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Orijinal araştırma (Original article)

The effectiveness of *Encarsia formosa* Gahan (Hym: Aphelinidae) against Glasshouse Whitefly (*Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae)) under glasshouse conditions

Sera Beyazsineği,*Trialeurodes vaporariorum* Westwood (Hem: Aleyrodidae),'ne karşı *Encarsia formosa* Gahan (Hym: Aphelinidae) yumurta parazitoitinin etkinliğinin sera koşullarında araştırılması

Doğancan KAHYA[1*] **Gordon PORT[2]**

Summary

Glasshouse whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) is one of the most economically important pests in terms of its damage to vegetable crops in glasshouse condition. Chemical control is still applied against this pest, but resistance has prevented the use of chemical control methods. In addition chemical pesticides pollute the environment. Biological control, therefore, is one of the viable alternative methods being use in the control of Glasshouse whitefly. The aim of this present study is to use of biological control against Glasshouse whitefly. The effectiveness of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) was observed between three different varieties of the tomato and cucumber (Moneymaker (tomato), Alicante (tomato) and Telegraph Improved (cucumber)) for this study The results obtained from present study indicated that the percentage of parasitism was 67% for Moneymaker, 67% for Alicante, and 62% for Telegraph Improved. While there was no significant difference in the percentage of parasitism, there was only a difference in the number of whitefly eggs laid between in variety of Alicante ((tomato) and Telegraph Improved (cucumber) in this study. According to the results, the biological control using *E. formosa* against Glasshouse whitefly can be used successfully on varieties of Moneymaker (tomato), Alicante (tomato), and Telegraph Improved (cucumber) for the control of Glasshouse whitefly in glasshouses.

Keywords: *Trialeurodes vaporariorum*, *Encarsia formosa*, Biological control, Glasshouse whitefly

Özet

Sera Beyazsineği *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) sera koşullarında yetiştirilen sebze ürünlerinde ekonomik açıdan zarara sebep olan önemli zararlı türler arasında yer almaktadır. Kalite ve verim açısından zarara sebep olan bu tür için kimyasal mücadele yaygın bir şekilde uygulanmaktadır. Ancak kimyasal mücadele bu zararlıda dayanıklılığa sebep olmakta ve kullanılan kimyasal pestisitlerin çevreye zarar verdiği bilinmektedir. Geliştirilen alternatif mücadele yöntemlerinden olan biyolojik mücadele bu zararlının kontrol altına alınmasında kullanılabilecek bir yöntemdir. Yapılan bu çalışmanın amacı, Sera Beyazsineğine karşı ticari olarak da kullanılan *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae)'nın etkinliğinin Moneymaker ve Alicante adlı domates ve Telegraph Improved adlı hıyar çeşitlerinde araştırılmasıdır. Bu projeden elde edilen sonuçlara göre parazitlenme oranlarında (Moneymaker için %67, Alicante için %67, Telegraph Improved için %62). bir farklılık gözlenmemiştir. Sadece Alicante ve Telegraph Improved çeşitlerinde Sera Beyazsineğinin bıraktığı yumurta sayısı açısından bir farklılık tespit edilmiştir. Sonuç olarak, *E. formosa*'nın Sera Beyazsineğine karşı bu çalışmada kullanılan Moneymaker ve Alicante isimli domates ve Telegraph Improved isimli hıyar çeşitlerinde sera koşullarında kullanılabilecek başarılı bir parazitoit olduğu görülmüştür.

Anahtar sözcükler: *Trialeurodes vaporariorum*, *Encarsia formosa*, Biyolojik mücadele, Sera Beyazsineği

[1] Biyolojik Control Research Institute, Yüreğir, Adana, Turkey
[2] University of Newcastle Upon Tyne, School of Biology, Newcastle Upon Tyne, United Kingdom
[*] Corresponding author (Sorumlu yazar) e-mail: kahyadogancan@gmail.com
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Introduction

The Glasshouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) is a significant pest for vegetables and many ornamental plants in glasshouses. According to some research results, 250 plant species have been identified as hosts for Glasshouse whitefly worldwide, and vegetables in particular (Osborne & Landa, 1992). The Glasshouse whitefly is a significant pest in economic terms with regard to glasshouse crops, due to the fact that this pest causes both direct and indirect damage, such as damaging leaves with honeydew, feeding on plant sap, and transmitting some plant viruses. Besides, moulds and fungi develop easily with Glasshouse whitefly (Mellor et al., 1997).

