontrol Agents (Eds. P. S. Grewal, R. U. Ehlers & D. I. Shapiro-Ilan). CABI Publishing, Oxon.

Urbaneja, A., R. Vercher, V. Navarro, F. Garcia Mari & J. L. Porcuna, 2007. La polilla del tomate, *Tuta absoluta*. Phytoma España, 194:16–23.

Usda-Aphis, 2011. Federal import quarantine order for host materials of tomato leafminer, *Tuta absoluta* (Meyrick), (Web page: http://www.aphis.usda.gov/import_export/plants/plant_imports/federal_order/downloads/2011/Tuta%20absoluta5-5-2011.pdf), (Date accessed: April 2014).

Webster, J. M., C. Chen & K. Hu, 2002. Bacterial metabolites. Entomopathogenic Nematology, 99-114.

Williams, E. C. & K. F. A. Walters, 1999. Foliar application of entomopathogenic nematode *Steinernema feltiae* against leafminers on vegetables. Biocontrol Science and Technology, 10: 61–70.

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*Original article (Orijinal araştırma)*

# Compositional analysis and toxicity of four plant essential oils to different stages of Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

Akdeniz un güvesi *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)'nın farklı gelişme evrelerine karşı dört uçucu bitki yağının toksik etkisi ve kimyasal yapısı

**Dilek PANDIR[1]*** **Hatice BAŞ[1]**

## Summary

Experiments were conducted to investigate the biological effects of essential oils from basil (*Ocimum basilicum* L.), paprika (*Capsicum annuum* L.), peppermint (*Mentha x piperita* L.) and rosemary (*Rosmarinus officinalis* L.) on the different stages of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Essential oils were obtained by Clevenger-type water distillation and analyzed by capillary gas chromatography-mass spectrometry. The doses of the essential oils applied were 0.1, 1, 5, 10, 20, 50 and 100 µl $L^{-1}$ air. The major compounds of the essential oils were detected as linalool (63.1%), capsaicin (35.4%), menthol (28.3%) and cineole (25.7%), in basil, paprika, peppermint, and rosemary oils, respectively. The essential oil of paprika caused the highest mortality of first instar larvae of *E. kuehniella* at a dose of 5 µl $L^{-1}$ air after 24 h exposure. Among the tested different stages, larvae of *E. kuehniella* were the most tolerant of essential. Basil, paprika, peppermint and rosemary oils exhibited toxicity to adult stages of *E. kuehniella* with 100% mortality obtained after 24 h at dose of 100, 5, 20 and 10 µl $L^{-1}$ air, respectively. Increasing the doses of essential oils resulted in increased toxicity to all stages of *E. kuehniella.* In conclusion, the four plants essential oils tested in this study have potential for use in the management of the stored-product pest, *E. kuehniella*.

**Keywords:** *Ephestia kuehniella*, essential oil, GC-MS, insecticidal activity, mortality

## Özet

Bu çalışma fesleğen, *Ocimum basilicum* L., nane, *Mentha x piperita* L., biberiye, *Rosmarinus officinalis* L., biber, *Capsicum annuum* L. uçucu yağlarının *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)'nın farklı gelişim evreleri üzerine gösterdikleri biyolojik etkinlikleri araştırmak için yapılmıştır. Uçucu yağlar Clevenger cihazında su distilasyonu yöntemiyle elde edilmiş olup, analizleri ise gaz kromatografisi kütle spektrometresi ile yapılmıştır. 0.1, 1, 5, 10, 20, 50, 100 µl $L^{-1}$ hava dozlarında uçucu yağ uygulanmıştır. Fesleğen, nane, biberiye ve biberde en çok bulunan bileşikler sırasıyla linalool (% 63.1), capsaicin (% 35.4), menthol (% 28.3) ve cineole (% 25.7)'dir. Biberin uçucu yağı 5 µl $L^{-1}$ hava dozda 24 saatte *E. kuehniella*'nın birinci dönem larvalarında en yüksek ölüme neden olmuştur. Test edilen farklı gelişme evreleri arasında bütün uçucu yağlara karşı en toleranslı gelişme evresi *E. kuehniella* larvası olmuştur. *E. kuehniella* ergininin %100 ölümü 24 saatte maruz bırakma süresinde fesleğen, nane, biberiye ve biber uçucu yağlarının sırasıyla 100, 5, 20, 10 µl $L^{-1}$ hava dozlarında elde edilmiştir. Uçucu yağların artan dozları *E. kuehniella*'nın bütün evrelerinde toksik etkinin artmasına neden olmuştur. Sonuç olarak bu çalışmada test edilen 4 bitki uçucu yağlarının depolanmış ürün zararlısı, *E. kuehniella*'nın mücadelesinde kullanılabilecek potansiyele sahip olabileceği görülmüştür.

**Anahtar sözcükler:** *Ephestia kuehniella*, uçucu yağ, GC-MS, insektisidal aktivite, ölüm

[1] Bozok University, Arts and Sciences Faculty, Biology Department, 66100 Yozgat-Türkiye
* Corresponding author (Sorumlu yazar) e-mail: durak77@gmail.com
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## Introduction

Fumigation is an economical method of using different gases and is still widely used to control stored-product insects in postharvest treatment because it is inexpensive, effective and does not damage stored grain (van Someren Graver, 2004; Azelmat et al., 2006). Pest control is often done with fumigants such as phosphine and methyl bromide. However, the usage of these fumigants has been restricted because of their effects on health and the environment, especially on the ozone layer (Bell, 2000) and development of insect resistance (Benhalima et al., 2004; Daglish et al., 2014). Methyl bromide has been banned in developed countries since 2005 and recently withdrawn worldwide from routine use as a fumigant under the directive of the Montreal Protocol on Substances that Deplete Ozone Layer (Schneider et al., 2003) except for quarantine, laboratory and pre-shipment purposes. Phosphine, as an actual alternative to methyl bromide, is also being criticized due to possible carcinogenic concerns (Alavanja et al., 1990), development of insect resistance (Benhalima et al., 2004; Daglish et al., 2014) and the requirements for long exposure periods (5 d or longer), which makes the chemical unsuitable for quarantine fumigation. Recently, there has been growing interest in research on the possibility of using essential oils as fumigants for controlling stored-product pests.

Aromatic plants are famous because of their nutritional and medicinal characteristics. Many of them, in particularly their essential oils, have a high level of efficiency in protecting crops and stored food against pests. The chemical composition of their essential oils can also vary depending on some parameters such as the geographical area, collecting season, distillation technique and plant part distilled (Lamiri et al., 2001), which has been found to have great influence on their insecticidal activities as a biopesticide (Batish et al., 2008; Boukhatem et al., 2014). Essential oils may have a role as pest control agents (Bachrouch et al., 2010 a,b). They are produced within plants as secondary metabolites. They give the characteristic odor and flavor to different parts of plants and are composed of 20 to 60 organic compounds (Bakkali et al., 2008). They show a broad spectrum of activity as ovicides, larvicides, adulticides, antifeedants, repellents and growth regulators (Nenaah et al., 2015) of insects.

*Ocimum basilicum* L., *Mentha x piperita* L. and *Rosmarinus officinalis* L., belong to the Lamiaceae. *O. basilicum is a* well-known annual culinary herb because it has a pleasant smell and taste. Large chemical variation exists within *O. basilicum* (Labra et al., 2004; De Masi et al., 2006; Telci et al., 2006; Carović-Stanko et al., 2011), which is influenced by several environmental factors. It has antibacterial, antifungal, insecticidal, and hepatoprotective activities and contains antimicrobial substances (Bernhardt et al., 2015). Peppermint oil is an important essential oil extracted mostly from leaves, flowers and stems of *M. x piperita* by steam distillation. Studies have shown that peppermint oil possesses strong antibacterial (Witkowska & Sowinska, 2013; Patra & Yu, 2014) antiviral (Schuhmacher et al., 2003; Schnitzler & Reichling, 2011) and antifungal (Edris & Farrag, 2003) activities, and has anti-inflammatory (Herro & Jacob, 2010; Fashner & Gitu, 2013) and antitumor (Hikichi et al., 2011) effects. *Rosmarinus officinalis,* mostly used as a food flavoring, is also known medicinally for its powerful antibacterial, antimutagenic effects, and as a chemopreventive agent (Oluwatuyi et al., 2004). According to the Peng et al. (2005), owing to its antioxidant properties, *R. officinalis* has been accepted as an antioxidant spice. Fresh peppers (*Capsicum annuum)* have antioxidant activities because of their content of vitamins C and E, provitamin A, carotenoids and phenolic compounds. They provide a benefits for human health, being protective against cancer, gastric ulcer, cardiovascular diseases, age-related macular degeneration and cataracts. The oxidation of cholesterol and docosahexaenoic acid has been prevented by peppers (Materska & Perucka, 2005; Sun et al., 2007).

*Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) is a storage pest that affects many cereals, including barley, maize, oats, rice, sorghum and wheat (bran, flour, grain, meal and semolina) (Karabörklü et al., 2011). *E. kuehniella* is found primarily in flour mills and bakeries. First instar larvae of *E. kuehniella* eat the embryo of whole kernels and larvae of the subsequent instars also attack the pericarp (Fraenkel & Blewett, 1946; Rathore et al., 1980). The development of larvae is faster on ground kernels (Kunike, 1938).

The present study was undertaken to investigate the effects of essential oils of aromatic plant species grown in Turkey against the different stages of the important stored-product pest, *E. kuehniella*. The chemical structures of essential oils derived from different parts of four Turkish plants; basil (*O. basilicum*), paprika (*C. annuum*), peppermint (*M. x piperita*) and rosemary (*R. officinalis*) were identified and their fumigant toxicities against eggs, larvae and adults of *E. kuehniella* examined.

## Materials and methods

### Insect culture

*Ephestia kuehniella* culture was obtained from the Biological Control Research Station, Adana and reared on a blend of about 1 kg wheat flour, 55 g yeast and 30 g wheat germ (Tunçbilek et al., 2009). Insect cultures were kept at 27±1ºC and 70±5% RH (14L:10D h photoperiod). One-day-old adults of *E. kuehniella* were collected daily, placed in plastic jars and their eggs were collected in a Petri dish.

### Plant material and analysis of the essential oils

Plants were collected in the middle of August 2014 from the fields of Aydin, Aegean Region, Turkey. Leaves and fruit were randomly collected and dried in the shade (20-25°C) for one week and then stored in cloth bags. Essential oils were extracted from plant materials by hydrodistillation using a modified Clevenger apparatus for 3 to 4 h at a laboratory scale. With this method, secondary plant metabolites (mainly terpenes and phenolic compounds) are obtained in a relatively pure fraction excluding most of primary metabolites (Ercan et al., 2013). Plant essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS). 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 $min^{-1}$ were used. The machine was coupled with a computer system that manages an ADAMS, NISTO5 and WILEY mass spectrum library.

### Toxicity of essential oil to *Ephestia kuehniella*

Fumigant toxicity tests were carried out in the 1 L glass jars containing larvae and adults of *E. kuehniella*. Six replicates consisted of ten adults and larvae were used for each dose of the essential oil. For fumigation test, filter papers were impregnated with the oils at a range of doses. Each impregnated filter paper was then attached to the underside of a jar lid. Larvae and adults of *E. kuehniella* were exposed to seven different doses of the essential oil (0.1, 1, 5, 10, 20, 50 and 100 µl $L^{-1}$ air) for 24 h, with no chemical given to a control group. For determining mortalities in each dose, adults and larvae were taken out from the jar and live and dead insects were checked with fine brush and counted. If adults and larvae were inactive, they were accepted as dead.

About 100 one-day-old eggs were pasted on egg cards with gum arabic for control and each doses and put into the glass jars. Different doses of essential oils (0.1, 1, 5, 10, 20, 50 and 100 µl $L^{-1}$ air) were applied on a filter paper and mortality of the eggs (unhatched) was counted after 24 h exposure.

### Data analysis

The mortality data obtained from bioassay tests were subjected to one-way analysis of variance (ANOVA) using SPSS 11.0 for Windows. The means were separated using the Tukey's multiple comparison procedure at a significance level of 0.05. A value of $p < 0.05$ was considered statistically significant.

