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SEVCAN ÖZTEMİZ

ALİ KÜDEN

SERPİL NAS

IŞILAY LAVKOR

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Efficacy of *Trichogramma evanescens* and *Bacillus thuringiensis* var. *kurstaki* in control of *Cydia pomonella* (L.) in Turkey

Sevcan ÖZTEMİZ[1,*], Ali KÜDEN[2], Serpil NAS[3], Işılay LAVKOR[3]
[1]Department of Plant Protection, Faculty of Agriculture and Natural Sciences, Düzce University, Düzce, Turkey
[2]Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Turkey
[3]Biological Control Research Institute, Adana, Turkey

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Abstract: This study was conducted to evaluate the effectiveness of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) release with as well as without *Bacillus thuringiensis* var. *kurstaki* (Berliner) in biological control of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in the Galaxy Gala apple variety grafted onto M9 rootstock in 2016. Four treatments were analyzed: releasing *T. evanescens* alone (TE), applying *B. thuringiensis* var. *kurstaki* (BT) alone, applying both (TE + BT), and a control (C) without any application at all. The experiment design entailed randomized blocks with four replicates. In each generation of the egg stage, 100,000 parasitoids per hectare were released twice (1440 parasitoids/plot), with an interval of 7–10 days. In the larval stage, BT was applied twice for the first generation and once for the second. Weekly counts were performed regularly. The decrease in egg count was 52.15% in TE, 58.99% in BT, and 65.46% in TE + BT plots. The decrease observed in larval numbers was 68%, 73.33%, and 94.66%, respectively. Egg parasitization rates varied between 58.64% and 69.79%. At harvest, fruit infestation rates were 9.66% and 8.33% in TE and BT plots and just 2.0% in the TE + BT plot, versus 34% in the control plot. Promising results were achieved in biological control of *C. pomonella* when TE was combined with BT. The combined treatment of both biological control agents significantly decreased the population of the pests and crop damage. The natural enemy alone was not effective in keeping the population below the economic threshold level.

Key words: Apple, biopesticide, biological control, codling moth, egg parasitoid, release

1. Introduction

The suitable climate and soil conditions of Turkey allow many fruit species to be grown, including pome and stone fruits, nuts, and subtropical fruits (Özongun et al., 2004; Ercişli, 2009; Erturk et al., 2010; Sarıdaş et al., 2016; Yazıcı and Şahin, 2016).

Apple (*Malus communis* L.) grows across a wide territory stretching from Anatolia to the southern Caucasus and is accustomed to a wide range of ecologies. In 2015, Turkey's apple production was 2,569,759 t while 34.9% of that amount was grown in the Mediterranean region of the country (http://tuik.gov.tr/). In this context, Turkey ranks third after China and the United States. While Turkey is among the top apple producers worldwide, its produce is considered subpar in terms of compliance with international standards. Modern methods and techniques are needed for the produce of the land to comply with changing demands in the market and to compete with other leading apple producers.

Among the pests that constitute a significant hazard in apple production, the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is the one that hits the apple orchards of Turkey the hardest. Pears, quince, and walnuts are also among its hosts. The larvae of the pest feed directly on the fruit, digging galleries inside and severely damaging fruit quality by eating the fleshy portion of the fruit as well as the kernel. In addition to the loss of fruit volume, the loss of quality and degradation caused by the pest leads to substantial decreases in the market value of the produce (Beers et al., 1993). Left unchecked, codling moth could destroy 60%–100% of apple crops (TAGEM, 1995; Yahodina and Yushchenko, 2015). Apple producers' favorite means of control against the pest is the use of chemicals (Madsen and Morgan, 1970; Niemezyk et al., 1996; Lacey and Unruh, 2005). Alternative control strategies are urgently needed to avoid the adverse effects of agrochemicals used in chemical control and to minimize the environmental problems. One such alternative

