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Evaluation of Entomopathogenic Bacteria Against *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae)*

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Abstract

Pseudaulacaspis pentagona (Targioni-Tozzetti) (Hemiptera: Diaspidae) which has a wide host range, is an important pest causing losses in yield. The insecticides are used for control against this pest but insecticidal control is difficult as scales protect themselves very effectively with hard, waxy armor. Also, the negative effects of the chemicals used in the control against benefical insects and with the increasing awareness on environmental issues, alternative methods were sought. Therefore, this study was performed to develop an alternative and effective control method for this pest by using biocontrol bacteria *Bacillus pumilus* (TV-67C), *Brevibacillus brevis* (CP-1) and *Bacillus megaterium* (TV-91C) under

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controlled conditions. The death adult number of the pest was recorded and mortality rate was calculated. All of the tested bacterial strains showed mortality rates from 41.68% to 89.04% against the white peach scale under controlled conditions. Consequently, our results indicated that especially *B. pumilus* strain TV-67C and *B. brevis* strain CP-1 can be used as biocontrol agents of *P. pentagona*.

Keywords: *Pseudaulacaspis pentagona*, White peach scale, Microbial control, Bacteria

1. Introduction

Pseudaulacaspis pentagona (Targioni-Tozzetti) (Hemiptera: Diaspidae) (the white peach scale) is one of the most damaging armoured scales. This pest is believed to have originated in China or Japan, although it was first reported in Italy in 1886 by Targioni (Brancsome, 2016). It is distributed throughout the World. *P. pentagona* threatened the silk industry in Italy because of killing mulberry trees and damaged the peach industry (Gossard, 1902; Van Duyn & Murphey, 1971) in the United States in the early 1900s (Gossard, 1902), too (Hanks & Denno, 1993). *P. pentagona* destroyed numerous peach orchards in Florida and south Georgia in the early part of this century (Brancsome, 2016). It is polyphagous species that infests mulberry, beside of various kinds of deciduous fruit trees, ornamental and wild plants (Ben-Doy *et al*., 2015). *P. pentagona* attacks twigs and branches of peach trees. It absorbs the sap and causes the leaves to deteriorate and the branches (Moussa *et al*., 2010).

The control of white peach scale is very hard because they cover themselves very effectively by their hard, waxy armor. Traditional control methods include insecticides as well as several pesticides. In general, control methods are applied to nymphs which is the most vulnerable periods of the pests (Brancsome, 2016), since adults are less affected from pesticide application due to their hard outer skeleton.

There are lots of studies in the world where natural enemies are used as biological control for this pest. *Encarsia berlesei*, *Chifocorus bipusrufatus* and *Cybocephalus fodori minor* are some important natural enemies and they have been effectively used in the control against this pest (Erkılıç & Uygun, 1995; Follett *et al*., 2015). However, efficiency of natural enemies is reduced in last few years since producers used broad spectrum insecticides which have negative effects on natural enemies and environment. The increasing adverse effects of pesticides on the environment and humans reveal that biological control has high importance (Carruthers & Hural, 1990; Inglis *et al*., 2001). That’s why, for the management of pests it is desired to develop long-lasting and effective biological control methods that are not harmful to the environment. The use of microbial agents is one of the methods for this pest control. Many entomopathogens such as *Bacillus* can be mass produced, formulated, and applied to pest populations in a manner analogous to chemical pesticides, i.e. as nonpersistent remedial treatments that are released inundatively (Bhattarai *et al*., 2016).

The present study was planned and conducted with the aim to determine the insecticidal effect of a totally three bacterial strains against *P. pentagona* which lead to economical losses on controlled conditions. According to the carried literature search, this research is the first research in literature where bacteria are used in the microbial control of *P. pentagona*.

2. Material and Methods

2.1 Host Plant, Harmful Insect and Bacterial Strains

Naturally infested (included *P. pentagona*) mulberry twigs (*Morus alba*) observed in Erzurum, Turkey (Figure 1a). Mulberry and *P. pentagona* were used as host plant and harmful insect in this study, respectively (Figure 1b).

Figure 1. Land view of trees damaged with *Pseudaulacaspis pentagona* (a), naturally infested mulberry twigs with *Pseudaulacaspis pentagona* (b)

Bacterial strains CP-1, TV-67C and TV-91C were isolated from *Ricania simulans*, *Rubus idaeus* and *Graminea* sp., respectively in previous studies (Table 1) (Göktürk *et al*., 2018; Erman *et al*., 2010) and defined in the MIS based on fatty acid methyl esters. Stock cultures of the bacterial strains were kept in Atatürk University Faculty of Agriculture Plant Clinical Laboratory (AUFAPCL).