## Results

### Chemical composition of essential oils

The chemical structures of the four essential oils quantified by GC-MS are shown in Table 1. Fourteen, 11, 22 and 17 main components were determined from basil, paprika, peppermint and rosemary oils, respectively. The five main components of essential oils of *O. basilicum* leaves were linalool (63.1%), methyl chavicol (15.1%), 1,8-cineole (4.34%), cadinene (2.56%) and isobornyl acetate (7.44%). The major compounds of *M. x piperita* oils were menthol (28.3%), methyl acetate (12.5%), menthone (15.5%), acetaldehyde (10.0%) and isovaleric aldehyde (9.62%). The major compounds of *R. officinalis* oils were pinene (13.0%), 1,8-cineole (25.7%), camphor (18.0%), camphene (12.8%), carenene (6.4%). The major compounds of *C. annuum* oils were capsaicin (35.4%), methyl chavicol (21.9%), dihydrocapsaicin (21.4%), nonivamide (8.66%) and 1-octadecanamine (3.21%).

Table 1. Composition of water extracts of tested aromatic plants, *Capsicum annuum, Mentha x piperita, Ocimum basilicum* and *Rosmarinus officinalis* essential oils assayed by gas chromatography-mass spectrometry

| Chemical Compounds | *Ocimum basilicum* | *Mentha x piperita* | *Rosmarinus officinalis* | *Capsicum annum* |
|---|---|---|---|---|
| | % | % | % | % |
| 1-Hexadecene | - | - | - | 0.05 |
| 1-Octadecanamine | - | - | - | 3.21 |
| 1,8-Cineole | 4.34 | 1.73 | 25.66 | - |
| 4-Hydroxy-4-methyl-2-pentanone | - | - | - | 8.74 |
| 9-Octadecenamide | - | - | - | 0.24 |
| Acetaldehyde | - | 10.02 | - | - |
| Cadinene | 2.56 | 0.36 | - | - |
| Camphene | - | - | 12.81 | - |
| Camphor | 1.19 | - | 18.03 | - |
| Capsaicin | - | - | - | 35.44 |
| Carenene | - | - | 6.36 | - |
| Carveol | - | 3.5 | - | - |
| Cymene | 0.37 | 0.35 | 3.19 | - |
| Dihydrocapsaicin | - | - | - | 21.35 |
| Eucalyptol | - | 1.15 | - | - |
| Germacrene-D | 2.33 | - | - | - |
| Heptadecane | - | - | - | 0.14 |
| Hexadecaienal | - | - | - | 0.17 |
| Isobornyl acetate | 7.44 | - | 9.64 | - |
| Isovalerianic acid | - | 1.48 | - | - |
| Isovaleric aldehyde | - | 9.62 | - | - |
| Limonene | 0.86 | 2.78 | 5.64 | - |
| Limonene oxide | 0.19 | 0.11 | 0.14 | - |

Table 1. (Continued)

| Chemical Compounds | *Ocimum basilicum* % | *Mentha x piperita* % | *Rosmarinus officinalis* % | *Capsicum annum* % |
|---|---|---|---|---|
| Linalool | 63.10 | 0.14 | 1.53 | - |
| Menthol | - | 28.26 | - | - |
| Menthone | - | 15.45 | - | - |
| Methyl acetate | - | 12.47 | - | - |
| Methyl chavicol | 15.14 | - | - | 21.88 |
| Myrcene | 0.21 | 0.82 | 0.90 | - |
| Neomenthol | - | 2.20 | - | - |
| Nonivamide | - | - | - | 8.66 |
| Oleic Acid | - | - | - | 0.12 |
| Pinene | 2.15 | 1.38 | 13.03 | - |
| Pinocarveol | - | - | 0.19 | - |
| Sabinene | 0.09 | 0.36 | 0.37 | - |
| Terpinen-4-ol | - | 0.45 | 1.77 | - |
| Terpinene | 0.03 | 0.03 | 0.49 | - |
| Terpinolene | - | 0.03 | 0.11 | - |
| Thujene | - | - | 0.14 | - |
| Valeraldehyde | - | 7.63 | - | - |

**Toxicity of essential oils to *Ephestia kuehniella***

The mortalities of *E. kuehniella* adults, larvae and eggs are shown in Figures 1 to 3. Toxicity of the essential oils to adults, larvae and eggs of *E. kuehniella* in descending order was *C. annuum* > *R. officinalis* > *M. x piperita* > *O. basilicum*.

**Effects of essential oil on adult mortality**

Mortality rates of adults increased with increasing doses of all essential oils (basil - $F = 59.0$, $df = 7$, $P < 0.05$; paprika - $F = 52.0$, $df = 7$, $P < 0.05$; peppermint - $F = 27.1$, $df = 7$, $P < 0.05$; and rosemary - $F = 57.6$, $df = 7$, $P < 0.05$). Basil and peppermint oils at 100 and 20 µl $L^{-1}$ air doses caused about 100% mortality, while rosemary and paprika oils caused 100% mortality at 10 $L^{-1}$ air or higher doses (Figure 1).

**Effects of essential oil on larvae mortality**

Dose of all essential oils had significant effects on larval mortality of *E. kuehniella*. (basil - $F= 52.8$, $df = 7$, $P < 0.01$; paprika: $F= 73.303$; $df = 7$, $P < 0.01$; peppermint - $F= 18.1$, $df = 7$, $P < 0.01$; and rosemary - $F = 68.4$, $df = 7$, $P < 0.01$). Larval mortality was proportional to the essential oil dose. Mortality rates for basil and peppermint oils at the 100 and 50 µl $L^{-1}$ air doses reached 86 and 57%, respectively while paprika and rosemary oils caused 100% mortality at 10 and 5 µl $L^{-1}$ air or higher dose (Figure 2).

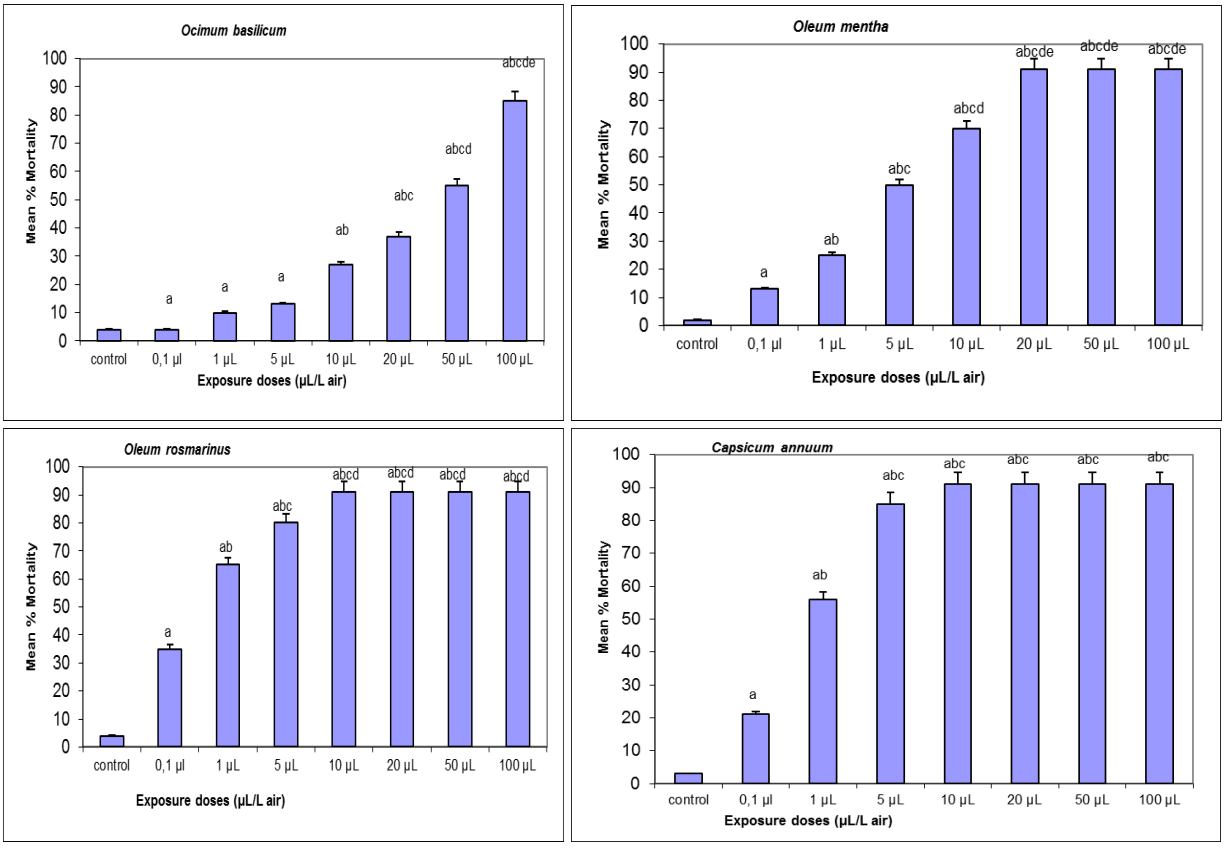


Figure 1. Percentage mortality of *Ephestia kuehniella* adults exposed to four essential oils at different doses for 24 h.

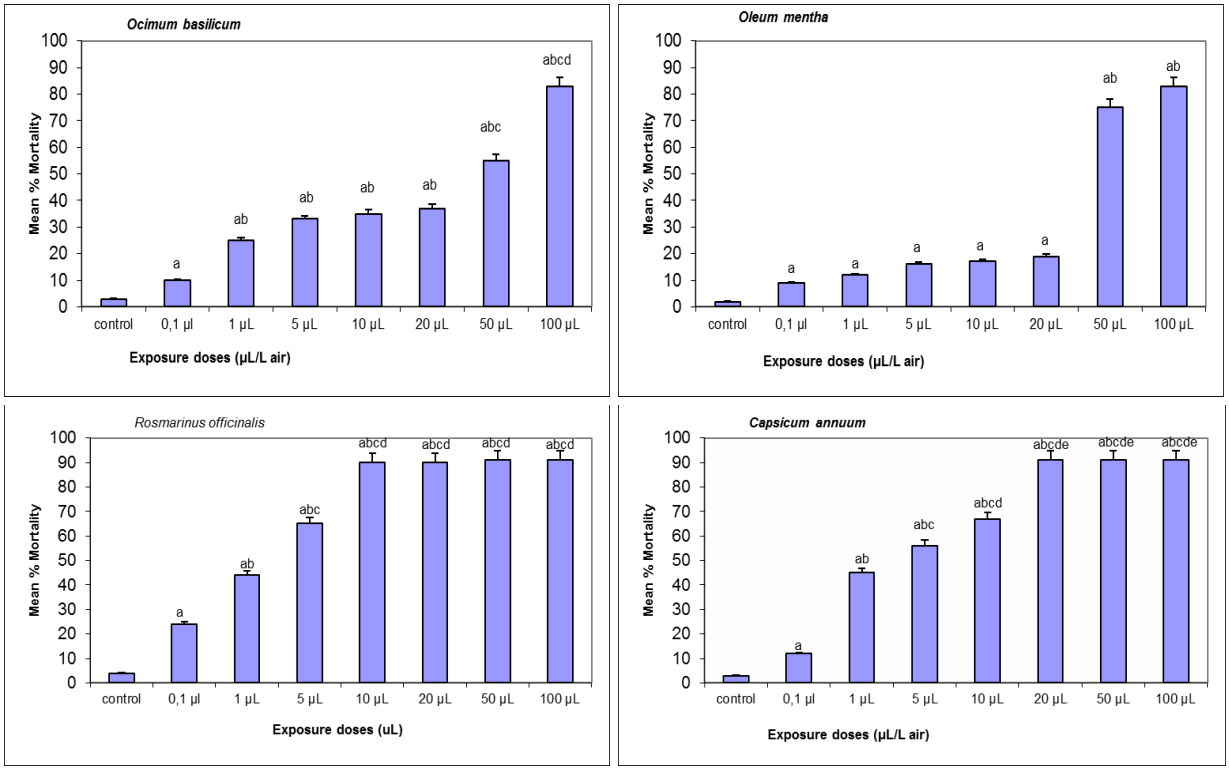


Figure 2. Percentage mortality of *Ephestia kuehniella* larvae exposed to four essential oils at different doses for 24 h.

### Effects of essential oil on egg mortality

The increasing dose of essential oils caused significant increase in mortality when *E. kuehniella* eggs were exposed to basil oil for 24 h (basil - F = 373, df = 7, P < 0.01). One hundred percent egg mortality was occured with oils of peppermint and rosemary (Figure 3). Paprika oil was the most effective essential oil against *E. kuehniella* eggs (F = 239, df = 7, P< 0.01).

