INSECTICIDAL EFFECT OF SOME BACTERIA ON CHERRY SLUGWORM (*CALIROA CERASI* (LINNAEUS, 1758) (HYMENOPTERA: TENTHREDINIDAE)

Fatih Dadasoglu[1,*], Onder Calmasur[2], Kenan Karagoz[1,3] and Recep Kotan[2]

[1] Agri Ibrahim Cecen University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, 04100 Ağrı, Turkey
[2] Ataturk University, Faculty of Agriculture, Department of Plant Protection, 25240 Erzurum, Turkey
[3] Agri Ibrahim Cecen University, Central Research and Application Laboratory, 04100 Ağrı, Turkey

ABSTRACT

In this study, 5 bacterial strains (*Bacillus cereus*, *Bacillus atrophaeus*, *Bacillus globisporus*, *Brevibacillus brevis,* and *Alcaligenes denitrificans* subsp. *xylosoxydans*), isolated from different insect species in the Eastern Anatolia region of Turkey, were identified on the basis of fatty acid methyl ester analysis and carbon source utilization profiles by using Microbial Identification System (MIS) and/or MicroPlate Systems (BIOLOG). Their insecticidal effects were tested on adult *Caliroa cerasi* on Petri dishes, *in vitro*. Seven days after application, all of the tested strains showed more or less insecticidal activity against this pest. The highest activities were obtained from *Brevibacillus brevis* strain FD-1 and *Bacillus cereus* strain FD-63 causing 86.7 and 100% mortality, respectively. These values were not statistically different from that of the positive control (Kingbo). The mortality rates of the other bacterial applications were lower than that of the positive control, but not that of the negative control. In conclusion, *Bacillus cereus* strain FD-63 and *Brevibacillus brevis* strain FD-1 are good candidates for use as biological control agents against this economically important pest.

KEYWORDS: Bacillus cereus, Brevibacillus brevis, biocontrol, biopesticide, Caliroa cerasi

1. INTRODUCTION

Cherry and sour cherry production of Turkey occupies an important place in worldwide production, and is still of considerable importance in terms of providing further fruit species and gene resources. In world production of sweet cherry (1,569,674,000 t), the highest share (22%) is held by Turkey (338,361,000 t), followed by USA (14%; 225,073,000 t) and others [1]. Unfortunately, there are many pest species causing significant economically losses for cherry and sour cherry cultivated in large areas both in our country and all over the world. Sawfly, one of the most important of these species, is widely distributed in Europe, Russia, Balkans, Turkey, Africa, Australia, United States and New Zealand. Literature records indicate that this pest causes severe economical losses [2-11].

Particularly, pear slugs, the larvae of the sawfly *Caliroa cerasi* (L.) (Hymenoptera: Tenthredinidae) representing a cosmopolitan pest, strip the surface tissue from leaves of *Pyrus* and *Prunus* species. Pear slugs feed on the upper surface of leaves, and typically avoid feeding on lower leaf surfaces. When feeding, pear slugs eat the tissues between leaf veins, but keep the veins themselves intact. This feeding pattern is known as skeletonization, gives leaves a lacy appearance, and if feeding is very extensive, the leaves may turn brown and shrivel [12, 13].

There are many insecticides for control of *Caliroa cerasi.* However, the problems of insecticide resistance and environmental/consumer health hazards associated with insecticide residues in plant material, have focused attention on alternative methods for controlling pests. The development of biocontrol agents may help to decrease the negative effects (i.e., residues, resistance and environmental pollution) of chemical pesticides that are commonly and extensively used for plant disease management in agriculture. There are many studies related to the biocontrol of pests using bacterial biological agents [14, 15], but there have been few studies on *Caliroa cerasi*.

