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Evaluation of the Effectiveness of Entomopathogens for the Control of Colorado Potato Beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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ABSTRACT

Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) damages potato, tomato, and eggplant, and is one of the most serious agricultural pests all over the world. Due to its resistance against chemical insecticides and some biopesticides, new biocontrol agents compatible with different ecological conditions are needed urgently for the integrated pest management programs of this pest. For this purpose, we studied the insecticidal effects of thirteen indigenous microbial isolates including entomopathogenic bacteria, fungi, and nematodes from our culture collection against *L. decemlineata* with screening and dose-response tests under laboratory conditions. *Bacillus thuringiensis* strain Xd3 caused 83% and 73% mortality against larvae and adults of the pest at 10^9 CFU mL^{-1} concentration within 10 days, respectively. While fungal isolate *Metarhizium anisopliae* Gg-12 yielded 98% mortality with 10^7 conidia mL^{-1} concentration on larvae at 15 days, mortality provided by Gg-12 on adults reached 100% at the same concentration and period. *Steinernema websteri* AS1 was determined as the most effective entomopathogenic nematode with 92% mortality within seven days on larvae. Based on probit analysis, the LC_{50} values of *B. thuringiensis* Xd3 against larvae and adults were calculated as, respectively, 1.73×10^6 and 1.69×10^7 CFU mL^{-1}, and that of *M. anisopliae* Gg12 were 1.18×10^4 and 6.2×10^3 conidia mL^{-1}, and that of *S. websteri* AS1 was 117 IJs mL^{-1}. Considering these results, the biopesticides developed from these isolates can be used safely and successfully in the pest management control programs of Colorado potato beetle.

Keywords: Microbial control, Bacillus thuringiensis, Metarhizium anisopliae, Steinernema websteri, Insecticidal activity

INTRODUCTION

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is the most important insect defoliator of potatoes (*Solanum tuberosum* L.) all over the world. Currently, it is a well-known pest in both commercial fields and home gardens. Its host range encompasses all members of the Solanaceous family, such as potato, tomato, pepper, eggplant, and weeds such all nightshades and buffalo bur. It can be a pesky pest defoliating whole potato fields in many parts of the world. Approximately 40 cm^2 leaves of potato can be devoured by both adults and larvae without discriminating among leaf tissues.

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Defoliation threshold levels are reported as 20% before tuber bulking, 10% during the first half of bulking, followed by 25% after bulking. Vine damage results in yield loss due to loss of foliage to support tuber growth, and misshaping of tubers is also possible. Severe damage may result in plant stunting as well. In addition to impressive feeding rates, the CPB is also characterized by high prolificacy, with one female laying 300–800 eggs (Harcourt, 1971). If not struggled, the beetles can cause up to 80-100% crop loss.