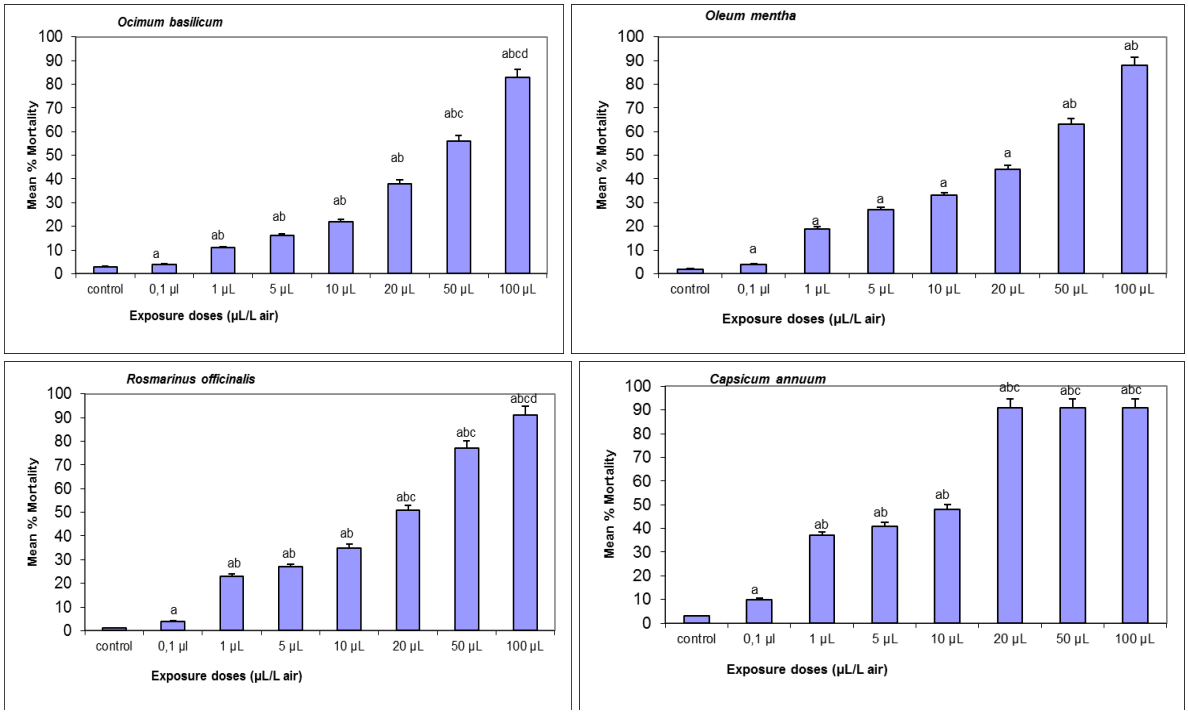


Figure 3. Percentage mortality of *Ephestia kuehniella* eggs exposed to four essential oils at different doses for 24 h.

## Discussion

Chemical composition of essential oils from different plants and seasonal variations in yield has been reported by Jemâa et al. (2012). Plants have different biological activity and can be used as insecticides and medicines (Razavi, 2012). Some essential oils have monoterpenes that effect acetylcholinesterase activity of the nervous system (Houghton et al., 2006). Chemical structure of essential oils of many plant species may be harmful to pests (Huang et al., 2000; Negahban & Moharramipour, 2007; Rajendran & Sriranjini, 2008). In this study, the plants used belong to the Lamiaceae and Solanaceae, and the essential oils they contain are widely used for medicinal, cosmetic, flavoring and general commercial applications. However, there has been little study of their insecticidal effect. The insecticidal activity of paprika and rosemary oils against *E. kuehniella* at the lowest dose was higher than basil and peppermint oils at the highest dose.

Use of synthetic insecticides is required for the management of insect pests but chemical-based insecticides may harm the environment. Insects have developed resistance against these insecticides and also some insecticides have threatened human health. Therefore, researchers must find alternative methods that are natural, inexpensive and environmentally friendly (Klein, 1976; Majumder et al., 2005). Therefore, some plants may be used as insecticidal, larvicidal and repellent agents (Rajashekar et al., 2010; Shahi et al., 2010; Ahmad et al., 2011; Kweka et al., 2011).

The essential oils of the four plants used in the present have had their chemical composition investigated by many researchers from many countries. Bernhardt et al. (2015) found that the main component of basil leaf oil was linalool (79.6%). Hu et al. (2015) reported that menthol (39.4%) was the major chemical in peppermint leaf oil. Wang et al., (2008) and Wesolowska et al. (2011) showed that the main component of rosemary and paprika fruit oils were 1,8-cineole and capsaicin (27.2 and 36.8%, respectively). Our results showed that the major component of leaf and fruit oil of basil, paprika peppermint, and rosemary were linalool (63.1%), capsaicin (35.4%), menthol (28.3%) and 1,8-cineole (25.7%), respectively (Table 1). Differences of component concentrations of essential oils can be due to differences in their collection areas.

Two studies have shown fumigant activity of many compounds on adults and to lesser extent larvae (Tunc et al., 2000; Isikber et al., 2006). The essential oils, or their major components, have been applied to eggs as fumigants and/or contact treatments in a range of studies (Shaaya et al., 1993; Ho et al., 1997; Huang et al., 1997; Obeng-Ofori et al., 1997; Obeng-Ofori & Reichmuth, 1997; Isikber et al., 2009), and the toxicity was dependent on the oil and insect stages tested. In this study, there were differences in the susceptibility of developmental stages of *E. kuehniella* to the four essential oils tested. Adults were more susceptible to the oils than eggs and larval stages.

Many essential oils from plants have been found to have insecticidal effects on stored-product pests (Negahban et al., 2007; Rajendran & Sriranjini, 2008; Ayvaz et al., 2009, 2010; Bachrouch et al., 2010a, b; Karabörklü et al., 2010, 2011). The main compound of essential oils of oregano and savory were determined as carvacrol by Ayvaz et al. (2010) and caused 100% mortality of *Plodia interpunctella* (Hubner) and *E. kuehniella* after 24 h exposure. In the present study, the fumigant activity of the *C. annuum* essential oil was significantly higher than that of other essential oils tested. At 10 µl $L^{-1}$ air dose of *C. annuum* essential oil for 24 h 100% of *E. kuehniella* larvae were killed. These results indicated that the essential oil of *C. annuum* had a significant fumigant activity against *E. kuehniella*. This toxicity against insects can be attributed particularly to its capsaicin, methyl chavicol and dihydrocapsaicin content.

Our results suggest that essential oils of basil, paprika, peppermint and rosemary are toxic to eggs, larvae and adults of *E. kuehniella*. One hundred percent mortality of different stages of *E. kuehniella* can be obtained using these essential oils. Therefore, these oils have potential for use in controlling insect pests in stored products. Use of essential oils in a control program could have both economic and ecological benefits. Further research is needed to obtain toxicity data for other stored-product insects, on penetration for bulk commodities and on effects on quality parameters of treated commodities.

## References

Ahmad, N., H. Fazal, B. H. Abbasi & M. Iqbal, 2011. In vitro larvicidal potential against Anopheles stephensi and antioxidative enzyme activities of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous*. Asian Pacific Journal of Tropical Medicine, 4: 169–175.

Alavanja, J. C. R., A. Blair & M. N. Masters, 1990. Cancer mortality in the U.S. flour industry. Journal of National Cancer Institute, 82: 840-848.

Ayvaz, A., O. Sağdıç, S. Karabörklü & I. Öztürk, 2010. Insecticidal activity of the essential oils from different plants against three stored product insects. Journal of Insect Science, 10: 1–13.

Ayvaz, A., S. Karabörklü & O. Sağdıç, 2009. Fumigant toxicity of five essential oils against the eggs of *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). Asian Journal of Chemistry, 21: 596–604.

Azelmat, K., D. El Garrouj, M. Mouhib & F. Sayah, 2006. Irradiation of Bouffegous dates: effects on chemical composition during storage. Postharvest Biology and Technology, 39: 217–222.

Bachrouch, O., J. M. Jemâa, A. W. Wissem, T. Talou, B. Marzouk & M. Abderraba, 2010a. Composition and insecticidal activity of essential oil from *Pistacia lentiscus* L. against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Journal of Stored Products Research 46 (4): 242–247.

Bachrouch, O., J. M. B. Jemaa, I. Chaieb, T. Talou & B. Marzouk, 2010b. Insecticidal activity of *Pistacia lentiscus* essential oil on *Tribolium castaneum* (Coleoptera: Tenebrionidae) as alternative to chemical control in storage. Tunisian Journal of Plant Protection, 5: 63–70.

Bakkali, F., S. Averbeck, D. Averbeck & M. Idaomar, 2008. Biological effects of essential oils – a review. Food and Chemical Toxicology, 46: 446–475.

Batish, D. R. H. P. Singh, R. K. Kohli & S. Kaur, 2008. Eucalyptus essential oil as a natural pesticide. Forest Ecology and Management, 256: 2166-2174.

Bell, C. H., 2000. Fumigation in the 21st century. Crop Protection, 19: 563-569.

Benhalima, H., M. Q. Chaudhry, K. A. Mills & N. R. Price, 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research, 40: 241-249.

Bernhardt, B., L. Sipos, Z. Kókai, A. Gere, K. Szabó, J. Bernáth & S. Z. Sárosi, 2015. Comparison of different *Ocimum basilicum* L. gene bank accessions analyzed by GC–MS and sensory profile. Industrial Crops and Products, 67: 498–508.

Boukhatem, M. N., F. M. Amine, A. Kameli, F. Saidi, K. Walid & S. B. Mohamed, 2014. Quality assessment of the essential oil from *Eucalyptus globulus* Labill of Blida (Algeria) origin. International Letters of Chemistry, Physics and Astronomy, 36: 303-315.

Carović-Stanko, K., Z. Liber, O. Politeo, F. Strikić, I. Kolak, M. Milos & Z. Šatović, 2011. Molecular and chemical characterization of the most widespread *Ocimum* species. Plant Systematics and Evolution, 294: 253–262.

Daglish, G. J., M. K. Nayak & H. Pavic, 2014. Phosphine resistance in *Sitophilus oryzae* (L.) from eastern Australia: inheritance, fitness and prevalence. Journal of Stored Products Research, 59: 237-244.

De Masi, L., P. Siviero, C. Esposito, D. Castaldo, F. Siano & B. Laratta, 2006. Assessment of agronomic: chemical and genetic variability in common basil (*Ocimum basilicum* L.). European Food Research and Technology, 223: 273–281.

Edris, A. E. & E. S. Farrag, 2003. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Nahrung, 47 (2): 117–121.

Ercan, S. F., H. Bas, M. Koc, D. Pandır & S. Oztemiz, 2013. Insecticidal activity of the essential oils from *Prangos ferulacea* (Umbelliferae) against different stages of *Ephestia kuehniella* (Lepidoptera: Pyralidae) and *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae) Turkish Journal of Agriculture and Forestry, 37 (6): 719-725.

Fashner, J. & A. C. Gitu, 2013. Common gastrointestinal symptoms: irritable bowel syndrome. FP. Essentials, 413: 16–23.

Fraenkel, G. & M. Blewett, 1946. Linoleic acid, vitamin E and other fat soluble substances in the nutrition of certain insects, *Ephestia kuehniella, E. elutella, E. cautella* and *Plodia interpunctella* (Lep.). Journal of Experimental Biology, 22: 172–190.

Herro, E. & S. E. Jacob, 2010. *Mentha piperita* (peppermint). Dermatitis, 21 (6): 327–329.

Hikichi, T., A. Irisawa, M. Sato, K. Watanabe, J. Nakamura, T. Takagi, T. Ikeda, R. Suzuki, H. Ohira & K. Obara, 2011. Utility of peppermint oil for endoscopic diagnosis of gastric tumors. Fukushima Journal of Medical Science, 57 (2): 60–65.

Ho, S. H., Y. Ma & Y. Huang, 1997. Anethole, a potential insecticide from *Illicium verum* Hook F., against two stored-product insects. International Pest Control, 39: 50-51.

Houghton, P. J., Y. Rena & M.J. Howes, 2006. Acetylcholinesterase inhibitors from plants and fungi. Natural Product Reports, 23: 181-199.