Glasshouses provide the most appropriate conditions for the growth of this pest. Mediterranean glasshouses are especially affected negatively by Glasshouse whitefly. Certain control methods are insufficient. For example resistance problem restricted the integrated control programs with insecticides (Fargues et al., 2003). In addition insecticides have a negative impact on non-target species such natural enemies (Gorman et al., 2002). According to conducted studies, biological control is one of the most alternative and effective methods for managing this pest in glasshouses (Bale et al., 2008). There are two main methods identified under biological control (Classical biological control and Augmentative Biological control) (Van Lenteren et al., 2003). Augmentative biological control is used prevalently in European Glasshouses. The release of *E. formosa* is particularly common bio-control methods used in glasshouses (Hoy, 2008).

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is a popular bio-control agent in biological control. It is used on vegetable crops and ornamental plants in glasshouses throughout the world. The success of *E. formosa* against Glasshouse whitefly has been proven by many studies. Approximately 15 host in aleyrodid genera is parasitized by *E. formosa*. Parasitized whitefly eggs turn black. *Encarsia formosa* is generally described as effective bio-control agent, but some factors affect the parasitism rate. Host plant is an important impact on the behaviours of parasitoids (Hoddle et al., 1998).

The Glasshouse whitefly is especially widespread in the temperate and subtropical zones. The life cycle of this pest is about 25-30 days at 21^{o}C. The optimum humidity is between 75-80% for the development of life cycle. The life cycles include the egg, four nymphal instars, and the adult stage. The fourth nymphal instar is known as 'pupae'. Feeding period and conversion to adult stage start with the fourth nymphal stage. Adults are triangular in shape because the basal sections of the wings are narrower than the distal position. Glasshouse whitefly female adults produce about 175-200 eggs on cucumber and tomato plants. Tomato, cucumber and eggplant are the common host plants in glasshouses (Capinera, 2008). Glasshouse whiteflies have direct and indirect impact on crops. These insects excrete honeydew and this cause the development of Sooty mould fungi on plants (Lei et al., 1998). Whitefly nymphs are sap-sucking phloem feeders. This situation cause more than 50% yield reduction. In addition, Whiteflies transmit many economically important viral pathogens (Byrne &Bellows, 1991). Tomato chlorosis virus (ToCV) and tomato infectious chlorosis virus (TICV) are transmitted by Glasshouse whitefly (Wisler et al., 1998).

The biological control of the Glasshouse whitefly with *E. formosa* is longstanding and popular method in glasshouses. This method has been introduced in European countries since 1930. When the insects developed resistance to pesticides, the importance of biological control with parasitoids became more popular and effective. Biological control with *E. formosa* is used in more than 20 countries which have a developed glasshouse industry (Van Lenteren et al., 1996). *Encarsia formosa* is asolitary, thelythokus endoparasitoid, and produces 8-10 eggs which are matured per day (Hoddle et al., 1998). Females are approximately 0,6 mm long and are yellow with a black abdomen and opalescent wings. The males are totally black, and are larger than females (Mahr et al., 2001).Tomato and cucumber are the main crops for the use of *E. formosa* in the control of the Glasshouse whiteflies. The fourth instar and prepupal nymphs of Glasshouse whitefly are the most appropriate stage for the parasitism of *E. formosa*. In addition, the rate of successful emergence in *E. formosa* is the highest in the recommended stages

(Hoddle et al., 1998). The colour of parasitized whitefly pupae changes to black in about 10 days. After that, the adults of *E.formosa* emerge 10 days later and the whitefly nymphs are killed (Mahr et al., 2001).