The use of chemicals for controlling the CPB began in 1864 (Gauthier *et al*., 1981) and still continues. Unfortunately, the intensive use of insecticides has resulted in the development of insecticide resistance to 52 different compounds including arsenicals, organochlorines, organophosphates, carbamates, and pyrethroids (Alyokhin *et al*., 2008). However, there are few old reports about the resistance of CPB to *B. thuringiensis* (Whalon *et al*., 1993; Alyokhin and Ferro, 1999). However, it does not mean that it will develop resistance to all *Bacillus thuringiensis* isolates because genotypic properties of the strains are different. Therefore, there is an increase in demand for alternative control methods including the use of microbial pesticides containing bacteria, fungi, and nematode (Anderson *et al*., 1989; Armer *et al*., 2004; Duan *et al*., 2004; Trdan *et al*., 2009). Up to now, many entomopathogenic microorganisms have been isolated from various habitats such as soil, air, plants, and insects and subsequently developed and used as microbial control agents (Unruh and Lacey, 2001; Batta, 2003; Secil *et al*., 2012; Shapiro-Ilan *et al*., 2012; Çakıcı *et al*., 2014; Mascarin and Jaronski, 2016; Sönmez *et al*., 2016; Eski *et al*., 2017; Tripathi and Gujar, 2017). Microbial pesticides based on *Bacillus thuringiensis* (*Bt*) have been used worldwide (Amalraj *et al*., 2000; Teera-Arunsiri *et al*., 2003; Eski *et al*., 2019; Devi *et al*., 2020). Delta-endotoxins produced in the sporulation phase of *Bt* have toxic effect on various insects. Especially, *Bt* var. *tenebrionis* have been used for management of coleopterans (Wraight and Ramos, 2005; Eski *et al*., 2017; Pérez *et al*., 2017). Fungi are the most commonly used microorganisms as biopesticides after bacteria. Although many entomopathogenic fungi have been identified, most of the commercially produced fungi are *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin that are relatively easy to mass-produce (Vega *et al*., 2009; Bruck, 2010). They have been extensively used for controlling many important pests including different lepidopteran, coleopteran, and dipteran species (Butt *et al*., 2013; Erler and Ates, 2015; Güven *et al*., 2015; Öztürk *et al*., 2015; Hussein *et al*., 2016; Ropek *et al*., 2019). However, another alternative strategy for microbial control of pests is utilization of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Guo *et al*., 2015; Patil *et al*., 2017). These nematodes are parasites of insects, killing them within a short time with the help of their associated symbiotic bacteria, and have a great potential as biological control agents of many insect pests (Laznik *et al*., 2009; Kepenekçi *et al*., 2016).

The aim of the present work was to assess the efficiency of indigenous entomopathogenic microorganisms isolated in Turkey against different stages of Colorado potato beetle in laboratory conditions.

MATERIALS AND METHODS

Origin of the Entomopathogenic Agents

Thirteen entomopathogenic agents that had very high lethal effects on some agricultural pests were isolated from some insect and soil samples in Turkey and used in the study. They were obtained from stock cultures of Microbiology Laboratory of the Department of Biology at Karadeniz Technical University, Trabzon, Turkey (Table 1).

Table 1. Entomopathogenic agents tested on CPB and their origin.

Agent	Species	Isolate	Origin	References
Bacteria	*Bacillus sphaericus*	Ar4	*Anoplus roboris*	Demir *et al.* 2002
	B. thuringiensis tenebrionis	Xd3	*Xyleborus dispar*	Sezen *et al.* 2008
	B. thuringiensis tenebrionis	Mm2	*Melolontha melolontha*	Sezen *et al*. 2007
	B. thuringiensis kurstaki	MnD	*Malacosoma neustria*	Kati *et al*. 2005
	B. thuringiensis kurstaki	BnBt	*Balaninus nucum*	Sezen and Demirbag 1999
Fungi	*Beauveria bassiana*	Mm-1	*Melolontha melolontha*	Unpublished data
	Beauveria pseudobassiana	Dm-5	*Dendroctonus micans*	Kocacevik *et al*. 2015
	Beauveria bassiana	Gg-1	*Gryllotalpa gryllotalpa*	Sönmez *et al*. 2016
	Myriodontum kerotinophylum	Gg-11	*Gryllotalpa gryllotalpa*	Sönmez *et al.* 2016
	Metarhizium anisopliae	Gg-12	*Gryllotalpa gryllotalpa*	Sönmez *et al.* 2016
Nematodes	*Steinernema feltiae*	ZET31	Soil	Erbaş *et al*. 2014
	Steinernema websteri	AS1	*Agrotis segetum*	Gökçe *et al*. 2015
	Heterorhabditis bacteriophora	ZET35	Soil	Erbaş *et al*. 2014

Insect Collection and Rearing Conditions

Laboratory culture of CPB was established from larvae and adults collected from potato plants in the fields without application of chemical or biological insecticides, near Trabzon. Rearing methods and conditions were similar to those used by Hare and Andreadis (1983) with some modification. Insects were reared on potato foliage in the disinfected plastic boxes (60×80 cm) for one generation in a growth chamber (25±2 ºC, 70±5% RH, 16:8 h light/dark photoperiod) before using them in the experiments.