Hu, G., X. Yuan, S. Zhang, R. Wang, M. Yang, C. Wu, Z. Wu & X. Ke, 2015. Research on choleretic effect of menthol, menthone, pluegone, isomenthone, and limonene in DanShu capsule. International Immunopharmacology, 24 (2), 191–197.

Huang, Y., J. M. W. L. Tan, R. M. Kini & S. H. Ho, 1997. Toxic and antifeedant action of nutmeg oil against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Journal of Stored Products Research, 33: 289-298.

Huang, Y., S. L. Lam & S. H. Ho, 2000. Bioactivities of essential oils from *Elletaria cardamomum* (L.) Maton to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). Journal of Stored Products Research, 36: 107–117.

Isikber, A. A., M. H. Alma, M. Kanat & A. Karci, 2006. Fumigant toxicity of essential oils from *Laurus nobilis* and *Rosmarinus officinalis* against all life stages of *Tribolium confusum*. Phytoparasitica, 34 (2): 167-177

Işıkber, A. A., N. Özder & O. Sağlam, 2009. Susceptibility of eggs of *Tribolium confusum*, *Ephestia kuehniella* and *Plodia interpunctella* to four essential oil vapors. Phytoparasitica, 37: 231–239.

Jemâa, J. M. B., S. Haouel, M. Bouaziz & M. L. Khouja, 2012. Seasonal variations in chemical composition and fumigant activity of five *Eucalyptus* essential oils against three moth pests of stored dates in Tunisia. Journal of Stored Products Research, 48: 61–67.

Karabörklü, S., A. Ayvaz & S. Yılmaz, 2010. Bioactivities of different essential oils against the adults of two stored product insects. Pakistan Journal of Zoology, 42: 679–686.

Karabörklü, S., A. Ayvaz, S. Yılmaz & M. Akbulut, 2011. Chemical composition and fumigant toxicity of some essential oils against *Ephestia kuehniella*. Journal of Economic Entomology, 104 (4): 1212-1219.

Klein, W., 1976. The future for insecticides, need and prospects. Metcalf RL, Mckelvey JJ, editors. Wiley Interscience, New York, USA, pp. 65.

Kunike, G., 1938. Die Feststellung des Nährwertes verschiedener Stoffe durch Frassversuche mit Vorratsschädlingen. Anzeiger Fur Schadlingskunde, 14: 101–105.

Kweka, E. J., M. Nyindo, F. Mosha & A. G. Silva, 2011. Insecticidal activity of the essential oil from fruits and seeds of *Schinus terebinthifolia*. Raddi against African malaria vectors. Parasites Vectors, 4: 129-138.

Labra, M., M. Miele, B. Ledda, F. Grassi, Mazzei & F. Sala, 2004. Morphological characterization: essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant Science, 167: 725–731.

Lamiri, A., S. Lhaloui, B. Benjilali & M. Berrada, 2001. Insecticidal effects of essential oils against Hessian fly, *Mayetiola destructor* (Say). Field Crops Research, 71: 9-15.

Majumder, P., H. A. Mondal & S. Das, 2005. Insecticidal activity of *Arum maculatum* tuber lectin and its binding to the glycosylated insect gut receptors. Journal of Agricultural and Food Chemistry, 53: 6725–6729.

Materska, M. & I. Perucka, 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). Journal of Agricultural and Food Chemistry, 9, 1750–1756.

Negahban, M. & S. Moharramipour, 2007. Fumigant toxicity of *Eucalyptus intertexta*, *Eucalyptus sargentii* and *Eucalyptus camaldulensis* against stored-product beetles. Journal of Applied Entomology, 131: 256–261.

Negahban, M., S. Moharramipour & F. Sefidkon, 2007. Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. Journal of Stored Products Research, 43: 123–128.

Nenaah, G. E., S. I. A. Ibrahim & B. A. Al-Assiuty, 2015. Chemical composition, insecticidal activity and persistence of three Asteraceae essential oils and their nanoemulsions against *Callosobruchus maculatus* (F.). Journal of Stored Products Research, 61: 9–16.

Obeng-Ofori, D. & C. H. Reichmuth, 1997. Bioactivity of eugenol, a major component of *Ocimum suave* (Wild.) against four species of stored product Coleoptera. International Journal of Pest Management, 43: 89-94.

Obeng-Ofori, D., C. H. Reichmuth, J. Bekele & A. Hassanali, 1997. Biological activity of 1,8 cineole, a major component of essential oil of *Ocimum kenyense* (Ayobaugira) against stored product beetles. Journal of Applied Entomology, 121: 237-243.

Oluwatuyi, M., G. W. Kaatz & S. Gibbons, 2004. Antibacterial and resistance modifying activity of *Rosmarinus officinalis.* Phytochemistry, 65: 3249–3254.

Patra, A. K. & Z. Yu, 2014. Effects of vanillin, quillaja saponin, and essential oils on in vitro fermentation and protein-degrading microorganisms of the Rumen. Applied Microbiology and Biotechnology, 98 (2): 897–905.

Peng, Y., J. Yuan, F. Liu & J. Ye, 2005. Determination of active components in rosemary by capillary electrophoresis with electrochemical detection. Journal of Pharmaceutical and Biomedical Analysis, 39: 431–437.

Rajashekar, Y., N. Gunasekaran & T. Shivanandappa, 2010. Insecticidal activity of the root extract of *Decalepis hamiltonii* against stored-product insect pests and its application in grain protection. Journal of Food Science and Technology, 47: 310–314.

Rajendran, S. & V. Sriranjini, 2008. Plant products as fumigants for stored-product insect control. Journal of Stored Products Research, 44: 126–135.

Rathore, Y. S., G. C. Sachan & A. K. Bhattacharya, 1980. Growth and development of three-stored grain insect on triticale. Scientific Culture, 46: 59–61.

Razavi, S. M., 2012. Chemical composition and some allelopathic aspects of essential oils of (*Prangos ferulacea* L.) Lindl at different stages of growth. Journal of Agricultural Science, 14: 349–356.

Schneider, S. M., E. N. Rosskopf, J. G. Leesch, D. O. Chellemi, C. T. Bull & M. Mazzola, 2003. Research on alternatives to methyl bromide: pre-plant and post-harvest. Pest Management Science, 59: 814-826.

Schnitzler, P. & J. Reichling, 2011. Efficacy of plant products against herpetic infections. H N O, 59(12): 1176–1184.

Schuhmacher, A., J. Reichling & P. Schnitzler, 2003. Virucidal effect of peppermint oil on the enveloped viruses herpes simplex virus type 1 and type 2 in vitro. Phytomedicine, 10: 504–510.

Shaaya, E., U. Ravid, N. Paster, M. Kostjukovsky, M. Menasherov & S. Plotkin, 1993. Essential oils and their components as active fumigants against several species of stored product insects and fungi. Acta Horticulturae, 344: 131-137.

Shahi, M., A. A. Hanafi-Bojd, M. Iranshahi, H. Vatandoost & M. Y. Hanafi-Bojd, 2010. Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). Journal of Vector Borne Diseases, 47: 185–188.

Sun, T., Z. Xu, C. T. Wu, M. Janes, W. Prinyawiwatkul & H. K. No, 2007. Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). Journal of Food Science, 72: 98–102.

Telci, I., E. Bayram, G. Yilmaz & B. Avci, 2006. Variability in essential oil composition of Turkish basils *(Ocimum basilicum* L.). Biochemical Systematics and Ecology, 34(6): 489–497.

Tunc, I., B. M. Berger, F. Erler & F. Daglı, 2000. Ovicidal activity of essential oils from five plants against two stored-product insects. Journal of Stored Products Research, 36: 161-168.

Tunçbilek, A. Ş., Ü. Canpolat & F. Sümer, 2009. Suitability of irradiated and cold-stored eggs of *Ephestia kuehniella* (Pyralidae: Lepidoptera) and *Sitotroga cerealella* (Gelechidae: Lepidoptera) for stockpiling the egg-parasitoid *Trichogramma evanescens* (Trichogrammatidae: Hymenoptera) in diapause. Biocontrol Science and Technology, 19: 127–138.

Van Someren Graver, J. E., 2004. Guide to fumigation under gas-proof sheets. Food and Agricultural Organization of the United Nations, Rome, 1-168.

Wang, W., N. Wu, Y. G. Zu & Y. J. Fu, 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chemistry, 108: 1019–1022.

Wesołowska, A., D. Jadczak & M. Grzeszczuk, 2011. Chemical composition of the pepper fruit extracts of hot cultivars *Capsicum annuum* L. Acta Scientiarum Polonorum Hortorum Cultus, 10(1): 171-184.

Witkowska, D., & J. Sowinska, 2013. The effectiveness of peppermint and thyme essential oil mist in reducing bacterial contamination in broiler houses. Poultry Science, 92(11): 2834–2843.

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*Orijinal araştırma (Original article)*

# Bazı Brassicaceae bitkilerinin *Meloidogyne arenaria* (Neal) ve *Meloidogyne incognita* (Kofoid & White) (Tylenchida: Meloidogynidae)'ya konukçuluk seviyeleri[1]

## Host suitability level of selected Brassicaceae plants for *Meloidogyne arenaria* (Neal) and *Meloidogyne incognita* (Kofoid & White) (Tylenchida: Meloidogynidae)

**Gökhan AYDINLI[2]** **Sevilhan MENNAN[3]***

## Summary

Biofumigation with *Brassica* spp. could be an alternative method to suppress root-knot nematodes. It is important to select poor or non-host *Brassica* genotypes for *Meloidogyne* spp. to investigate the efficiency of biofumigation. To determine potential biofumigant plants, 40 *Brassica* genotypes were screened for host suitability level to *Meloidogyne arenaria* (Neal) and *Meloidogyne incognita* (Kofoid & White) in pot experiments in 2010 and 2011. Seedlings of each genotype were inoculated with 2000 or 0 root-knot nematode's eggs per plant. Pots were arranged in a completely randomized block design with 5 replicate in a controlled greenhouse at 20±1°C for 60 days. Experiment was repeated once. Host suitability was based on the gall index, egg masses index and nematode developmental stage. As a result of host suitability level studies, 12 genotypes for *M. incognita* and 9 of these 12 genotypes for *M. arenaria* were found to be as poor host. According to all parameters, poor hosts might be selected to search and use for their biofumigation potentially.

**Key words:** *Brassica* spp., *Meloidogyne arenaria, Meloidogyne incognita*, host level

## Özet

*Brassica* türlerinin kullanıldığı biyofumigasyon, kök-ur nematodlarının baskılanmasında önemli bir alternatif olabilmektedir. *Brassica* genotiplerinden konukçu olmayan ya da zayıf konukçuların seçilmesi, biyofumigasyon etkinliklerinin araştırılmasında önemlidir. Biyofumigasyon potansiyeli araştırılacak bitkilerin belirlenmesi için, 2010 ve 2011 yıllarında 40 *Brassica* genotipinin *Meloidogyne arenaria* (Neal) ve *Meloidogyne incognita* (Kofoid & White)'ya olan konukçuluk seviyeleri saksı denemeleri ile araştırılmıştır. Her bir genotipin fideleri 2000 veya 0 kök-ur nematodu yumurtası ile bulaştırılmıştır. Tesadüf blokları deneme deseninde, 5 tekerrürlü yürütülen denemede bitkiler 60 gün 20±1°C sıcaklıktaki kontrollü serada yetiştirilmiş ve 1 kere tekrarlanmıştır. Genotiplerin konukçuluk seviyeleri, ur skalası, yumurta kümesi skalası ve nematodun kök dokusu içindeki gelişimine dayandırılarak belirlenmiştir. Çalışmalar sonucunda, *Brassica* türlerinden 12 genotipin *M. incognita*'ya, bu 12 genotipden 9'unun ise *M. arenaria*'ya karşı, düşük konukçuluk seviyesinde olduğu tespit edilmiştir. Tüm parametreler değerlendirildiğinde, düşük seviyedeki konukçu olarak saptanan genotiplerin, biyofumigasyon potansiyellerinin araştırılması ve bu amaçla kullanılması önerilebilmektedir.