* Correspondence: s_oztemiz@hotmail.com

strategy is the use of biological control agents. More than 100 species of parasitic wasps have been described for *C. pomonella*. *Trichogramma* species are important biological control agents (Hassan, 1993; Zimmermann, 2004; Consoli et al., 2010). Among these, nine or more species of *Trichogramma* are listed (Pinto et al., 2002). Hundreds of studies were produced over a period of 70 years on the inundative release of *Trichogramma* species against codling moth. However, the literature is consistent about the low efficacy of such approaches, suggesting significant problems in their utilization for biological control of this pest (Lacey and Unruh, 2005). Although a number of studies were conducted on codling moth in Turkey (İren and Gürkan, 1971; Bulut and Kılınçer, 1986, 1992), none investigated the effectiveness of releasing beneficial insects for controlling the pest. The efficacies of these biological control agents against *C. pomonella*, as well as the dose of parasitoids to be released on apple and a number of other criteria, are unknown in Turkey. The present study intends to close this gap; reduce the amount of pesticides used in apple production; lower production costs, leading to more economical production; increase natural predator populations; and protect the environment and human health by using biological control agents in practice (Knight et al., 1997; Epstein et al., 2000). This study also aimed to determine the effectiveness of biopesticide *Bacillus thuringiensis* var. *kurstaki* (BT) when applied at the larval stage. BT is the most widely used biopesticide (Lacey et al., 2001). *C. pomonella* larvae are also susceptible to toxins produced by BT (Rang et al., 2000; Konecka et al., 2015). It has been reported that this microbial insecticide against codling moth can be used in apple orchards (Morris et al., 1994; Vogt, 1995; Sauphanor and Delorme, 1996; Malik et al., 2002; Ertürk, 2016). It has also been recorded by many researchers that the use of *Trichogramma* releases with other control methods should be emphasized in biological control (Stinner, 1977; Smith, 1996). The most widely explored combination involves the use of BT with *Trichogramma* releases. Trials have shown that this combination can provide greater suppression of pests (Mertz et al., 1995; Lacey and Unruh, 2005; Jalali and Singh, 2006). Against this background, this study intends to evaluate the efficacy of releasing *T. evanescens* and *B. thuringiensis* var. *kurstaki* alone as well as in combination with each other to control *C. pomonella* in apple orchards of Turkey.

2. Materials and methods

2.1. Material

The main materials of the study are apple trees, *C. pomonella*, *T. evanescens*, laboratory host *Ephestia kuehniella* Zeller, *Bacillus thuringiensis* var. *kurstaki* (commercial bacterial insecticide - Delfin WG), climate rooms, production cages, release bags, pheromone traps, and laboratory materials such as glass tubes and petri dishes.

2.2. Methods

Production studies were carried out in laboratory and climate rooms, and release studies were carried out in an apple orchard.

2.2.1. Rearing of insects

Trichogramma evanescens used in the trials was obtained from a laboratory of the Biological Control Research Station, Adana, Turkey, reared on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs according to the methodology described by Hassan (1981). All parasitoids were produced at the same time, and newly parasitized fresh eggs were used. The laboratory provided a temperature of 25 ± 1 °C, 60%–80% relative humidity, and a photoperiod of 16 h of light and 8 h of dark (Öztemiz, 2001).

2.2.2. *Trichogramma evanescens* release with the application of *Bacillus thuringiensis* var. *kurstaki*

2.2.2.1. Features of trial sites

The study was carried out at Pozantı/Pozmer (Pozantı Agricultural Research and Application Center) in the province of Adana. The center is in Pozmer Alpu village, located at a distance of 5 km from the Pozantı district center at an altitude of 1100 m. The center's coordinates are 37°28′N (13.3644) and 34°52′E (50.9412).

2.2.2.2. Features of apple varieties

The Galaxy Gala apple variety grafted onto M9 rootstock used in the trial was introduced from New Zealand. Its fruits are medium in size, while the flesh is creamy, hard, juicy, aromatic, and sweet. It is considered one of the best autumnal varieties. The pollinators used are Fuji, Jersey Mac, Golden Delicious, Braeburn, and Granny Smith.

2.2.2.3. Experimental design

The experimental design employed randomized complete blocks with four replicates. Four treatments were applied on each parcel constituting such complete blocks: releasing *T. evanescens* alone (TE), applying *B. thuringiensis* var. *kurstaki* (BT) alone, applying both together (TE + BT), and a control (C) without any application or release (see Table 1). Each parcel had nine trees at about 40 m^2. Samples were taken from four trees selected in the center of each plot. A pheromone trap was used to determine the release time, as shown in Figure 1.

The intervention was applied to each generation of the pest during the egg stage at a dosage of 100,000 parasitoids/ha twice (1440 parasitoids/plot) with an interval of 7–10 days (Radzivilovskaya and Salikhov, 1987; Damianov et al., 2014). It was done with release cards containing parasitized eggs of different developmental stages. Climatic data were recorded with a datalogger at the trial site. In addition, data from the meteorological station were evaluated, as shown in Figure 2. A degree-day model was used and

Table 1. Application details in biological control of *Cydia pomonella*.