Preparation of Entomopathogenic Bacteria and Bioassay

A loopful of bacteria from glycerol stocks was streaked onto nutrient agar plates, incubated at 30 °C overnight. Then, a single colony was transferred into nutrient broth medium (AppliChem, Germany) and incubated in a shaker at 120 rpm for 24 h at 30 °C. After incubation, bacterial density was determined by spectrophotometer at 600 nm and adjusted to 10^9 CFU mL^{-1} (Ben-Dov *et al*., 1995). One mL bacterial suspensions of each isolate was spread on the fresh potato leaves and air-dried. Then, thirty 3rd instar larvae and adults were placed in disinfected plastic boxes (25×50 cm) and fed with contaminated leaves, separately. Experiments were replicated three times. The control was made by feeding the 3rd instar larvae and adults on untreated potato leaves. Experiments were conducted at 25±2 °C and 70% RH on a 16:8 photoperiod for 15 days. Besides, dose-response experiments were performed using different concentration (from 10^9 to 10^6 CFU mL^{-1}) of the most effective isolate. Bioassays were also repeated three times on different durations.

Preparation of Entomopathogenic Fungi and Bioassay

Entomopathogenic fungi from stock culture were inoculated onto potato dextrose agar with 1% yeast extract and incubated at 25 °C for 10-15 days. Fungal spores were harvested and transferred to 10 mL of sterile 0.01% Tween 80. The conidial suspensions were filtered through two layers of sterile muslin into sterile plastic tubes and were vortexed for 2 minutes. The concentrations of conidial suspension were adjusted to 10^7 conidia mL^{-1} using a Neubauer hemocytometer and suspensions with higher germination rates than 97% were used for bioassay. Spore suspensions were applied to 3rd instar larvae and adults separately, by spraying with a mini hand sprayer. The control group was treated with sterile water with 0.01% Tween 80. Insects were fed with disinfected potato leaf that was replaced daily. Experiments were carried out with 10 insects per fungal isolate and repeated 3 times. Experiments were conducted at 25 °C under 16:8 h L:D photoperiod. Mortality was checked daily for 15 d and cadavers were surface sterilized with 1% sodium hypochlorite for 30 s, followed by 3 rinses with sterile distilled water. Then, they were placed on wet filter paper in sterile Petri dishes, sealed with parafilm and incubated at 25 °C to induce sporulation and mycosis on cadavers. The isolate causing the highest mortality was used in dose-response experiments. Different concentrations (from 10^8 to 10^5 conidia mL^{-1}) were prepared and bioassays were conducted as described above. All bioassays were repeated three times on different occasions.

Preparation of Entomopathogenic Nematodes and Bioassay

To assess the efficacy of nematodes on the late last instar larvae (one day before pre-pupa) and adults of CPB, plastic cups (4×4 cm) were filled with ten grams of sterilized-sandy soil whose moisture content was adjusted to 7% by adding distilled water. Then, nematode suspensions containing 1000 IJs mL^{-1} were inoculated in the cups. The treated cups were kept at room temperature for 1 hour and insects were placed on the sand surface. Control cups were prepared adding 1 mL of distilled water without nematodes. Experiments were performed with 30 larvae and adults per treatment. The experiments were repeated three times on different dates. The cups were incubated at 25 °C. Seven days after treatment, the sandy soil in each box was poured out and mortality was recorded. All dead larvae were placed individually onto White traps and the emergence of IJs from larvae was recorded (White, 1927). Dead insects were also dissected under the stereomicroscope to ascertain that mortality resulted from nematode infection. After the screening test, dose experiments (250, 500, 1000, and 2000 IJs mL^{-1}) were carried out using the most effective isolate. Bioassays were also repeated three times on different durations.