As mentioned above, host plant species and variety are also the most important factors affecting the efficiency of the *E. formosa*. Some species are better host plants than others in terms of the control of Glasshouse whitefly using *E. formosa*. For example, the release of *E. formosa* causes the population of the Glasshouse whitefly to decline by almost 40% in tomato glasshouses. On the other hand, cucumber is more suitable host for the development of whiteflies, but is worse than tomato with regard to paraistoid foraging (Hoddle et al., 1998).

Predators and pathogens have been used for biological control of Glasshouse whitefly (Van Lenteren et al., 1996). For example, *Macrolophus caliginosus* Wgn. (Hemiptera: Miridae) is an important predator of Glasshouse whitefly. This predator generally prefer eggs and nymphs of whitefly. However, this species can cause damage to fruit by feeding on it, if the whitefly population is limited. In addition, *Orius* spp. (Hemiptera: Anthocoridae) are used commercially against Glasshouse whitefly. But, short days and lower temperatures have negative impact on these predators (Mahr et al., 2001). *Lecanicillium lecanii* is significant pathogens for control of Glasshouse whitefly. However, this fungus needs high humidity and humidity has to remain high for at least 10 hours per day for the germination of spores and infection (Mahr et al., 2001).

The objectives of this study are to determine the effectiveness of *E. formosa* in its use against Glasshouse whitefly, *T. vaporariorum*, on three different varieties namely Moneymaker (tomato), Alicante (tomato), and Telegraph Improved (cucumber) by comparing three varieties in terms of difference between the percentages of parasitism.

Materials and Methods

Forty-eight tomato (36) and cucumber (12) plants were sown during the experiment. Two tomato varieties (Moneymaker and Alicante) and one cucumber variety (Telegraph Improved) were used during the experiment. Seeds were sown in pots in a glasshouse in the University of Newcastle Upon-Tyne. Prior to experiments, the plants were transferred to a growth room (24 ^{o}C 18:6 L:D). Perforated plastic bags (bread bags) were used to protect individual plants from other insects. The plants were watered regularly.

The Glasshouse whiteflies were sourced from a culture at Newcastle University. Whitefly adults were maintained on grown aubergine plants in a mesh cage in a growth room and the plants were watered regularly. The Whitefly culture comprised approximately 200 Glasshouse whitefly adults. The adults were transferred to the experiment plants using a suction device (pooter). After transferring the adults, the infested tomato and cucumber plants were put in the growth room, and each covered with a plastic bag.

The parasitic wasps (*E. formosa*) used for this experiment were ordered via www. Ladybirdplantcare.co.uk. The commercial name of these products is Bioline (Syngenta) and Encsure fc (bcp CERTIS). The parasitoid cards include the pupae of *E. formosa* with each card having approximately 100 parasitoid pupae. Each treatment plant used 1 card for the experiment, and therefore 24 cards were used during the investigation. The parasitoid pupae were stuck to the cards, and the cards were hung in the middle of the plants for the proper spread of the parasitoids.

Cards were protected from direct sunlight during storage, as direct sunlight affects the efficiency of the parasitoid cards. Fresh parasitoid cards were used to be fully effective. The *E. formosa* cards remained on the tomato and cucumber plants until the black parasitized egg was seen under the leaves. The minimum temperature is 18^{o}C for the activity of the parasitoid, so the temperature of the growth room was kept the higher than 18^{o}C (temperature remained between 20^{o}C and 24^{o}C during this process). At the end of this process, cards were removed from the tomato and cucumber plants in order to measure the efficiency of the parasitoid cards.

The design of experiment

Six tomato and six cucumber plants were used for each experiment. Three experiments have been established during the project. Three plants were used for the control, and three plants were used for the treatment. At the beginning of the process, Glasshouse whiteflies were transferred from aubergine plants to the tomato and cucumber plants. Six whitefly adults were transferred to plants, and the plants were bagged and placed in the growth room. These plants remained in the growth room (for approximately 24 hours) for oviposition.