Table 1. Identification and similarity indexes (SIM) and hypersensitivity (HR) of biocontrol bacterial strains used in the study

Strain No	Isolated from	MIS Identification results	SIM	HR	References
CP-1	*Ricania simulans*	*Brevibacillus brevis*	0.650	-	Erman *et al*., 2010
TV-67C	*Rubus idaeus*	*Bacillus pumilus*	0.630	-	Göktürk *et al*., 2018
TV-91C	*Graminea* sp.	*Bacillus megaterium*	0.474	-	Göktürk *et al*., 2018

SIM: Similarity, -: Negative effect; HR: Hypersensitivity

2.2 Preparation of Bacterial Suspensions

The bacterial suspensions were prepared according to Tozlu *et al.* (2019) and the bacteria density was adjusted at ($1x10^8$ CFU/ml) by a spectrophotometry, sing nutrient broth medium (NB) and transferred to sterile spray bottles.

2.3 Determination of the Bacterial Strains Insecticidal Effect Under Controlled Conditions

The effectiveness of the bacterial strains against *P. pentagona* was tested using the mulberry twigs under controlled condition. Naturally infested with *P. pentagona* the mulberry twigs were brought to AUFAPCL. Twigs were divided into groups each included average of 20 adults of *P. pentagona* and placed in polyethylene lined plastic boxes (19x12.5x7 cm). About 180 adults of *P. pentagona* were sprayed by the three of bacterial suspensions (10^8 cfu/ml) (60 adults/each bacterial suspention) and 60 adults of *P. pentagona* were sprayed by NB for control treatment. All plastic boxes were incubated at 25±2 ^{0}C, in 65-70% RH and under a photoperiod regime of 16:8 (light: darkness). The final evaluation of the trial was carried out and the mortality rates were determined during 18 days. The number of dead insects and mortality rates (%) of *P. pentagona* were recorded once every six days. Mortality rate was calculated with the following formula;

$$\text{Mortality rate (\%)} = \frac{100 \text{ x the number of dead adults in treatment}}{\text{Total adult in treatment}}$$

Re-isolation from the adult insects that were determined to be infected according to the Koch Postulates was performed and the entomopathogenic bacterial strains were recovered. Experiment was carried out in 4 repetitions for each combination.

2.4 Analysis of Results

The data of study were analyzed using the JMP 5.0 program and apllications differences were determined according to ANOVA results and “LSMeans Differences Student's” multiple comparison test.

3. Results

The effects of *B. brevis, B. pumilus* and *B. megaterium* strains on *P. pentagona* adults were tested. The results showed that all the bacteria have insecticidal activity in controlled coditions. The adults started to die in all applications except the control on the sixth day (Figure 2). The dead adult number according to days during the 18 days follow-up period are given in Figure 2. The percentages of dead adults were 74.94% (TV-67C), 72.27% (CP-1) and 27.29 (TV-91C) during 6 days, 92.17% (TV-67C), 82.17% (CP-1) and 42.5% (TV-91C) during 12 days and 100% (TV-67C), 95.24% (CP-1) and 55.25% (TV-91C) during 18 days (Figure 2).

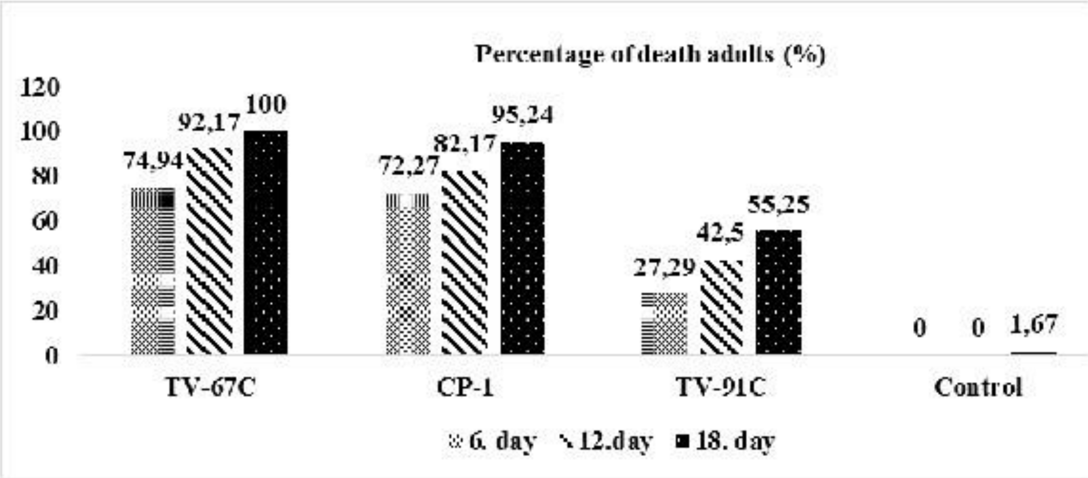

Figure 2. *Pseudaulacaspis pentagona* dead adults number according to days during the 18 days follow-up period