The aim of this study is to investigate the insecticidal effect of 5 bacterial strains (for details see Summary), isolated from different insect species in the Eastern Anatolia region of Turkey, on *Caliroa cerasi.*

2. MATERIALS AND METHODS

2.1. Isolation and cultivation of the bacterial strains

Bacterial strains were tested for their insecticidal activities against *Caliroa cerasi* on Petri plates, *in vitro*. They were isolated from larvae of *Yponomeutida evonymella* and *Malacosoma neustria* collected from the East-

* Corresponding author

ern Anatolia region of Turkey. The bacterial cultures were grown on nutrient agar (NA) for routine use, and maintained in Luria Broth (LB) with 15% glycerol at -80 °C for long-term storage [16], at the Department of Plant Protection, Faculty of Agriculture, Atatürk University.

2.2. Identification of the bacterial strains by microbial identification system (MIS)

Identification of the tested bacterial strains was confirmed by using MIS systems. Preparation and analysis of FAMEs from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA). FAMEs were separated by gas chromatography (HP-6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0.2 mm x 0,25 µm with cross-linked 5% phenyl methyl silicone). FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package [17].

2.3. Identification of the bacterial strains by Biolog microplate system (BIOLOG)

Identification of the tested bacterial strains was confirmed by using BIOLOG systems. One or two days before the inoculation of Biolog GN2 and GP2 plates, bacterial strains were streaked on TSA or BUG agar plates. Each well of Biolog GN2 or GP2 micro-titer plates was inoculated with 125 µl of the Gram-negative or positive bacterial suspension, respectively, adjusted to the appropriate density (10^8 cfu/ml) and incubated at 27 °C for 24 and 48 h. The development of color was automatically recorded using a microplate reader with a 590-nm wavelength filter. Identification (Biolog Microlog 34.20 database) and ASCII file output of test results, applying the automatic threshold option, were performed using BIOLOG420/ Databases/ GN601 and GP601 KID software [18]. Carbon source utilization rates of the strains were estimated as percentages.

2.4. Hypersensitivity tests (HR)

All of the bacterial strains were tested for hypersensitivity on tobacco plants (*Nicotina tabacum* L. var. Samsun). The bacterial suspension (10^8 cfu/ml) prepared in sterile distilled water and infiltrated into the inter-costal area of the leaves of tobacco plants by using a 3-cc syringe (Becton Dickinson, Franklin Lakes, NJ, USA). The inoculated plants were incubated in a completely randomized design on the greenhouse bench for 24–48 h at 20–28 °C. The presence of rapid tissue necrosis at the inoculation site was recorded within 24–48 h after infiltration. This test was repeated, at least three times, for each strain. For HR tests, sterilized distilled water (sdH_2O) was used as a negative control [16].

2.5. Bioassay

Tryptic Soy Agar (TSA, Oxoid) and Tryptic Soy Broth (TSB, Oxoid) plates were used in the experiments. All bacterial isolates were incubated in TSA at 27 °C for 24 h. After incubation period, a single colony was transferred to 500-ml flasks containing TSB, and grown aerobically in the flasks on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Germany). The bacterial suspension was then diluted in sterile distilled water (sdH_2O) to a final concentration of 10^8 cfu/ml with a turbidimeter. The leaves of sour cherry trees were added to each of the Petri plates to feed the insects. The prepared solutions were transferred to sterile glass injection containers, and the suspensions were sprayed onto Petri plates. Ten insects, collected from naturally affected leaves, were released in each Petri plate. The plates were sealed with parafilm and transferred to desiccators containing 40 ml sdH_2O. When plates were removed from the desiccators, the mortality was recorded after 1, 4 and 7 days. The assay was designed in randomized complete blocks with three replications; 1% kingbo and sterile media TSB were used as the positive and negative control, respectively.

2.6. Data analysis

In order to determine significant differences in toxicity among the insecticidal activities, analysis of variance (ANOVA) was carried out using the SPSS 18.0 statistical software package. The results showed significant differences at the $P<0.01$ level.