After 24 hours, whiteflies were removed from control and treatment plant, and returned to the whitefly culture for use in another experiment. The plants with whitefly eggs were left for approximately 16 days until the whitefly nymphs reached third nymphal instar. This is the most suitable age for the use of the parasitoid in the control of Glasshouse whitefly (*T. vaporariorum*). One parasitoid card was used for each treated plant.

The experiment was designed as below:

Moneymaker (tomato) + six whitefly adult + *Encarsia formosa*

Alicante (tomato) + six whitefly adult +*Encarsia formosa*

Telegraph Improved (cucumber) + six whitefly adult + *E. formosa*

Control was designed as below:

Moneymaker (tomato) + six whitefly adult

Alicante (tomato) + six whitefly adult

Telegraph Improved (cucumber) + six whitefly adult

Three experiments were carried out during the course of the investigation. Forty-eight different results were obtained for three experiments (24 for treatment, 24 for control). Figure 1 explains the important stages of the experiment during the investigation.

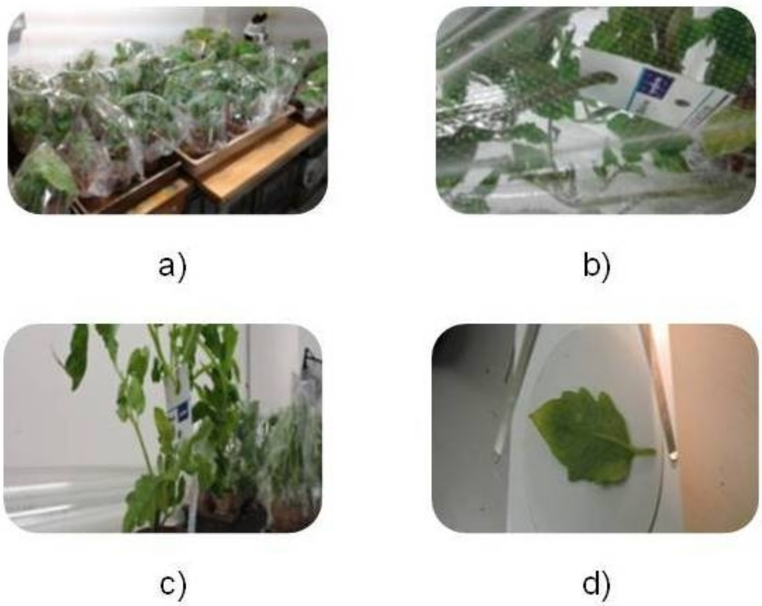

Figure 1: Important stages in the experiment: a) Whitefly infestation, b) the use of parasitoid cards, c) counting whitefly eggs, d) counting parasitized eggs

Data analysis

The effectiveness of parasitoid cards between three varieties was assessed using descriptive statistical tests. Normality tests were initially applied to data on parasitism rate and then one-way ANOVA and Tukey (Post-hoc) tests were applied to analyse the data using IBM SPSS Statistic 21 programme. In addition, independent sample t-test was used in determining significant differences between treatment and control plants in terms of total number of eggs.

Results

Total egg count

There is no significant difference ($F_{2.088}$, $p > 0.05$) identified in the treatment and control plants in terms of whitefly eggs (Treatment: 22.58 and Control: 22.25). The Independent sample t-test was applied in order to determine differences between the treatment and control plants.

Table 1 shows the collected and assessed data during the experiment in terms of whitefly eggs The mean number of total eggs was also calculated in respect of three varieties separately. There was significant difference ($F_{4.036}$, $p < 0.05$) between Moneymaker, Alicante, and Telegraph Improved in relation to total egg count (Moneymaker: 22.58 Alicante: 21.17 Cucumber: 24.50). Tukey HSD test was applied in order to determine difference within varieties. Alicante (tomato) and Telegraph Improved (cucumber) had difference in terms of laying eggs. There was no difference identified between Moneymaker-Alicante and Moneymaker- Telegraph Improved.