Data Analysis

Mortality rates were corrected for control mortality, using Abbott's formula (Abbott, 1925). The data were subjected to two-way ANOVA and subsequently means were separated using the least significant difference (LSD) test (p=0.05). In addition, median lethal concentration (LC_{50}) were determined by probit analysis (Finney, 1971). Survival curves were generated as a function of the observation time through the Kaplan-Meier method (Kaplan and Meier, 1958). All statistical analyses were performed using SPSS version 20 software (IBM Inc., Armonk, NY, USA).

RESULTS AND DISCUSSION

Some entomopathogenic agents isolated from various insects and soil samples in Turkey were tested to determine their

efficacy in the biological control of CPB. Among the isolates used, *B. thuringiensis tenebrionis* Xd3 (*Btt*-Xd3) was determined as the most effective bacterium against both larvae and adults with 83% and 73% mortality with 10^9 CFU mL^{-1}, respectively. Mortality of other bacteria to CPB larvae was found to range from 24-75%. However, mortality of the adults was lower than larval and ranged from 13-51% (Figure 1). While 73% of *Btt*-Xd3 treated larvae were killed, mortality of CPB feeding on the control potato leaf disks was 3% for the duration of the bioassay, indicating that all mortality resulted from the ingestion of the *Bt* spore and crystals. It is known that insect originated *Btt*-Xd3 produces cry3 endotoxin, which is efficacious against Coleopterans (Tatar, 2008). Dose-response experiments using *Btt*-Xd3 isolate showed that mortality rates increased with dose increases (Figure 2). However, we found that there was statistically no difference in the insecticidal activity of *Btt*-Xd3 between 10^8 and 10^9 CFU mL^{-1} concentration ($p>0.05$). We think that it depends on the amounts of ingested bacteria. In addition, the insecticidal activity of *Btt*-Xd3 was different against larvae and adults ($p<0.05$). Median lethal concentrations of *Btt*-Xd3 isolate were also estimated by probit analysis and calculated as 1.73×10^6 and 1.69×10^7 CFU mL^{-1} for larvae and adults, respectively (Table 2). Also, LT_{50} values of *Btt*-Xd3 (10^9 CFU mL^{-1}) for larvae and adults were determined as 8.04 and 8.83 days, respectively, using Kaplan-Meier method. This difference is because the coleopteran larvae are more susceptible than adults against toxins. Zehnder and Gelernter (1989) indicated that virulence of the toxin decreases with the increasing age of the pest. They tested the M-ONE, which is a liquid formulation containing 4.5% *B. thuringiensis* var. *san diego* against 2nd and 3rd instar larvae of CPB and they found that 98% and 52% mortality were observed, respectively. Haffani *et al*. (2001) expressed the *cry*3Aa3 gene in an *E. coli* expression system and tested the efficiency against third-instar larvae of the CPB using the leaf disk method. They found that the LD_{50} of Cry3Aa3 protein was 672.9 ng per individual. Kryukov *et al*. (2009) reported that *B. thuringiensis* ssp. *morrisoni* strain 2495 caused 60% mortality of middle-aged larvae of CPB at 5×10^7 spores mL^{-1} concentration under laboratory conditions. *Bt* subsp. *kurstaki* (strain EG2424) and *Bt* subsp. *tenebrionis* (serotype H8a, 8b) were tested on the larvae of CPB and caused 74% and 60% mortality (Ghassemi-Kahrizeh and Aramideh, 2015). Ferro and Gelernter