	Biological control agents	Application dosages	Release/ application time	Application frequency
1	*T. evanescens*	100,000 parasitoids/ha (1440 parasitoids/plot)	21.04.2016 01.05.2016 16.07.2016 27.07.2016	During the egg period of the pest; 2 releases per 7–10 day interval for each offspring
2	*B. thuringiensis var. kurstaki* 32,000 IU/mg	100 g/100 L water (2 g/tree)	12.05.2016 27.05.2016 22.07.2016	During the larval stage of the pest; 3 applications in total, 2 for the first generation, 1 for the second generation
3	*T. evanescens* + *B. thuringiensis* var. *kurstaki* 32,000 IU/mg	100,000 parasitoids/ha (1440 parasitoids/plot) + 100 g/100 L water (2 g/tree)	21.04.2016 01.05.2016 16.07.2016 27.07.2016 12.05.2016 27.05.2016 22.07.2016	During the egg period of the pest; 2 releases per 7–10 day interval for each offspring + during the larval stage of the pest; 3 applications in total, 2 for the first generation, 1 for the second generation
4	Control	-	-	-

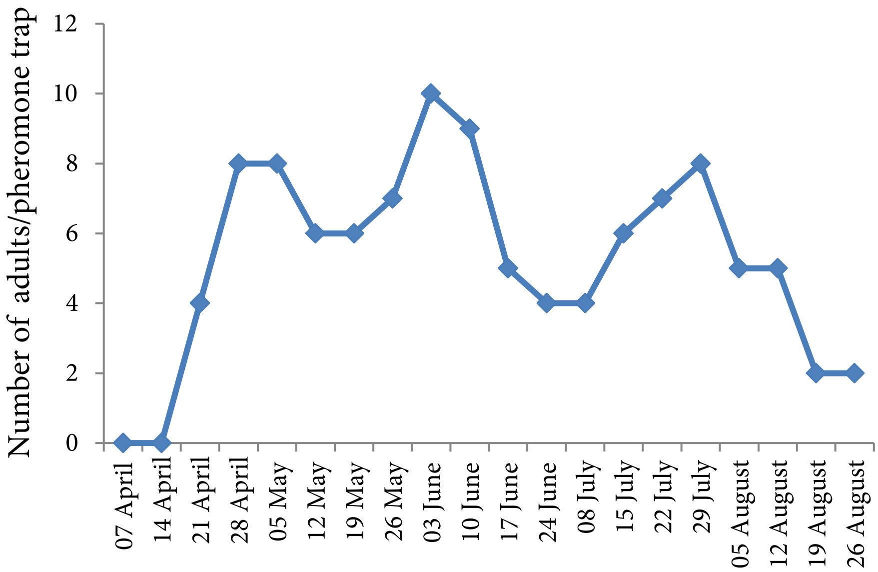

Figure 1. Number of codling moth adults caught in pheromone traps in 2016.

effective temperature sums were calculated to determine application time against larvae. It is theoretically assumed that if the first codling moth adult was caught in a trap and found 150 days later, the first larvae started to exit and the second larvae emerged when at 800 days (Ahmad et al., 1995; TAGEM, 2008). Thus, BT was applied twice against the first generation, once against the second generation, and thrice against the larval stage of the pest. Weekly counts were performed regularly. No intervention was applied to the control plot. The application area was a total of 0.7 ha. The distance between the parcels was at least 100 m. The application details are given in Table 1.

2.2.3. Evaluation

In order to determine the rate of parasitization of *C. pomonella* eggs, 25 leaves from each of the four trees selected from the center of each plot, amounting to a total of 100 leaves, were collected, and parasitized and nonparasitized eggs were recorded. Before the harvest, 100 fruit samples collected from each plot were counted, and damaged and healthy fruit rates were recorded. During