**Anahtar sözcükler:** *Brassica* spp., *Meloidogyne arenaria, Meloidogyne incognita*, konukçuluk seviyesi

[1] Bu çalışma TÜBİTAK-TOVAG 109O544 nolu proje ile desteklenmiş ve 31st International Symposium of the European Society of Nematologists (23-27 September 2012, Adana, Turkey)'de poster bildirisi olarak kısa özeti basılmıştır.
[2] Bozok Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, 66200, Yozgat
[3] Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, 55139, Samsun
*Corresponding author (Sorumlu yazar) e-mail: smennan@omu.edu.tr
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## Giriş

Geçtiğimiz yüzyılın büyük bir bölümünde, özellikle yüksek girdili ürünlerin kullanıldığı ve tarımın yoğun yapıldığı alanlarda, kök-ur nematodlarını kontrol altına almak için başvurulan yöntemlerin başında kimyasal ilaç kullanımı gelmektedir (Nyczepir & Thomas, 2009). Nematod mücadelesinde kullanılan kimyasalların büyük bir bölümünün geniş etkili fumigantlar olması, insan ve çevre sağlığı açısından önemli risklere neden olmaktadır. Özellikle 21. yüzyılın başlarına kadar, yaygın olarak kullanılan ve nematodların yanı sıra toprak kökenli hastalık ve zararlıları da etkili bir şekilde kontrol altına alan metil bromidin, ozon tabakasını inceltici etkisinden dolayı pek çok ülkede yasaklanması ile çevreye dost sürdürülebilir mücadele alternatifleri üzerinde durulmaya başlanmıştır (Monfort et al., 2007; Lopez-Perez et al., 2010).

Hayvansal ve bitkisel orijinli organik maddelerin toprakta ayrışması sırasında çıkan gazların toksik etkisinden yararlanılarak geliştirilen biyofumigasyon uygulamaları, kimyasal fumigantlara en iyi alternatif olarak görülmektedir (Mennan & Katı, 2010). Son yıllarda, bitki paraziti nematodların yanı sıra toprak kökenli patojenleri ve yabancı otları kontrol altına almak için *Brassica* grubu bitkilerin kullanıldığı biyofumigasyon uygulamaları ile ilgili çalışmalar oldukça artmıştır (Brown et al., 1991; McFadden et al., 1992; Mojtahedi et al., 1993; Angus et al., 1994; Boydston & Hang, 1995; Ploeg & Stapleton, 2001; Zasada & Ferris, 2004; Monfort et al., 2007; Lopez-Perez et al., 2010; Fourie et al., 2016). Bu bitkilerin toprağa karıştırılması sırasında, dokularında bulunan glikosinat bileşikleri, gaz haline geçerek biyosidal etkiye sahip izotiyosiyanatları üretir (Brown et al., 1991; Fan et al., 2008). *Brassica* grubu bitkiler örtücü bitki, tuzak bitki, toprağın organik içeriğini arttırıcı yeşil gübre ve tüketim amaçlı olmak üzere çok yönlü kullanım özelliğine sahiptirler (Halbrendt, 1996; Melakeberhan et al., 2008; Mennan & Katı, 2010). Biyofumigasyon amacıyla kullanılan örtücü bitkiler ile ilgili en önemli sorun, mücadele edilecek nematod türüne olan konukçuluk seviyeleridir. Özellikle kök-ur nematodu gibi geniş konukçu dizisine sahip nematodların mücadelesinde kullanılacak bitkilerin, nematod türüne olan konukçuluk seviyesinin bilinmesi gerekmektedir. Toprak sıcaklığının nematodun gelişimi için uygun olduğu durumlarda, örtücü bitki olarak hassas olanların kullanılması, bitkinin toprağa karıştırılmadan önceki yetiştirme sürecinde, nematodun bitkide üremesine ve bunun sonucunda topraktaki popülasyonunun artırmasına neden olacaktır. Bu durum, mücadelede için istenilen başarının elde edilmesini engelleyecektir. Bu nedenle, kullanılacak bitkilerin nematodun konukçusu olmayan veya zayıf konukçu olanlardan seçilmesi gerekmektedir (Stirling & Stirling, 2003; Pattison et al., 2006; Edwards & Ploeg, 2014).

*Brassica* tarımı ile özdeşleşen Samsun ilinde önemli zarara neden olan kök-ur nematodlarının mücadelesi için, yörede yaygın tarımı yapılan *Brassica* genotiplerinin, biyofumigasyon potansiyelininin değerlendirilmesi oldukça önemlidir. Bu çalışma ile biyofumigasyon potansiyeli olan bitkilerin belirlenmesi için 40 *Brassica* genotipinin bölgede ve ülkemizde olduğu kadar dünyada da en yaygın görünen 2 kök-ur nematodu türü *Meloidogyne arenaria* Neal ve *Meloidogyne incognita* Kofoid & White (Tylenchida: Meloidogynidae)'ya karşı konukçuluk seviyeleri araştırılmıştır.

## Materyal ve Yöntem

Çalışmanın ana materyalini; Samsun ili, Bafra ve Çarşamba ilçelerinde gerçekleştirilen arazi çalışmaları ile sağlanan kök-ur nematodları *M. arenaria* ve *M. incognita* (Irk 2)'ya ait seri kültürler ve yörede yaygın olarak tarımı yapılan 40 *Brassica* genotipi oluşturmaktadır (Çizelge 3).

**Denemelerde kullanılan toprak ve tohumların sterilizasyonu ve fidelerin yetiştirilmesi**

Denemelerin tamamında ve nematodların seri kültür üretiminde kullanılan toprak ve kum karışımı (2:1) otoklavda 121°C'de 60 dakika tutulup 24 saat bekletildikten sonra yeniden aynı sıcaklık ve sürede tutularak sterilize edilmiştir.

Kök-ur nematodu popülasyonlarının seri kültür üretiminde, hassas domates çeşidi (Falcon, May Tohum) kullanılmıştır. Denemelerde kullanılan bitkilerin tohumları, ekimden önce % 3 NaOCl (sodyum hipoklorit) solüsyonunda 5 dakika yüzey sterilizasyonuna tabi tutulmuştur. Beyaz ve kırmızı baş lahana, brokoli, brüksel lahanası, karnabahar, turp ve roka bitkilerinin yörede en fazla üretilen çeşitlerine ait tohumları satın alınmıştır. Kolza, şalgam, hardal genotiplerine ait tohumlar Prof. Dr. Fatih Seyis'den (Recep Tayyip Erdoğan Üniversitesi, Rize), yeşil yaprak lahana genotipleri ise Prof. Dr. Ahmet Balkaya'dan (Ondokuz Mayıs Üniversitesi, Samsun) temin edilmiştir. Ayrıca denemelere, seri kültür için kullanılan domates çeşidi hassas konukçu olarak ilave edilmiştir. Genotiplerin tohumları steril topraklar içeren tepsilerde fide haline getirilmiştir. Fideler steril toprak ve kum bulunan plastik saksılara (300 cc) 1 adet olacak şekilde şaşırtılmış ve 20±1°C sıcaklıktaki sera içerisine yerleştirilmiştir.

**Kök ur nematodlarına ait yumurta inokulumlarının elde edilmesi ve bitkilere bulaştırılması**

*Meloidogyne arenaria* ve *M. incognita* (Irk 2) ile bulaşık hassas domates çeşidinden oluşan seri kültürlerden, yeteri kadar bitki sökülerek kökleri dikkatli bir şekilde yıkanmıştır. Kökler küçük parçalara ayrılarak içerisinde % 0,5 NaOCl bulunan erlene yerleştirilmiş ve 3 dakika çalkalanmıştır. Bu solüsyon sırasıyla 200 ve 500 mesh eleklerden geçirilmiş ve alttaki eleğin üzerinde kalan yumurtalar piset yardımıyla, cam behere toplanmıştır (Hussey & Barker, 1973). Elde edilen yumurta solüsyonundan 1ml'lik hacimler 10 defa çekilerek stereo mikroskop altında sayılmış ve gerekli inokulum hesaplanmıştır. Fideler şaşırtıldıktan 7 gün sonra, bitki başına 2000 yumurta, saksıda açılan küçük kuyucuklara, enjektör yardımıyla verilmiştir. Bulaştırmalardan sonra, saksılar sera (20±1°C) içinde tutulmaya devam edilerek, günlük bakımları yapılmıştır. Denemeler, 2010 ve 2011 yıllarında 5 tekerrürlü olarak tesadüf blokları deneme desenine göre yürütülmüş olup 1 kez tekrarlanmıştır.

**Denemelerin değerlendirilmesi ve verilerin analizi**

Yapay bulaştırmalardan 60 gün sonra denemedeki bitkiler sökülerek kökler yıkanmış ve 0-5 ur skalasına göre değerlendirilmiştir (Çizelge 1; Kinloch, 1990). Doku içerisindeki nematodların sayı ve dönemlerinin tespiti için kökler asit fuksin solüsyonu (3,5 gr asit fuksin+250 ml asetik asit+750 ml saf su) ile boyanmıştır (Bybd et al., 1983). Köklerde tespit edilen yumurta kümeleri sayılarak 0-5 skalasına göre değerlendirilmiştir (Çizelge 2; Taylor & Sasser, 1978). Ayrıca, konukçuluk seviyesinin doğru şekilde değerlendirilmesi ve biyofumigasyon çalışmaları için uygun genotiplerin seçilebilmesi amacıyla denemeye alınan genotiplerin % RS (Relative Susceptibility= Test edilen bitkideki toplam nematod sayısı / hassas kontrol bitkisindeki toplam nematod sayısı x 100) değerleri belirlenmiştir (Teklu et al., 2014).

Çizelge 1. Köklerin değerlendirilmesinde kullanılan 0-5 ur skalası (Kinloch, 1990)

| Kökteki Urlanma Oranı | Ur Skalası |
|---|---|
| Ur yok | 0 |
| Az sayıda küçük urlu | 1 |
| <%25 urlu | 2 |
| %25-50 urlu | 3 |
| %50-75 urlu | 4 |
| >%75 urlu | 5 |

Çizelge 2. Köklerdeki yumurta kümesi sayısının değerlendirilmesinde kullanılan yumurta kümesi skalası (Taylor & Sasser, 1978)

| Yumurta Kümesi Sayısı / Kök | Yumurta Kümesi Skalası |
|---|---|
| 0 | 0 |
| 1-2 | 1 |
| 3-10 | 2 |
| 11-30 | 3 |
| 31-100 | 4 |
| >100 | 5 |

Araştırmada incelenen özelliklerin, genotiplere ve kök-ur nematodu türlerine göre farklılıkların belirlenmesinde veri yapısına bağlı olarak tesadüf blokları deneme deseni (Two-way ANOVA) uygulanmıştır. Denemeler sonunda elde edilen tüm veriler SAS istatistik programında değerlendirilmiştir. Bitki köklerinden elde edilen dişi ve toplam nematod değerleri analiz edilmeden önce $\log_{10}(x+1)$ transformasyonuna tabi tutulmuştur. Genotipler arasındaki farklılıklar Tukey çoklu karşılaştırma testine göre belirlenmiştir. Aynı bitki genotipinin kök-ur nematodu türlerine gösterdiği reaksiyonda, farklılık olup olmadığı ise t testine göre değerlendirilmiştir.

## Araştırma Sonuçları

Samsun ilinde yaygın olarak yetiştirilen 40 *Brassica* genotipinin kök-ur nematodları *M. arenaria* ve *M. incognita*'ya karşı konukçuluk seviyelerini belirlemek amacıyla 2010 ve 2011 yıllarında yürütülen 2 denemede, elde edilen veriler arasında istatiksel anlamda fark bulunmadığından, sonuçlar birleştirilerek verilmiştir. Denemeye alınan 40 genotipin 24'ü hem yumurta kümesi skalası hem de ur skalası değerlerine göre her iki nematod türüne de benzer reaksiyon göstermiştir (Çizelge 3; P≤0,05). Buna karşın, yumurta kümesi skalası bakımından 9 genotipin, ur skalası bakımından ise 10 genotipin, bu iki nematod türüne gösterdiği reaksiyon birbirinden farklıdır. *Meloidogyne arenaria* ile bulaştırılan 16, *M. incognita* ile bulaştırılan 18 genotipte yumurta kümesi tespit edilmemiştir. Her iki kök-ur nematodu türünde de yumurta kümesi tespit edilmeyen genotip sayısı ise 14'dür. Denemeye alınan brokoli, brüksel lahanası, karnabahar ve çin lahanası genotiplerinin tamamında yumurta kümesi skalası 0 olarak tespit edilmiştir. Turp ve roka genotiplerinde ise sadece *M. arenaria* ile bulaştırılan bitkilerin köklerinde, çok az sayıda yumurta kümesine rastlanmıştır. Bu iki bitki türüne ait genotipler, aynı zamanda köklerinde en düşük ur skalası tespit edilen genotiplerdendir. Her iki nematod türü için de ur skalası değeri 1 ve altında olan diğer genotipler ise şalgam ve brokolidir. Diğer *Brassica* gruplarına kıyasla genel olarak beyaz baş lahana ve hint hardalı genotiplerinin tamamı, her iki nematod türünün de yüksek seviyede yumurta kümesi oluşturmasına izin vermiştir. *Meloidogyne arenaria* ile bulaştırılan 13 genotip ve *M. incognita* ile bulaştırılan 15 genotipin ur skala değeri, hassas konukçu olarak kullanılan domateste tespit edilen ur skala değerinden istatiksel olarak farksızdır (P≤0,05). *Meloidogyne arenaria* ile bulaştırılan bu 13 genotipin tamamı *M. incognita*'ya da aynı reaksiyonu gösterirken, beyaz baş lahananın B-2 genotipi ve etiyopya hardalının CGN03980 genotipi *M. arenaria* ile bulaştırıldığında, *M. incognita'*nın aksine hassas çeşite göre önemli derecede daha az ur skalasına sahip olmuşlardır.