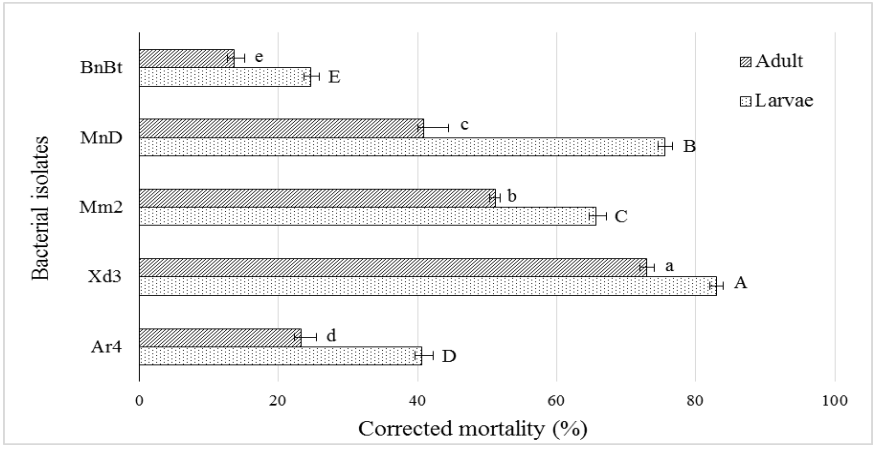

Figure 1. Mortality rates of bacterial isolates on CPB 15 days after application of 10^9 CFU mL^{-1} concentration. Different letters represent statistically significant differences between mortalities according to the least significant difference (LSD) ($p<0.05$). Bars show standard deviation.

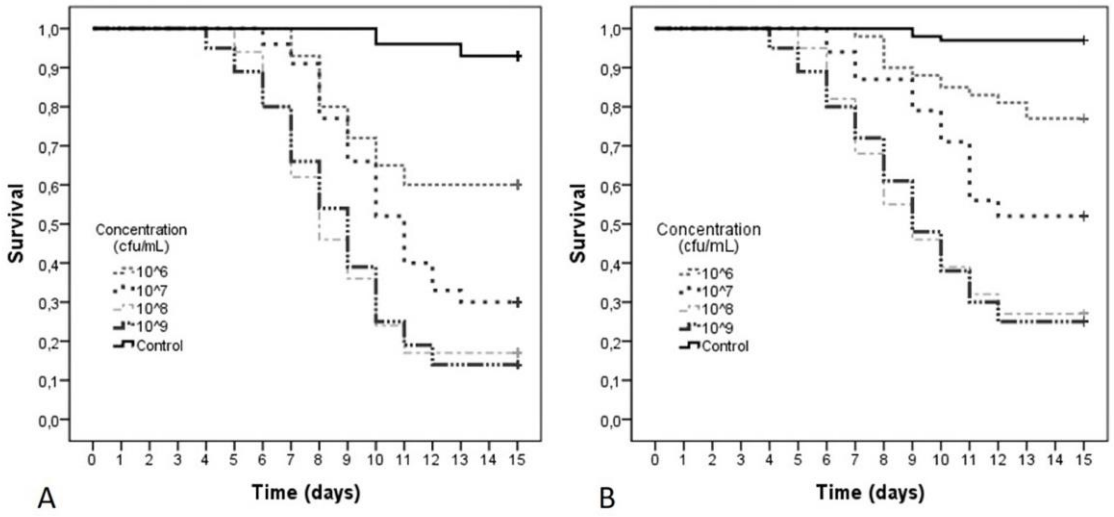

Figure 2. Survival graphs of *L. decemlineata* treated with different doses of *B. thuringiensis* strain Xd3 against larvae (A) and adults (B). Data are combined from three experimental runs. Control: sterile 0.01% Tween 80.

(1989) used 10% (wt/wt) spray-dried technical powder containing *B. thuringiensis* var. *san diego* spores and crystals against the larvae of CPB and they reported that ingestion of *Bt* resulted in dramatic reduction in feeding activity and 74% mortality was observed 96 hours after application. Similarly, *Btt*-Xd3 had a high insecticidal effect on both larvae and adults, and it can be a good candidate for controlling CPB.