The insecticidal activities of bacterial strains tested against *P. pentagona* adults on mulberry twigs are given in Table 2. According to adults percentage mortality, the difference between applications was found to be statistically significant (F: 123.1; $p<0.01$). Among the tested three bacteria, *B. pumilus* and *B. brevis* were the most effective strains causing 89.04% and 83.23% (insignificantly different) mortality rate in the adult of *P. pentagona*, respectively. *B. megaterium* strain showed 41.68% (which was significantly different from the other two bacteria) mortality rate (Table 2).

Table 2. The insecticidal effect of three different bacterial suspensions against adults of *Pseudaulacaspis pentagona* under controlled condition

Applications	**Mean death adult ratio (%) ± SD**
TV-67C (*B. pumilus*)	89.04±12.82 A
CP-1 (*B. brevis*)	83.23 ±11.52 A
TV-91C (*B. megaterium*)	41.68 ±13.998 B
Control	0.56±0.96 C
CV	**13.90**
LSD	**7.21**

Mean values Mean ± Standard deviation: in the same column by the same letter are not significantly different to the test of LS Means Differences Student's ($p<0.01$)

The effects of the most effective bacterial strains *B. pumilus* TV-67C strain and control treatment against *P. pentagona* adults were given in Figure 3. TV-67C bacterial strain melted the *P. pentagona* adults armour within 6 days.