Bu verilere ilaveten, genotiplerin konukçuluk durumlarını ortaya koyabilmek amacıyla, hassas domatesteki toplam nematod sayısına göre hesaplanan RS (%) değerinden yararlanılmıştır (Çizelge 4). Kolzanın Elvis ve hint hardalının PI181033 genotipi *M. arenaria,* asya hardalının PI426414 genotipi ise *M. incognita* ile bulaştırıldığında, genotiplerinin kökleri içerisinde tespit edilen toplam nematod sayısı domatesten daha yüksektir.

Çizelge 3. *Meloidogyne arenaria* ve *Meloidogyne incognita* ile bulaştırılan (2000 yumurta/bitki) *Brassica* genotiplerinin kontrollü serada (20±1°C) 60 gün yetiştirilmesi ile bitki köklerinde oluşan yumurta kümesi skalası ve ur skalası değerleri[1]

| Bitki Türü | Genotip | Yumurta Kümesi Skalası[2] | | Ur Skalası[3] | |
|---|---|---|---|---|---|
| | | *M. arenaria* | *M. incognita* | *M. arenaria* | *M. incognita* |
| *Brassica oleracea* var. *acephala* (Yaprak lahana) | S-8 | 1,40 f-i* | 0,40 de | 3,90 a-e | 4,60 a-c |
| | S-19 | 0,60 g-i | 0,30 de | 2,50 e-k | 2,50 e-i |
| | S-38 | 0,00 i | 0,00 e | 3,00 d-i | 3,30 c-f |
| | 55 TE 13 | 0,20 g-i* | 0,00 e | 3,70 a-e | 4,30 a-d |
| | 55 TK 12 | 0,00 i | 0,00 e | 3,10 d-h | 3,10 d-g |
| *Brassica oleracea* var. *capitata* subvar. *alba* (Beyaz baş lahana) | B-2 | 3,60 a-c* | 3,70 ab | 3,50 b-f* | 4,90 ab |
| | B-9 | 2,30 c-f | 3,10 b | 1,70 h-n* | 3,50 b-e |
| | 166 | 3,00 b-e | 2,70 bc | 2,20 f-l* | 3,30 c-f |
| | 201 | 3,30 bc | 3,90 ab | 1,10 k-o* | 3,00 d-g |
| | Yalova | 3,50 a-c | 3,10 b | 4,40 a-d | 4,30 a-d |
| *Brassica napus* (Kolza) | B2 | 0,00 i* | 0,80 de | 5,00 a | 5,00 a |
| | B13 | 0,00 i | 0,00 e | 2,50 e-k | 2,80 e-h |
| | Elvis | 3,20 b-d | 3,20 b | 4,70 ab | 4,60 a-c |
| | Jura | 0,00 i | 0,00 e | 5,00 a | 4,90 ab |
| | Oase | 3,70 a-c | 3,70 ab | 5,00 a | 4,50 a-c |
| *Brassica rapa* (Yumrulu şalgam) | PI 352802 | 1,60 e-h | 1,20 c-e | 0,70 m-o | 2,20 e-k |
| | PI 352811 | 0,10 hi | 0,30 de | 2,10 f-m | 2,00 f-l |
| | PI 426720 | 0,60 g-i | 0,50 de | 2,00 g-m* | 1,30 i-o |
| *Brassica rapa* subsp. *trilocularis* (Şalgam) | PI 426422 | 1,00 f-i | 1,10 c-e | 0,50 no | 0,40 no |
| | PI 459017 | 1,70 d-g | 1,30 c-e | 1,20 j-o | 1,10 j-o |
| *Brassica oleraceae* convar. *botrytis* subvar. *cymosa* (Brokoli) | Batavia | 0,00 i | 0,00 e | 1,00 l-o* | 0,50 m-o |
| | Bejo | 0,00 i | 0,00 e | 1,50 j-n* | 1,00 k-o |
| | Semito | 0,00 i | 0,00 e | 0,90 l-o | 0,70 l-o |
| *Brassica campestris* subsp. *pekinensis* (Çin lahanası) | Çin lahanası | 0,00 i | 0,00 e | 1,50 j-n | 1,80 g-m |

Çizelge 3. (devamı)

| Bitki Türü | Genotip | Yumurta Kümesi Skalası[2] | | Ur Skalası[3] | |
|---|---|---|---|---|---|
| | | *M. arenaria* | *M. incognita* | *M. arenaria* | *M. incognita* |
| *Brassica tournefortii* (Asya hardalı) | Amos | 0,00 i | 0,00 e | 3,70 a-e | 4,30 a-d |
| | PI426414 | 3,40 bc | 3,30 b | 4,90 ab | 4,90 ab |
| | PI426415 | 3,70 a-c | 3,80 ab | 5,00 a | 4,80 ab |
| *Brassica oleracea* var. *capitata* subvar. *rubra* (Kırmızı baş lahana) | Mahrenkopf | 1,50 e-i | 0,60 de | 3,20 c-g | 2,00 f-l |
| | Zencibaş | 4,00 ab* | 2,50 b-d | 4,90 ab* | 4,20 a-d |
| *Brassica juncea* (Hint hardalı) | PI181033 | 4,00 ab | 3,20 b | 4,90 ab | 4,70 ab |
| | PI311734 | 2,50 b-f | 3,40 b | 4,60 a-c | 4,60 a-c |
| *Brassica carinata* (Etiyopya hardalı) | CGN03980 | 0,00 i* | 0,30 de | 2,60 e-j* | 4,50 a-c |
| | CGN0422 | 0,00 i | 0,00 e | 1,60 i-n* | 1,80 g-m |
| *Brassica oleraceae* convar. *oleraceae* var. *gemmifera* (Brüksel lahanası) | Arzuman | 0,00 i | 0,00 e | 1,70 h-n | 1,50 h-n |
| | Davlin | 0,00 i | 0,00 e | 2,60 e-j | 2,40 e-j |
| *Brassica oleraceae* convar. *botrytis* subvar. *botrytis* (Karnabahar) | Pampana | 0,00 i | 0,00 e | 1,20 j-o | 1,20 i-o |
| | Snowball | 0,00 i | 0,00 e | 2,60 e-j | 2,20 e-k |
| *Raphanus sativus* (Turp) | Beyaz-Semito | 0,30 g-i* | 0,00 e | 0,80 l-o | 0,70 l-o |
| | Kırmızı | 0,40 g-i* | 0,00 e | 0,90 l-o | 1,00 k-o |
| *Eruca sativa* (Roka) | İstanbul | 0,20 g-i* | 0,00 e | 0,20 o | 0,20 o |
| *Solanum lycopersicum* (Domates) | Falcon | 5,00 a | 5,00 a | 5,00 a | 5,00 a |

[1] Denemeler 5 tekerrürlü olup, her iki deneme arasında farklılık olmaması nedeniyle elde edilen veriler birleştirilerek verilmiştir. Tukey HSD testine göre sütun içerisinde aynı harfe sahip değerler P≤0,05 göre istatistiksel olarak birbirinden farksızdır.
[2] 0-5 yumurta kümesi skalası = 0: yumurta kümesi yok; 1: 1-2 yumurta kümesi; 2: 3-10 yumurta kümesi; 3: 11-30 yumurta kümesi; 4: 31-100 yumurta kümesi; 5: 100'den fazla yumurta kümesi (Taylor ve Sasser, 1978).
[3] 0-5 ur skalası= 0: ur yok; 1: az sayıda küçük urlar; 2: <%25 urlu; 3: %25-50 urlu; 4: %50-75 urlu; 5: >%75 urlu (Kinloch, 1990).
* t testine göre aynı lahana genotipinin nematod türlerine olan reaksiyonu önemli derecede farklıdır (P≤0,05).

RS değeri %10'un altında olan genotiplerin tamamının yumurta kümesi skalası da 1'den azdır. Yumrulu şalgam (PI352811 ve PI426720), brokoli (Semito), etiyopya hardalı (CGN0422), brüksel lahanası (Arzuman), karnabahar (Snowball), turp (Beyaz-Semito ve Kırmızı) ve roka (İstanbul) genotipleri, her 2 nematod türüne de düşük seviyede konukçudur. Kolza (B13), brokoli (Batavia) ve kırmızı baş lahana (Mahrenkopf) genotipleri ise sadece *M. incognita*'ya zayıf konukçuluk göstermiştir.

Çizelge 4. *Meloidogyne arenaria* ve *Meloidogyne incognita* ile bulaştırılan (2000 yumurta/bitki) *Brassica* genotiplerinin kontrollü serada (20±1°C) 60 gün yetiştirilmesiyle köklerde elde edilen dişi sayısı, toplam nematod sayısı ve konukçu indeksi (RS)[1]

| Bitki Türü | Genotip | Dişi /g kök[2] | | Toplam nematod /g kök[2] | | RS (%)[3] | |
|---|---|---|---|---|---|---|---|
| | | *M. arenaria* | *M. incognita* | *M. arenaria* | *M. incognita* | *M. arenaria* | *M. incognita* |
| *Brassica oleracea* var. *acephala* (Yaprak lahana) | S-8 | 21,53 b-d | 18,15 cd | 29,00 b-d | 22,70 cd | 87,35 | 69,42 |
| | S-19 | 19,97 b-f | 11,11 d-i | 29,04 b-d | 26,70 bc | 87,47 | 81,65 |
| | S-38 | 12,25 b-i | 8,56 d-i | 15,17 b-h | 15,29 c-i | 45,69 | 46,76 |
| | 55 TE 13 | 9,01 d-j | 9,26 d-i | 12,93 b-h | 12,50 d-k | 38,95 | 38,23 |
| | 55 TK 12 | 4,92 g-l | 2,02 i-n | 6,85 d-l | 4,15 h-m | 20,63 | 12,69 |
| *Brassica oleracea* var. *capitata* subvar. *alba* (Beyaz baş lahana) | B-2 | 13,52 b-h | 17,71 c-e | 14,77 b-h | 18,40 c-g | 44,49 | 56,27 |
| | B-9 | 15,64 b-i | 19,17 cd | 16,59 b-g | 20,75 c-e | 49,97 | 63,46 |
| | 166 | 17,51 b-e | 16,69 c-e | 17,87 b-f | 17,47 c-f | 53,83 | 53,43 |
| | 201 | 11,73 b-i | 20,49 cd | 12,37 b-h | 21,17 cd | 37,26 | 64,74 |
| | Yalova | 15,86 b-h | 3,84 f-l | 15,86 b-h | 3,84 h-m | 47,77 | 11,74 |
| *Brassica napus* (Kolza) | B2 | 33,07 bc | 27,84 bc | 33,07 bc | 27,84 bc | 99,61 | 85,14 |
| | B13 | 2,68 i-m | 2,07 i-n | 3,90 f-m | 3,14 i-n | 11,75 | 9,60* |
| | Elvis | 36,76 ab | 14,87 c-f | 36,76 ab | 14,97 c-i | 110,72 | 45,78 |
| | Jura | 20,37 b-h | 11,38 d-j | 20,51 b-e | 11,58 d-k | 61,78 | 35,41 |
| | Oase | 17,97 b-g | 13,59 c-h | 17,97 b-f | 13,59 c-i | 54,13 | 41,56 |
| *Brassica rapa* (Yumrulu şalgam) | PI 352802 | 4,32 f-k | 3,78 f-l | 5,11 e-m | 4,77 f-l | 15,39 | 14,59 |
| | PI 352811 | 2,54 i-m | 2,05 i-n | 2,79 h-m | 2,12 j-o | 8,40* | 6,48* |
| | PI 426720 | 2,90 i-m | 3,12 h-l | 3,02 h-m | 3,25 j-o | 9,10* | 9,94* |
| *Brassica rapa* subsp. *trilocularis* (Şalgam) | PI 426422 | 3,62 h-l | 4,30 f-l | 3,94 f-m | 4,57 g-l | 11,87 | 13,98 |
| | PI 459017 | 4,43 e-k | 6,92 e-k | 4,57 e-m | 7,15 e-l | 13,77 | 21,87 |
| *Brassica oleraceae* convar. *botrytis* subvar. *cymosa* (Brokoli) | Batavia | 0,00 m | 0,00 n | 3,46 h-m | 3,25 j-o | 10,42 | 9,94* |
| | Bejo | 0,00 m | 0,00 n | 3,59 g-m | 4,67 h-m | 10,81 | 14,28 |
| | Semito | 0,00 m | 0,00 n | 1,66 j-m | 0,09 o | 5,00* | 0,28* |
| *Brassica campestris* subsp. *pekinensis* (Çin lahanası) | Çin lahanası | 0,25 lm | 2,75 l-m | 3,96 f-m | 7,83 d-k | 11,93 | 23,94 |