3. RESULTS

The MIS and BIOLOG identification results of the tested bacterial strains and their hypersensitivity test results on tobacco plants are shown in Table 1. According to the MIS/BIOLOG results, bacterial strains isolated from larvae of *Yponomeutida evonymella* were identified as *Alcaligenes denitrificans* subsp. *xylosoxydans*/ *Alcaligenes faecalis* (strain FD-61), *Bacillus globisporus*/ *Bacillus amyloliquefaciens* (strain FD-64), *Bacillus cereus* GC subgroup B/*Bacillus amyloliquefaciens* (strain FD-63) and *Bacillus atrophaeus*/ *Bacillus licheniformis* (strain FD-17); from larvae of *Malacosoma neustria* they were identified as *Brevibacillus brevis*/ *Bacillus subtilis* (strain FD-1). Hypersensitivity test results of all strains were negative on tobacco leaves. Carbon source utilization rates of the tested bacterial strains changed from 17.89 to 33.68% (Table 1). Average mortality number of *Caliroa cerasi* at 1, 4 and 7 days, and the insecticidal effects of the tested bacterial strains on *Caliroa cerasi* are summarized in Fig. 1 and Table 2, respectively. The results show that these strains are toxic and have a significant insecticidal effect on *Caliroa cerasi*. According to the results of ANOVA, the insecticidal effect was significant ($P< 0.01$) (Table 2). On the first day, a high mortality rate (40%) was observed from positive control (1% Kingbo). Low mortality rates (0.0%) were observed with negative control (TSB), *Brevibacillus brevis* strain FD-1, *Bacillus atrophaeus* strain FD-17, *Alcaligenes denitrificans* subsp. *xylosoxydans* strain FD-61, *Bacillus cereus* GC subgroup

TABLE 1 - MIS and BIOLOG identification results of bacterial strains, their similarity index (SI), hypersensitivity test (HR) results and carbon source utilization rates (CSU, %)

Strain no	MIS results	SI	BIOLOG results	SI	HR	CSU	Isolated from
FD-1	*Brevibacillus brevis*	62	*Bacillus subtilis*	34	-	17.89	Larvae of *Malacosoma neustria*
FD-17	*Bacillus atrophaeus*	45	*Bacillus licheniformis*	34	-	26.31	
FD-61	*Alcaligenes denitrificans* subsp. *xylosoxydans*	55	*Alcaligenes faecalis*	74	-	33.68	Larvae of *Yponomeutida evonymella*
FD-63	*Bacillus cereus* GC subgroup B	24	*Bacillus amyloliquefaciens*	17	-	25.26	
FD-64	*Bacillus globisporus*	34	*Bacillus amyloliquefaciens*	30	-	25.26	

-: Hypersensitivity test result was negative on tobacco plant.

TABLE 2 - The mortality rate of *Caliroa cerasi with* bacterial application measured after 1, 4 and 7 days under laboratory conditions.

Applications of bacteria	Mortality rate					
	First day		Fourth day		Seventh day	
FD-1 *Brevibacillus brevis*	0.0	a	46.7	b	86.7	c*
FD-17 *Bacillus atrophaeus*	0.0	a	33.3	ab	33.3	b
FD-61 *Alcaligenes xylosoxydans* ssp. *xylosoxydans*	0.0	a	40.0	b	43.3	b
FD-63 *Bacillus cereus* GC subgroup B	0.0	a	53.3	b	100.0	c
FD-64 *Bacillus globisporus*	0.0	a	33.3	ab	36.7	b
Control (+) Kingbo %1	40.0	b	100.0	c	100.0	c
Control (-) TSB	0.0	a	0.0	a	0.0	a

* Values followed by different letters are significantly different (P<0.01).