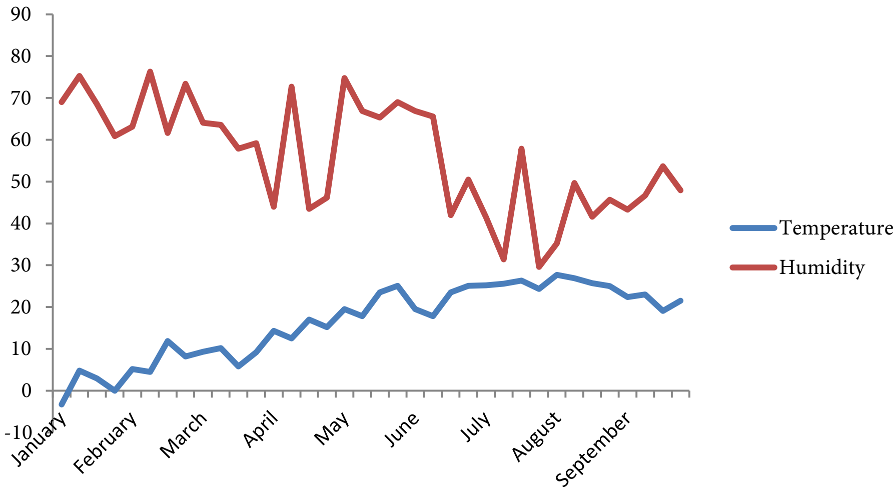

Figure 2. Mean temperature and humidity values in the experimental plot in 2016.

the harvest, in turn, 300 fruit samples were taken from each plot, and the rates of damaged and healthy fruits were calculated (Damianov et al., 2014). In addition, the number of harmful larvae in each plot and yield were also assessed. Analysis of variance (ANOVA) (Zar, 1999) was performed with reference to the data gathered. The mean values were then subjected to LSD test at 1% significance level (Fischer, 1954; Zar, 1999). Egg and larvae count results were evaluated according to the Henderson–Tilton method (Karman, 1971). It was observed that, in comparison to the control plot, egg and larvae counts were reduced in all the plots that were subjected to treatment. The total damaged fruit percentage (%) was assessed according to the Abbott formula (Abbott, 1925). The application was deemed successful if the rate of total spillage and total damage was less than 2% (Andermatt et al., 1988; TAGEM, 2008).

3. Results and discussion

The egg parasitization rates of *C. pomonella* were found to be 58.64% in TE and 69.79% in TE + BT plots (Table 2). In a similar study, it was reported that an effect of up to 63% could be achieved with *Trichogramma* species and races (Hassan, 1986). In another study, *Trichogramma* release against *C. pomonella* provided an average efficiency of 56.58% when used in combination with a biological pesticide (Xu et al., 2014). The statistical analysis of the *C. pomonella* eggs ($F = 42.39$, $LSD = 58.74$, $d.f. = 15$, $P < 0.01$) and parasitized eggs ($F = 69.01$, $LSD = 22.63$, $d.f. = 15$, $P < 0.01$) led to a picture emphasizing two distinct groups. The parasitism rates ($F = 220.29$, $LSD = 11.23$, $d.f. = 15$, $P < 0.01$), on the other hand, led to a picture with three distinct groups, as shown in Table 2.

The falls recorded in egg counts were 52.15% in TE, 58.99% in BT, and 65.46% in TE + BT plots. The larvae numbers, in turn, fell by 68%, 73.33%, and 94.66%, respectively. Similar results were reported by Lundgren et al. (2002) and Blatt et al. (2016). Damage reduction is equivalent to the 50%–70% reported in other studies (Hassan et al., 1988; Hassan, 1993). The number of larvae fell due to the fall in the number of eggs. Moreover, in the first stage, the larvae were found to be very sensitive

Table 2. The parasitization rates of *Cydia pomonella* eggs along with the number of eggs and larvae of the pest in different applications (mean ± SE).

Applications	*C. pomonella* larvae count	*C. pomonella* egg count	*C. pomonella* parasitized egg count	Parasitization rate (%)
TE	24 ± 0.81 b*	133 ± 14.69 b	78 ± 6.68 a	58.64 ± 3.10 b
BT	20 ± 0.81 b	114 ± 8.99 b	4 ± 1.76 b	3.50 ± 1.61 c
TE + BT	4 ± 0.81 c	96 ± 8.57 b	67 ± 8.58 a	69.79 ± 3.22 a
C	75 ± 2.38 a	278 ± 12.24 a	0 ± 0.00 b	0 ± 0.00 c

TE: *Trichogramma evanescens*, BT: *Bacillus thuringiensis* var. *kurstaki*, C: control
* The difference between means shown with different letters is significant $P < 0.01$ (LSD).