Çizelge 4. (devamı)

| Bitki Türü | Genotip | Dişi /g kök[2] | | Toplam nematod /g kök[2] | | RS (%)[3] | |
|---|---|---|---|---|---|---|---|
| | | *M. arenaria* | *M. incognita* | *M. arenaria* | *M. incognita* | *M. arenaria* | *M. incognita* |
| *Brassica tournefortii* (Asya hardalı) | Amos | 9,94 c-j | 4,53 f-l | 11,77 b-j | 4,53 h-m | 35,45 | 13,85 |
| | PI426414 | 20,97 b-d | 46,30 a | 20,97 b-e | 46,30 a | 63,16 | 141,59 |
| | PI426415 | 8,23 d-j | 7,95 e-k | 8,23 b-j | 7,95 e-l | 24,78 | 24,31 |
| *Brassica oleracea* var. *capitata* subvar. *rubra* (Kırmızı baş lahana) | Mahrenkopf | 5,23 g-k | 1,62 i-n | 6,28 d-l | 2,51 j-o | 18,92 | 7,68* |
| | Zencibaş | 7,51 d-j | 3,28 g-l | 9,00 b-j | 3,74 h-m | 27,11 | 11,44 |
| *Brassica juncea* (Hint hardalı) | PI181033 | 43,71 a | 15,28 d-h | 43,89 a | 15,33 c-i | 132,20 | 46,88 |
| | PI311734 | 11,71 b-i | 5,57 f-l | 11,90 b-i | 5,57 h-m | 35,84 | 17,03 |
| *Brassica carinata* (Etiyopya hardalı) | CGN03980 | 4,38 e-k | 18,32 c-g | 4,63 e-m | 18,63 c-i | 13,95 | 56,97 |
| | CGN0422 | 0,41 k-m | 1,29 j-n | 0,58 m | 1,82 k-o | 1,75* | 5,57* |
| *Brassica oleraceae* convar. *oleraceae* var. *gemmifera* (Brüksel lahanası) | Arzuman | 0,05 m | 0,00 n | 1,15 k-m | 0,50 m-o | 3,46* | 1,53* |
| | Davlin | 2,80 i-m | 3,80 f-l | 6,95 c-k | 9,63 d-j | 20,93 | 29,45 |
| *Brassica oleraceae* convar. *botrytis* subvar. *botrytis* (Karnabahar) | Pampana | 0,00 m | 0,00 n | 17,18 b-g | 21,37 cd | 51,75 | 65,35 |
| | Snowball | 0,00 m | 0,00 n | 1,97 i-m | 2,22 k-o | 5,93* | 6,79* |
| *Raphanus sativus* (Turp) | Beyaz Semito | 1,86 j-m | 0,28 mn | 2,10 j-m | 0,28 no | 6,33* | 0,86* |
| | Kırmızı | 1,46 k-m | 1,33 k-n | 1,59 k-m | 1,33 l-o | 4,79* | 4,07* |
| *Eruca sativa* (Roka) | İstanbul | 0,90 k-m | 1,77 l-n | 1,15 lm | 1,77 l-o | 3,46* | 5,41* |
| *Solanum lycopersicum* (Domates) | Falcon | 33,20 bc | 32,70 b | 33,20 bc | 32,70 b | 100,00 | 100,00 |

[1] Denemeler 5 tekerrürlü olup, her iki deneme arasında farklılık olmaması nedeniyle elde edilen veriler birleştirilerek verilmiştir. Tukey HSD testine göre sütun içerisinde aynı harfe sahip değerler P≤0,05 göre istatistiksel olarak birbirinden farksızdır.
[2] Analiz edilmeden önce verilere $\log_{10}(x+1)$ transformasyonu uygulanmıştır. Toplam nematod sayısına yumurta dönemi dahil değildir.
[3] Test edilen bitkideki toplam nematod sayısı / kontrol (domates) bitkisindeki toplam nematod sayısı x 100
*RS<%10 olan genotipler

RS değeri (>%10) yüksek olmasına rağmen yumurta skalası düşük (<1) olarak tespit edilen genotipler bulunmaktadır. Kolza (B2 ve Jura), brokoli (Bejo), asya hardalı (Amos), etiyopya hardalı (CGN03980), brüksel lahanası (Davlin), karnabahar (Pampana), yaprak lahana genotipleri (S-8 hariç) ve çin lahanası her iki nematod türüne de bu şekilde reaksiyon göstermişlerdir. Bu genotiplerden brokoli (Bejo), karnabahar (Pampana) ve çin lahanası, düşük ur skala değerlerine (1,00-1,80) sahip olurken, diğerlerinde ur skala değerleri de (2,40-5,00) yüksektir. Kökünde yumurta kümesi oluşturmamış olan bu 3 genotipin düşük ur skalasına sahip olmasının nedeni, kökte nematodların büyük bir bölümünün 2. larva döneminde olmasıdır.

Yumurta skalası (>1) ve RS değeri (>%10) yüksek olan genotipler ise iyi konukçu olarak değerlendirilebilir. Böylece beyaz baş lahana, şalgam ve hint hardalı türlerine ait genotiplerin tamamı, kolza, yumrulu şalgam, asya hardalı ve kırmızı baş lahana genotiplerinden bazıları her iki nematod türüne de iyi konukçu olarak saptanmıştır.

## Tartışma

*Brassica* genotiplerinin konukçuluk durumu nematod türlerine hatta aynı türün farklı popülasyonlarına göre değişiklik gösterebilir (Khan & Khan, 1991; McSorley & Frederick, 1995; Melakeberhan et al., 2006; Edwards & Ploeg, 2014). Bu nedenle, kontrol altına alınmak istenen nematod popülasyonunun teşhisinin tür seviyesinde yapılması bu mücadele stratejisinin optimizasyonu açısından önemlidir (Edwards & Ploeg, 2014). Çalışmada kullanılan genotiplerinden bazılarının yumurta kümesi skalası ve ur skalası nematod türlerine göre önemli farklılıklar göstermektedir. Buna karşın, RS değerleri dikkate alındığında zayıf konukçu olarak değerlendirilenler (B13, Batavia ve Mahrenkopf hariç) her 2 nematod türü için de aynı genotiplerdir. Bitkilerin konukçuluk seviyelerini belirlemek için Sasser et al. (1984) üreme faktörü ile birlikte ur skalası değerlerini de dikkate almakta ve düşük ur skalasına (≤2) sahip olanlar dayanıklı olarak değerlendirilmektedir. Buna göre, çalışmada RS ve yumurta kümesi skalası dikkate alınarak zayıf konukçu olarak tespit edilen genotipler (Snowball hariç) ur skalası bakımdan da dayanıklı olarak reaksiyon göstermektedir. Bu itibarla, bu genotiplerin biyofumigasyon çalışmalarında öncelikli olarak tercih edilmesi gerekmektedir.

Çalışmada zayıf konukçu olarak tespit edilen rokanın farklı bir çeşidi (Nemat) daha önce değişik araştırıcılar tarafından farklı kök-ur nematodu türlerine karşı denenmiş ve benzer sonuçlar elde edilmiştir (Curto et al., 2005; Melakeberhan et al., 2006; Kokalis-Burella et al., 2013; Edwards & Ploeg, 2014; Kruger et al., 2015). Bu çalışmada olduğu gibi, Curto et al. (2005) *M. incognita*'nın, Melakeberhan et al. (2006) ise *M. hapla*'nın bu bitki üzerinde hayat döngülerini tamamlama sürelerinin oldukça uzun olduğunu ve çok az dişi bireyin gelişebildiği tespit etmişlerdir. Her iki araştırıcı da bu bitkinin aynı zamanda bu özelliğinden yararlanılarak tuzak bitki olarak da kullanılabileceğini bildirmişlerdir. Nematodun köke girişine izin veren fakat gelişip üremesine engel olan bitkiler, tuzak bitki olarak adlandırılmaktadır (Gardner & Caswell-Chen, 1994; Melakeberhan et al., 2008; Mennan & Katı, 2010). Bu çalışmada, RS değeri yüksek olmasına rağmen köklerinde yumurta kümesi oluşturmayan genotipler tespit edilmiştir. Köklerine çok sayıda nematod giriş yapabildiği halde, deneme süresince hayat döngüsünü tamamlayamayan ve kökünde dişi birey tespit edilmeyen karnabahar genotiplerinden Pampana ve brokoli genotiplerinden Bejo, her iki nematod türüne çok iyi birer tuzak bitki olarak görülebilir. *Meloidogyne incognita*'ya zayıf konukçu olarak tespit edilen brokoli genotiplerinden Batavia da, *M. arenaria* türüne karşı tuzak bitki potansiyeline sahiptir. Köklerinde dişi bireyler tespit edilmesine rağmen, yumurta oluşumunun hiç rastlanmadığı veya nadiren rastlandığı genotipler de biyofumigasyon çalışmalarında kullanılabilme potansiyelindedir. Fakat bu şekilde reaksiyon gösteren genotipler, biyofumigasyon amacıyla kullanılırken bitkilerin toprağa karıştırılmadan önceki yetiştirme süresi ve toprak sıcaklığı göz önünde bulundurulmalıdır.

Bu çalışmada olduğu gibi, daha önce bazı araştırıcılar tarafından turp bitkisinin farklı genotipleri, değişik kök-ur nematodu türlerine karşı zayıf konukçu olarak tespit edilmiştir (Curto et al., 2005; Pattison et al., 2006; Edwards & Ploeg, 2014). Buna karşın, Gardner & Caswell-Chen (1994) ve McSorley & Frederick (1995) ise denemeye aldıkları turp çeşitlerinin kök-ur nematodlarına hassas reaksiyon gösterdiğini tespit etmişlerdir. Turp ve roka dışında çalışmada kullanılan brokoli ve karnabahar genotiplerinin hepsi zayıf konukçu veya tuzak bitki şeklinde konukçu reaksiyonu göstermiş olup, biyofumigasyon çalışmalarında kullanılmaya adaydır. Farklı kök-ur nematodu türlerine Cruciferea familyasına ait kültür bitkilerinin konukçu reaksiyonunu araştıran McSorley & Frederick (1995), *M. arenaria* ve *M. incognita* (ırk 1)'ya en düşük ur ve yumurta kümesi skalası gösteren kültür bitkilerinden birinin brokoli olduğunu ve kök-ur nematodunun popülasyon yoğunluğunu azaltmak için tarımsal üretim

sistemleri içinde kullanılabileceğini ifade etmiştir. Buna karşın, aynı araştırıcılar karnabahar çeşidinin ise bizim sonuçlarımızın aksine denemeye alınan kök-ur nematodlarının hepsine hassas olduğunu bildirmişlerdir. Khan & Khan (1991) ise *M. incognita*'nın farklı ırklarına karşı, denemeye aldığı 37 karnabahar çeşidinden 13 tanesinin ırklara spesifik olarak dayanıklılık gösterdiğini tespit etmiştir. Edwards & Ploeg (2014) da elde ettiğimiz sonuçlara benzer olarak, brokolinin farklı bir çeşidinin *M. incognita*'ya zayıf konukçu olduğunu bildirmiştir. Brokoli bitkisinin, toprağa karıştırılmasıyla gerçekleştirilen biyofumigasyon uygulamalarında nematod popülasyonunu azalttığı bilinmektedir (Lopez-Perez et al., 2010; Kaşkavalcı & Duran Akkurt, 2012). Bu itibarla, bu çalışmada kullanılan brokoli bitkilerinin biyofumigasyon çalışmalarında kullanılarak, nematod popülasyonu üzerine etkileri değerlendirilmelidir.