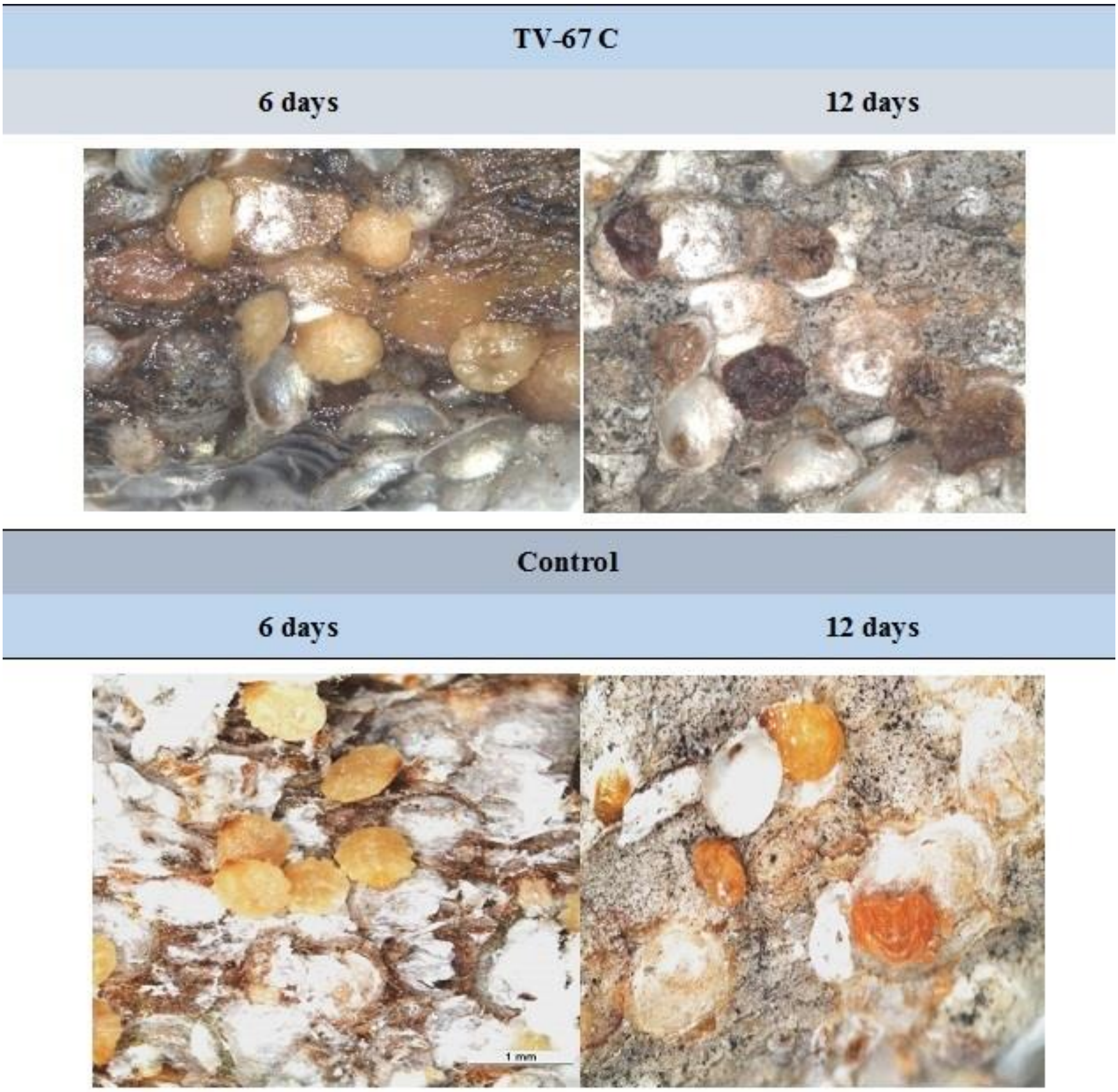

Figure 3. The most effective bacterial strains TV-67C (*Bacillus pumilus*) and control treatment

4. Discussion

Entomopathogens are one of the most effective factors regulating pest populations. Throughout the world, many biopesticides are widely used in greenhouse products, ornamental plants, stored products, forest products, vegetable and fruit garden products as biological control of pests (Lacey *et al*., 2001). *Bacillus* species are the most important species for microbial control. It is known that *Bacillus* species particularly *Bacillus thuringiensis, B. brevis, B. cereus, B. circulans, B. megaterium* and *B. subtilis* are used for biotechnological and industrial applications (Xu & Côté, 2003; Rooney *et al*., 2009). *B. pumilus* which has a high effect against *P. pentagona* in this study is found everywhere. It is stated in previoulsly studies that some of the *B. pumilus* isolates result fungicidal activity (Bottone & Peluso, 2000; Lehman *et al*., 2001), some result strong antibacterial activity (Aunpad & Na-Bangchang, 2007), some promotes plant growth, (Raunpach & Kloepper, 1998; Yan *et al*., 2002; Joo *et al*., 2005; Forchetti *et al*., 2007), also some others are used as probiotics (Green *et al*., 1999; Caldini *et al*., 2002; Duc Le *et al*., 2004). *B. pumilus* was first identified by Heins as insect pathogen against *Diabrotica undecimpunctata* and *Spodoptera exigua*. In one study was reported that *B. pumilus* was highly toxic to the larvae of *Ceratitis capitata*. It was also pointed out that it is important to develop biotechnological study strategies to reduce economic losses caused by *C. capitata* (Molina *et al*., 2010). Rishad *et al*. (2017) reported that *B. pumilus* exhibited high chitinase activity and the enzyme also exhibited biopestisidal role against larvae of *Scirpophaga incertulas*, a serious agricultural pest of rice, too. Another bacterial species *B. brevis* produced different antibiotics such as gramicidin S (Edwads & Seddon, 2001), toadadine (Song *et al*., 2012), ethylparaben (Che *et al*., 2015) and enzyme such as chitinase (Ahmad & Omar, 2014; Minghui *et al*., 2015). Therefore, it has antimicrobial effects against soil-borne pathogens such as *Fusarium* (Ahmad & Omar, 2014) and and insecticidal effects against pests such as *Bruchus dentipes* (Tozlu *et al*., 2011; Blibech *et al*., 2012), *Spodoptera littoralis* (Sakr, 2017) and *Halyomorpha halys* (Tozlu *et al*., 2019).

B. megaterium, which is 41.68% effective in this study, is the centre of many studies showing that it is a potential biocontrol agent against plant diseases (Tozlu *et al*., 2019; Trivedi & Pandey, 2008; Matar *et al*., 2009; Velmurugan *et al*., 2009). In addition, *B. megaterium* tested against insecticidal activity against *Agrotis segetum* larvae was found to be effective in 30% in another study (Sevim *et al*., 2010). This species is obtained from the stomach of the pest *Limonius canus* LeConte (Coleoptera: Elateridae) an important potato pest (Lacey *et al*., 2007). Furthermore, this species can produce amylase hydrolytic enzyme responsible for carbohydrate digestion and this makes species to have more importance (Parasanna *et al*., 2014).

As far as we know, this is the first study related to the microbial control of *P. pentagona*. 3 bacterial isolates were tested against *P. pentagona* under controlled conditions and all of the bacterial isolates tested effected in varying percentages.

5. Conclusion

It needs to be considered that use of intensive chemicals for combating this pest results adverse effects on natural enemies, predators and parasitoids, of this pest. That’s why, it is very important to include effective and resistant biopesticides which has no toxic effects on neither environment nor human health in control system against *P. pentagona* in places where natural enemies are absent or less active. This study emphasizes that a risk-free microbial agents can be successfully applied to *P. pentagona* instead of chemical pesticides in integrated pest management studies.

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