Table 1: The assessment of number of total whitefly egg within three varieties during the experiment

Variety	Mean egg number ± Standard error
Moneymaker (Tomato)	22.28±0.832 **ab**
Alicante (Tomato)	21.17± 0.663 **b**
Telegraph Improved (Cucumber)	24.50± 0.883 **a**

*The number of total egg for Alicante and Telegraph Improved indicates significant difference (P-value<0.05), as determined by One-way ANOVA and Tukey's test

Parasitism

There was no significant difference ($F_{2.024}$, $p > 0.05$) between parasitized nymphs between varieties (Moneymaker: 15.33 Alicante: 13.56 and Cucumber: 16.17). In addition, There was no significant differences ($F_{2.972}$, $p > 0.05$) between Moneymaker(tomato variety), Alicante (tomato variety), and Telegraph Improved (cucumber variety) with regard to the number of unparasitized nymphs (Moneymaker: 7.56 Alicante: 6.44 Cucumber: 9.83).

The parasitism rates were also assessed to determine difference between three varieties. Table 2 shows the collected and assessed data during the experiment in terms of parasitism. There was no significant difference ($F_{0.587}$, $p > 0.05$) between Moneymaker, Alicante, and Telegraph Improved in terms of rate of parasitism (Moneymaker: 67% Alicante: 67% Telegraph Improved (cucumber): 62%). Although parasitoid cards remained on the plants with shorter period than recommended in the technical information, the experiment results provide evidence for the success of the *E. formosa* in controlling the Glasshouse whitefly (*T. vaporaiorum*) on tomato and cucumber.

Table 2: The assessment of parasitism rate (%) within three varieties during the experiment

Variety	**Parasitism rate (%)±Standard error**
Moneymaker (Tomato)	67.37±0.03394 **a**
Alicante (Tomato)	67.90±0.03944 **a**
Telegraph Improved (Cucumber)	62.53±0.02383 **a**

The parasitism rate for each variety indicates no significant difference (P-value > 0.05), as determined by one-way ANOVA and Tukey's test

Discussion

This study has set out to prove the effectiveness of the *E. formosa* against Glasshouse whitefly. According to experiment results, the effectiveness of parasitism on two varieties of the tomato and one variety of cucumber was investigated and compared. The subjects such as host plant relationship in terms of paraistoid and whitefly, parasitism potential of *E. formosa*, and the quality of host plant were discussed with the results of experiment in this section. The effectiveness of parasitoid cards is demonstrated as being approximately 90% in the technical information sheet (Syngenta, 2014). However, the result of present experiment indicated that it was about 70% for three varieties.

According to previous studies, Glasshouse whitefly was easily affected by quality of host plant (Van Roermund et al., 1997). The effect of genotypes did not impact the percentage of oviposition between tomato varieties significantly (Van Roermund et al., 1997). The experiment is in line with those of Van Roermund et al. (1997). The mean numbers of total egg count are similar for both. Different tomato varieties did not affect the oviposition rate in present experiment (22.28 for Moneymaker and 21.17 for Alicante). On the other hand, Hoddle et al. (1998) reported that cucumber is more suitable host plant for development of Glasshouse whiteflies. In addition, tomato was a less suitable host plant for the Glasshouse whitefly. These results are parallel to those of present study. Cucumber has more suitable conditions for oviposition of Glasshouse whitefly since the egg count was higher than Moneymaker and Alicante in the experiment (22.28 for Moneymaker, 21.17 for Alicante and 24.50 for Cucumber).

The effectiveness of *E. formosa* is proved against Glasshouse whitefly because glasshouse environment was identified as the most suitable environment for this parasitoid (Cooper & Oetting, 1987). The parasitism rate increased in higher temperatures. Lower temperatures have a negative impact on predomination of parasitic wasps. According to Burnett, 24^{o}C is the appropriate temperature for the parasitism. If the temperature falls below 24^{o}C, the population of Glasshouse Whitefly and *E. formosa* have same percentages (Cooper & Oetting, 1987). The success of experiment echoed that of Cooper & Oetting (1987), because temperature remained 24^{o}C during the experiment. The mean parasitism was about 66.36% for this experiment. 14.88 eggs (the mean number of parasitized eggs) were parasitized in 22.42 eggs (the mean number of total eggs).