Table 3. Average yield (kg), number of damaged and undamaged fruits and percentage of damaged fruits (%) with different application characteristics (Mean ± S.E.).

Applications	Tree fruit yield (kg)	Number of undamaged fruits	Number of damaged fruits	Rate of damaged fruit (%)
TE	31.875 ± 0.66 b*	271 ± 1.59 b	29 ± 1.58 b	9.66 ± 0.52 b
BT	33.637 ± 0.73 b	275 ± 0.90 b	25 ± 0.91 b	8.33 ± 0.30 b
TE + BT	37.617 ± 0.95 a	294 ± 0.81 a	6 ± 0.81 c	2.00 ± 0.27 c
C	22.142 ± 0.62 c	198 ± 1.50 c	102 ± 1.47 a	34.00 ± 0.49 a

TE: *Trichogramma evanescens*, BT: *Bacillus thuringiensis* var. *kurstaki*, C: control
* The difference between means shown with different letters is significant $P < 0.01$ (LSD).

and suffered high mortality rates due to rain, wind, and predatory species, leading to a substantial fall in the number of larvae.

At harvest, fruit infestation rates were 9.66% and 8.33% on TE and BT plots and 2.0% on the TE + BT plot, respectively, compared to 34% on the control plot as shown in Table 3. Similar results were achieved in another study performed in Romania, where the percentage of damaged fruits was found to be 12.00% and 12.66% on the plot for which the agent was released compared to 26.33% and 30.66% on the control plot in 2009 and 2010, respectively. That study also reduced the number of sprayings, replacing it with an intervention successfully used in integrated pest management (Damianov et al., 2014). In another study, the rate of fruit damage was found to be 8.7% in the experimental plot compared to 26% in the control plot (Radzivilovskaya and Salikhov, 1987). When the reductions in fruit contamination are compared with the control plot, TE was found to achieve a 71.56% reduction, while BT achieved 75.49% and the TE + BT plot presented a 94.11% decrease. In Ukraine, it was reported that when *T. dendrolimus* was released against *C. pomonella*, fruit infestation rates were just 18.1% compared to 48.6% in the control plots (Yahodina and Yushchenko, 2015). Therefore, the literature concurs with the findings of the present study. When the damaged fruit counts ($F = 994.41$, $LSD = 6.152$, d.f. = 15, $P < 0.01$) and yield ($F = 72.88$, $LSD = 3.54$, d.f. = 15, $P < 0.01$) were examined statistically, three different groups were formed. The highest yield was found on TE + BT plots (37.617 kg/tree), followed by BT (33.637 kg/tree), and TE (31.875 kg/tree) plots, respectively, as shown in Table 3. A negative significant relationship ($r = 0.970$) was found between the number of damaged fruits and yield, suggesting that the number of damaged fruits has a significant impact on yield. Fruit falling before the harvest was also examined. The infestation rate was 2% on plots where only one agent was applied and 1% on the plot where both agents were applied, compared to 4% on the control plot. Intervention is considered successful if total damage is less than 2% (Andermatt et al., 1988; TAGEM, 2008). In the study, this rate of acceptable economic loss threshold was achieved on the plot where both agents were applied. The damage level of 1%–3% is equivalent to that reported from other studies (Hassan et al., 1988; Hassan, 1993). Since the Galaxy Gala used in the experiment is an early variant and the harvesting was done in the second half of August, only the damage caused by the first offspring was significant. It has been reported that there is a significant difference between early-maturation and late-maturation varieties in terms of the level of damage; the rate of damaged fruits for the early varieties was 38%, reportedly rising as high as 77% for late varieties (İşçi, 2008). Therefore, in the case of early apple varieties, it is advisable to increase the releases against the first generation of pests if necessary. Depending on the length of the oviposition period of the pest, a third release could be appropriate. The same strategy should be followed for the late apple varieties against the second generation of pests.

In conclusion, this is the first such study in Turkey presenting the case that biological control methods that have no negative effects on the environment and human health are effective alternatives to chemical control (Lacey and Unruh, 2005; Holmes et al., 2016). Promising results were achieved in the biological control of *C. pomonella* when *Trichogramma* releases were combined with *B. thuringiensis* var. *kurstaki*. There is a need for further studies to determine and establish optimal release strategies with different pest densities and apple varieties. To control the pest effectively it is necessary to combine all available control measures within integrated pest management strategies in apple orchards in Turkey.

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