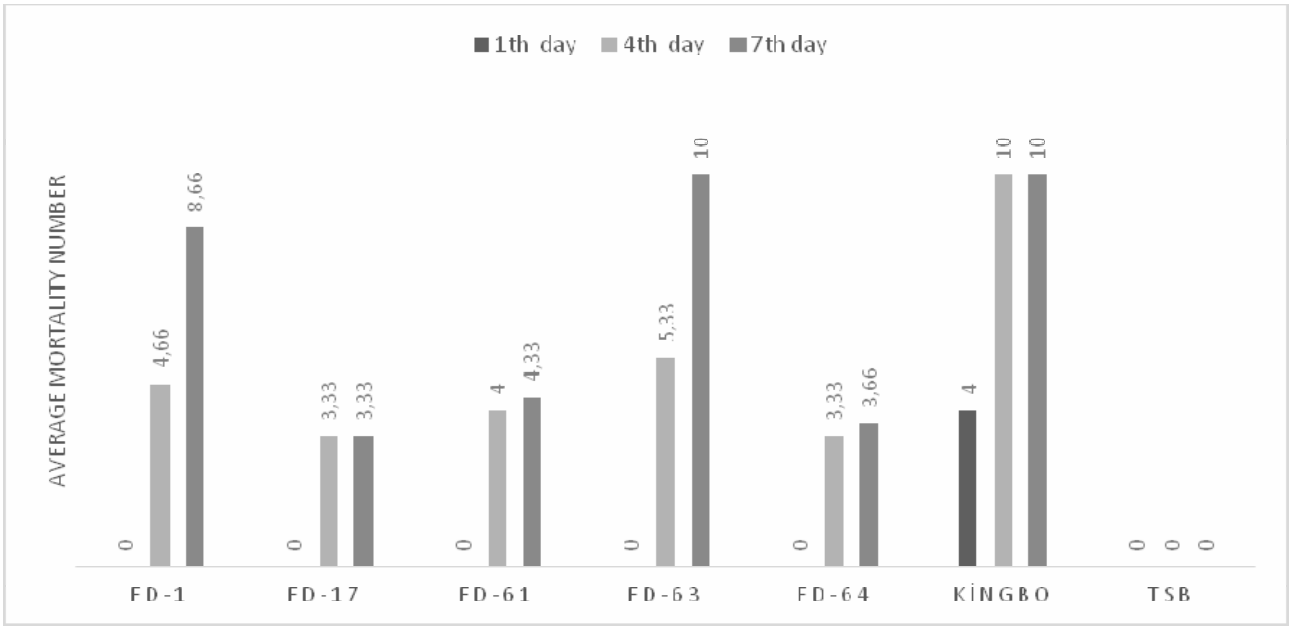

FIGURE 1 - Average mortalitiy number of *Caliroa cerasi* at 1, 4 and 7 days.

B strain FD-63, and *Bacillus globisporus* strain FD-64. On the 4th day, the highest and lowest mortality rates were observed from positive control (1% Kingbo) and negative control, respectively. All of the bacterial applications caused mortality but the mortality rates of *Bacillus atrophaeus* strain FD-17 and *Bacillus globisporus* strain FD-64 were not different from the negative control. The mortality rates of *Brevibacillus brevis* strain FD-1, *Alcaligenes denitrificans* subsp. *xylosoxydans* strain FD-61 and *Bacillus cereus* GC subgroup B strain FD-63 were changed from 40 to 53.3% on the fourth day. Their insecticidal activities were different from the negative control. The 4th day mortality rate of *Bacillus cereus* GC subgroup B strain FD-63 in the bacterial applications was found to be the highest. But, its activity was lower than that of positive control (1% Kingbo). On the 7th day, all of the bacterial applications showed insecticidal activity in a range of 36.7 to 100%. The 7th day mortality rates of *Brevibacillus brevis* strain FD-1 and *Bacillus cereus* GC subgroup B strain FD-63 were statistically indifferent

from that of positive control (1% Kingbo). *Bacillus atrophaeus* strain FD-17, *Alcaligenes denitrificans* subsp. *xylosoxydans* strain FD-61 and *Bacillus globisporus* strain FD-64 caused insecticidal activities on *Caliroa cerasi* but their mortalities were not statistically different from the negative control.