Çalışmada kullanılan *Brassica* türlerinin arasında kök-ur nematoduna karşı gösterilen reaksiyonda farklılık tespit edildiği gibi aynı türün genotipleri arasında da farklılık bulunmaktadır. Beyaz baş lahana, şalgam ve hint hardalı genotiplerinin tamamı ise tutarlı bir şekilde her iki nematod türüne de iyi konukçu olarak saptanmıştır. Curto et al. (2005) ve Edwards & Ploeg (2014) tarafından *M. incognita* için konukçuluk durumu değerlendirilen hint hardalı genotiplerinin gösterdiği reaksiyonlar, bu çalışmadaki sonuçlar ile benzerdir. Pattison et al. (2006) ise bu bitki türüne ait genotipleri, *M. arenaria*'nın 2 farklı popülasyonuna karşı zıt reaksiyon gösterdiğini tespit etmiş ve popülasyonlardan birinin denemede dayanıklı kontrol olarak kullanılan sorgum ile aynı reaksiyona, diğer popülasyonun ise hassas kontrol olarak kullanılan domates ile aynı reaksiyona sahip olduğunu belirtmiştir. Bu itibarla, aynı nematod türünün farklı bölgelerdeki popülasyonlarının, konukçu bitkilere olan davranışlarının da farklı olabileceği unutulmamalıdır. Popülasyonun özelliğine göre uygulanacak yerel mücadele programları, hem kök-ur nematodlarının kontrol altında tutulmasına yardımcı olacak hem de birim alandan en yüksek verimin alınmasını sağlayacaktır. Bu durum pek çok kaynakta da ifade edilmiş olup, alana özelleşmiş (site specific) mücadele olarak tanımlanmaktadır (Melakeberhan, 2008; Melakeberhan et al., 2012; King & Taberna, 2013; Liu et al., 2014).

Sonuç olarak, *Brassica* türlerinden oluşan 12 genotipin *M. incognita*'ya, bu genotiplerden 9'unun ise *M. arenaria*'ya karşı biyofumigasyon çalışmalarında kullanılabilecek düşük konukçuluk seviyesine sahip olduğu tespit edilmiştir. Biyofumigasyon için tercih edilecek bitkinin, yüksek glikosinat içeriğine ve fazla miktarda biyokütle oluşturma yeteneğine sahip olması da istenir (Kirkegaard & Sarwar, 1998; Morra & Kirkegaard, 2002; Monfort et al., 2007). Özellikle tuzak bitkilerin sahip olduğu glukosinat içeriği sayesinde, bu şekilde bir etki gösterdiği düşünülmektedir (Mennan & Katı, 2010). Melakeberhan et al. (2006), tuzak bitki olarak rokanın, hassas domates bitkisinin %10'u kadar bile nematodun konukçusu olmasının, topraktan çok sayıda nematodu güvenli bir şekilde uzaklaştırmaya yeteceğini bildirmişlerdir. Tuzak bitkilerin mücadele yapılacak alanda belli bir süre yetiştirildikten sonra yeşil gübre uygulaması şeklinde gerçekleştirilecek biyofumigasyon çalışmalarında, bu bitkilerin toprağa karıştırılmadan önce çok sayıda nematoda konukçuluk ederek topraktaki popülasyonu belli bir seviyenin altına düşürecek olması, biyofumigasyonun etkinliğini artırabilecektir. Bu nedenle, deneme süresince tuzak bitki gibi reaksiyon gösterenler de biyofumigasyon çalışmalarında tercih edilebilir.

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## Yararlanılan Kaynaklar


Angus, J. F., A. Gardner, J. A. Kirkegaard & J. M. Desmarchelier, 1994. Biofumigation: Isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. Plant and Soil, 162 (1): 107-112.

Boydston, R. A. & A. Hang, 1995. Rapeseed (*Brassica napus*) green manure crops suppress weeds in potato (*Solanum tuberosum*). Weed Technology, 9 (4): 669-675.

Brown, P. D., M. J. Morra, J. P. McCaffrey, D. L. Auld & L. Williams III, 1991. Allelochemicals produced during glucosinolate degradation in soil. Journal of Chemical Ecology, 17 (10): 2021-2034.

Bybd, D. W., Jr. T. Kirkpatrick & K. R. Barker, 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology, 15 (1): 142-143.

Curto, G., E. Dallavalle & L. Lazzeri, 2005. Life cycle duration of *Meloidogyne incognita* and host status of Brassicaceae and Capparaceae selected for glucosinolate content. Nematology, 7 (2): 203-212.

Edwards, S. & A. Ploeg, 2014. Evaluation of 31 potential biofumigant Brassicaceous plants as hosts for three *Meloiodogyne* species. Journal of Nematology, 46 (3): 287-295.

Fan, C. M., G. R. Xiong, P. Qi, G. H. Ji & Y. Q. He, 2008. Potential biofumigation effects of *Brassica oleracea* var. *caulorapa* on growth of fungi. Journal of Phytopathology, 156 (6): 321-325.

Fourie, H., P. Ahuja, J. Lammers & M. Daneel, 2016. Brassicacea-based management strategies as an alternative to combat nematode pests: A synopsis. Crop Protection, 80: 21-41.

Gardner, J. & E. P. Caswell-Chen, 1994. *Raphanus sativus*, *Sinapis alba* and *Fagopyrum esculentum* as hosts to *Meloidogyne incognita*, *Meloidogyne javanica* and *Plasmodiophora brassicae*. Journal of Nematology, 26 (4S): 756-760.

Halbrendt, J. M., 1996. Allelopathy in the management of plant-parasitic nematodes. Journal of Nematology, 28 (1): 8-14.

Hussey, R. S. & K. R. Barker, 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter, 57 (12): 1025-1028.

Kaşkavalcı, G. & H. Duran Akkurt, 2012. Organik domates tarımında Kök-ur nematodları (*Meloidogyne* spp.)'na karşı savaşta bazı yöntemlerin birlikte kullanım etkinlikleri. Türkiye Entomoloji Dergisi, 36 (3): 413-422.

Khan, A. A. & M. W. Khan, 1991. Reaction of cauliflower cultivars to *Meloidogyne javanica* and races of *Meloidogyne incognita.* Nematropica, 21 (2): 161-166.

King, B. A. & J. P. Taberna, 2013. Site-specific management of *Meloidogyne chitwoodi* in Idaho potatoes using 1,3-dichloropropene; approach, experiences, and economics. Journal of Nematology, 45 (3): 202-213.

Kinloch, R. A., 1990. "Screening for Resistance to Root-Knot Nematodes, 16-23". In: Methods for Evaluating Plant Species for Resistance to Plant-Parasitic Nematodes (Ed: Starr, J. L.). The Society of Nematologists, Hyattsville, 86 pp.

Kirkegaard, J. A. & M. Sarwar, 1998. Biofumigation potential of brassicas- I. Variation in glucosinolate profiles of diverse field-grown Brassicas. Plant Soil 201 (1): 71-89.

Kokalis-Burelle, N., D. M. Butler & E. N. Rosskopf, 2013. Evaluation of cover crops with potential for use in anaerobic soil disinfestation (ASD) for susceptibility to three species of *Meloidogyne*. Journal of Nematology, 45 (4): 272-278.

Kruger, D. H. M., J. C. Fourie & A. Malan, 2015. Control potential of Brassicaceae cover crops as green manure and their host status for *Meloidogyne javanica* and *Criconemoides xenoplax*. South African Journal of Enology and Viticulture, 36 (1): 165-174.

Liu, Z., T. Griffin & T. L. Kirkpatrick, 2014. Statistical and economic techniques for site-specific nematode management. Journal of Nematology, 46 (1): 12-17.

Lopez-Perez, J. A., T. Roubtsova, M. C. Garcia & A. Ploeg, 2010. The potential of five winter-grown crops to reduce root-knot nematode damage and increase yield of tomato. Journal of Nematology, 42 (2): 120-127.

McFadden, W., J. Potter & J. E. Brandle, 1992. Use of *Brassica* spp. as green manure for biological control of *Pratylenchus penetrans*. Phytopathology, 82 (2): 246.

McSorley, R. & J. J. Frederick, 1995. Responses of some common Cruciferae to root-knot nematodes. Journal of Nematology, 27 (4S): 550–554.

Melakeberhan, H., 2008. Nutrient use efficiency and precision management of nematodes-concepts and possibilities. Nematologia Brasileira, 32 (1): 1-12.

Melakeberhan, H., A. Xu, A. Kravchenko, S. Mennan & E. Riga, 2006. Potential use of arugula (*Eruca sativa*) as a trap crop for *Meloidogyne hapla*. Nematology, 8 (5): 793-799.

Melakeberhan, H., S. Mennan, M. Ngouajio & T. Dudek, 2008. Effect of *Meloidogyne hapla* on multi-purpose use of oilseed radish (*Raphanus sativus*). Nematology, 10 (3): 375-379.

Melakeberhan, H., D. Douches & W. Wang, 2012. Interactions of selected potato cultivars and populations of *Meloidogyne hapla* adapted to the Midwest U.S. soils. Crop Science, 52: 1132-1137.

Mennan, S. & T. Katı, 2010. Bitki paraziti nematodlar ile mücadelede biofumigasyon. Anadolu Tarım Bilimleri Dergisi, 25 (2):120-134.

Mojtahedi, H., G. S. Santo, J. H. Wilson & A. N. Hang,1993. Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. Plant Disease, 77 (1): 42-46.

Monfort, W. S., A. S. Csinos, J. Desaeger, K. Seebold, T. M. Webster & J. C. Diaz-Perez, 2007. Evaluating *Brassica* species as an alternative control measure for root-knot nematode (*M. incognita*) in Georgia vegetable plasticulture. Crop Protection, 26 (9): 1359-1368.

Morra, M. J. & J. A. Kirkegaard, 2002. Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biology and Biochemistry, 34 (11): 1683-1690.

Nyczepir, A. P. & S. H. Thomas, 2009. "Current and Future Management Strategies in Intensive Crop Production Systems, 412-443". In: Root-Knot Nematodes (Eds: Perry, R. N., M. Moens & J. L. Starr). CAB International, Wallingford, 488 pp.

Pattison, A. B., C. Versteeg, S. Akiew & J. Kirkegaard, 2006. Resistance of Brassicaceae plants to root-knot nematode (*Meloidogyne* spp.) in northern Australia. International Journal of Pest Management, 52 (1): 53-62.

Ploeg, A. T. & J. J. Stapleton, 2001. Glasshouse studies on the effects of time, temperature and amendment of soil with broccoli plant residues on the infestation of melon plants by *Meloidogyne incognita* and *M. javanica*. Nematology, 3 (8): 855-861.

Sasser, J. N., C. C. Carter & K. M. Hartman, 1984. Standardization of Host Suitability Studies and Reporting of Resistance to Root-Knot Nematodes. Cooperative Publication of The Department of Plant Pathology, North Carolina State University and the United States Agency for International Development, Raleigh, North Carolina, U.S.A., 7 pp.

Stirling, G. R. & A. M. Stirling, 2003. The potential of Brassica green manure crops for controlling root-knot nematode (*Meloidogyne javanica*) on horticultural crops in a subtropical environment. Australian Journal of Experimental Agriculture, 43 (6): 623-630.

Taylor, A. L. & J. N. Sasser, 1978. Biology, Identification and Control of Root-Knot Nematodes (*Meloidogyne* spp.). A Cooperative Publication of the Department of Plant Pathology, North Carolina State University and the United States Agency for International Development, Raleigh, NC, USA, North Carolina State University Graphics, 111 pp.

Teklu, M. G., C. H. Schomaker & T. H. Been, 2014. Relative susceptibilities of five fodder radish varieties (*Raphanus sativus* var. *oleiformis*) to *Meloidogyne chitwoodi*. Nematology, 16 (5): 577-590.

Zasada, I. A. & H. Ferris, 2004. Nematode suppression with brassicaceous amendments: Application based upon glucosinolate profiles. Soil Biology and Biochemistry, 36 (7): 1017-1024.