According to Sütterlin & Van Lenteren (1997), leaf hairiness is important factor for walking activity of paraistoids and oviposition of Glasshouse whitefly. However, Sütterlin & Van Lenteren (1997) showed that, rate of paraisitsm was not affected by leaf hairiness. Although leaf hairiness has effect on walking activity and oviposition, this did not limit the use of *E. formosa* in the control of Glasshouse whitefly. The experiment result was in accordance with the findings of Sütterlin & Van Lenteren (1997). The average value of the parasitism was around 66,36%. In addition each variety (Moneymaker (tomato), Alicante (tomato) and Telegraph Improved (cucumber) demonstrated positive result for parasitism, with the percentage being above 60% (67% for Moneymaker, 67% for Alicante and 62% for Telegraph Improved (cucumber)). Although tomato and cucumber varieties have hairy leaves, the percentage of parasitism was not negatively affected in the experiment.

Host plant quality is also one of the most important factors for biological control of Glasshouse whitefly with *E. formosa* because it can easily affect the inoculative releases. According to previous studies, cucumber is not good host plant for *E. formosa*. Although Glasshouse whitefly population grows fast on cucumber, parasitism result is insufficient for biological control. The cucumber's characteristics are not suitable for *E. formosa*. The leaves are large and hairy, and the honeydew remains on the leaves for a long time (Van Lenteren et al., 1996). In addition, the surface of cucumber leaves have a negative impact on parasitoid foraging (Hoodle et al., 1998).

Although tomato is not suitable host plant for whitefly development, the leaves of tomatoes are more appropriate in terms of the foraging behaviour of parasitoid. Previous studies investigated the percentage of parasitism on different tomato varieties. According to results, 12 tomato varieties were investigated and there is fewer differences between tomato varieties with regard to parasitism rate (Hoddle et al., 1998). Another study on tomatoes concluded that the proportion of parasitism by *E. formosa* to be in region of 40-70% (Van Roermund et al., 1997).

The results of the experiment back up previous studies. For example, the mean number of total eggs (22.28 for Moneymaker, 21.17 for Alicante and 24.50 for Telegraph Improved (cucumber)) was the highest on cucumber in present experiment. The cucumber is more suitable than tomatoes in terms of whitefly development. On the other hand, the percentage of parasitism on tomato varieties is higher than that on cucumber (67.37% for Moneymaker, 67.90% for Alicante and 62.53% for Telegraph Improved (cucumber)). These results match those of with Hoddle et al. (1998) 's and Van Roermund et al. (1997). The parasitism rates of two tomatoes varieties (Moneymaker and Alicante) were almost equal in the experiment. In addition the average of parasitism rate was 68% on tomatoes. Similar result regarding to the parastisim rate on tomatoes is reported by Van Roermund et al. (1997). The percentage of parasitism on tomatoes was higher than that on cucumber. Similarly Hoddle et al. (1998) reported tomato varieties are more suitable than cucumber in terms of foraging behaviour in this experiment.

The greenhouse environment is an important factor for increasing success of the natural enemies. Glasshouses are contained areas, and so provide isolation from the spread of other pests, providing convenient conditions for the release and activity of biological control agents. Natural enemies are introduced on limited pests, so as to increase the success of the biological control in glasshouses (Van Lenteren, 2000). The results of the experiment are in line with those produced by Van Lenteren (2000), as the experiment was carried out under stable condition; every plant was bagged, only Glasshouse whitefly was introduced to tomato and cucumber plants, and the conditions of growth room were stable during the experiment. These conditions are important evidence for obtaining successful results at the end of the experiment.

This research concludes that, owing to the efficacy rate of *E. formosa*, this is a successful bio-control agent against the Glasshouse whitefly, *T. vaporariorum*, in the glasshouse conditions. The parasitism result for tomato and cucumber are almost similar to each other. *Encarsia formosa* can be used effectively against Glasshouse whitefly in glasshouses.

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