The highest mortality rates were observed from *Brevibacillus brevis* strain FD-1 and *Bacillus cereus* GC subgroup B strain FD-63. The insecticidal activities of the rest of bacterial applications were significantly different from both negative and positive control (1% Kingbo)

4. DISCUSSION AND CONCLUSION

In this study, 5 bacterial strains were identified as *Bacillus*, *Brevibacillus* and *Alcaligenes* species on the basis of FAME analysis and carbon source utilization profiles by using MIS and BIOLOG. Their insecticidal effects were tested on adult individuals of *Caliroa cerasi.* When the MIS results of the tested bacterial strains were compared to the BIOLOG identification results, MIS results of all strains, except strain FD-1, were confirmed by BIOLOG at the genus level but not species level. The results show that MIS and BIOLOG alone are accurately enough to serve as a primary method for identifying entomopathogenic *Bacillus* species, at least in genus level. The 5 bacterial species identified are known as insect pathogenic bacteria, and are eligible for the possible use to control insect pests. The development of natural or biological insecticides will help to reduce the negative effects, such as residue formation, resistance development and environmental pollution, of synthetic insecticides.

The best-known example of entomopathogenic bacteria is *Bacillus* genus [19]. The *Bacillus*-based biological control agents (BCAs) are among the best-known, most widely developed and safe alternatives to chemical pesticides for control of insect pests [20]. Insecticides are relatively easy to use and have generally provided effective pest control; it is likely that they will always be a component of pest management programs.

Unfortunately, insecticides have some undesirable attributes. They usually present some degree of hazard to the applicator and other people who may come in contact with them; they can leave residues that some find unacceptable; they can contaminate soil and water and affect wildlife, aquatic life, and other non-target organisms; they can interfere with beneficial organisms, such as pollinating insects and natural enemies of pests; and insects can develop resistance to insecticides, effectively eliminating those materials as pest management options [21-23]. Therefore, considering the deleterious effects of insecticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of pests. The use of microorganisms as biocontrol agents is one of the first choices for pest control [23]. Another important alternative measure to chemical insecticides is biological control measure which involves the regulation of pest population using natural control agents, such as predators and nematodes. But the safety offered by microbial insecticides is their greatest strength.

In this study, we observed that *Bacillus cereus* GC subgroup B strain FD-63 and *Brevibacillus brevis* strain FD-1 were the most effective strains. For the 7th day, the mortality rates of *Bacillus cereus* GC subgroup B strain FD-63 and *Brevibacillus brevis* strain FD-1were recorded to be 100.0 and 86.7%, respectively. This was the highest effectiveness in the bacterial applications and statistically indifferent from that of the positive control (1% Kingbo). On the7th day, the mortality rates in *Bacillus atrophaeus* strain FD-17, *Bacillus globisporus* strain FD-64 and *Alcaligenes xylosoxydans* ssp. *xylosoxydans* strain FD-61 applications were observed to be 33.3, 36.7 and 43.3%, respectively. These mortality rates were different from the negative and the positive control, being better than negative but worse than positive control. On the first day, bacterial strains were not effective. But, the mortality rates of strains on the fourth and seventh day were recorded from 33.3 to 100%. Generally, the insecticidal activities of tested bacterial strains on the seventh day were higher than on first and fourth day. We think that some strains need time to adapt to the environmental conditions, and 24 or 48 h probably were not enough to produce spores for the *Bacillus* species. The results of experimental infections are very promising but all results were provided under laboratory conditions. Some treatments of these isolates should be carried out under storage conditions.

Carbon source utilization rates of the tested bacterial strains changed from 17.89 to 33.68%. This is very important for potential entomopatogenic bacteria to adapt easily to the environmental conditions. Our results showed that *Bacillus cereus* GC subgroup B strain FD-63 and *Brevibacillus brevis* strain FD-1 have insecticidal activity against *Caliroa cerasi*.

In conclusion, it is considered that these strains can be used against *Caliroa cerasi* as potential biocontrol agents. This study matters to highlight the successful usage of an environment-friendly, natural, and for health of humans and other livings, risk-free product against *Caliroa cerasi* in substitution for the chemical pesticides that are intensely used and harmful for environment, natural balance and human health.

The authors have declared no conflict of interest

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CORRESPONDING AUTHOR

Fatih Dadasoglu
Department of Molecular Biology and Genetics
Faculty of Science and Letters
Agri İbrahim Cecen University
Agri 04100
TURKEY

Phone: +90 472 2154073
Fax: +90 472 215 6554
E-mail: f-dadas@hotmail.com