Metarhizium anisopliae Gg-12 (*Ma*-Gg12) isolate was detected as the most effective fungal isolate against both larvae and adults of the pest (p<0.05). It showed 98% and 100% mortality against larvae and adults, respectively. In addition, other isolates showed also high mortality rates between 59% and 93% (Figure 3). Dose-response experiment of *Ma*-Gg12 showed that the rise of the application dose caused higher mortality in a short time (Figure 4). LT_{50} values of *Ma*-Gg12 (10^8 conidia mL^{-1}) for larvae and adults were determined as 5.18 and 5.73 days, respectively. In addition, LC_{50} of *Ma*-Gg12 was determined as 1.18×10^4 conidia mL^{-1} for the larvae and 6.2×10^3 conidia mL^{-1} for the adults (Table 2). The rate of mycosis (over 90%) in cadavers was determined to confirm that the mortality was caused by fungal infection. It is known that the genus *Metarhizium* is an important group of entomopathogenic fungi that is widely used for control of leaf beetles. *Metarhizium anisopliae* KTU-60 tested against *Agelastica alni* (L.) (Coleoptera: Chrysomelidae) at 10^7 conidia mL^{-1} concentration showed 100% mortality on 3rd instar larvae, 90% mortality was recorded on the adults (Sonmez *et al.*, 2017). The other strain of *M. anisopliae* (CG321) caused 100% larval mortality on *Cerotoma arcuata* Olivier (Coleoptera: Chrysomelidae) (Teixeira and Franco, 2007). The efficiency of *Metarhizium* against CPB was also reported in previous studies. Akhanaev *et al*. (2017) tested *M. robertsii* strain P-72 against 3rd instar larvae of CPB at 10^6 conidia mL^{-1} concentration and it showed 80% mortality. *M. robertsii* strain R-72 led to 86% mortality within 12 days after the application of 7×10^5 conidia mL^{-1} (Yaroslavtseva *et al*. 2017). Kryukov *et al*. (2009) reported that when the CPB were infected with *M. anisopliae* strain R-72-kh, 100% mortality rate was observed on days 14. In our study, *Ma*-Gg12 also displayed

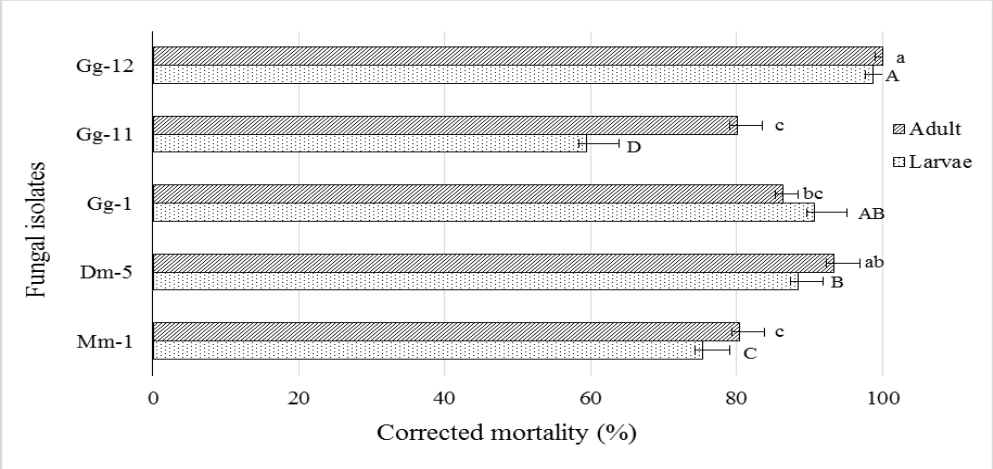

Figure 3. Mortality rates of fungal isolates on CPB 15 days after application of 10^7 conidia/ml fungal suspension. Different letters represent statistically significant differences among treatments with respect to mortality according to the least significant difference (LSD) test (p<0.05). Bars show standard deviation.

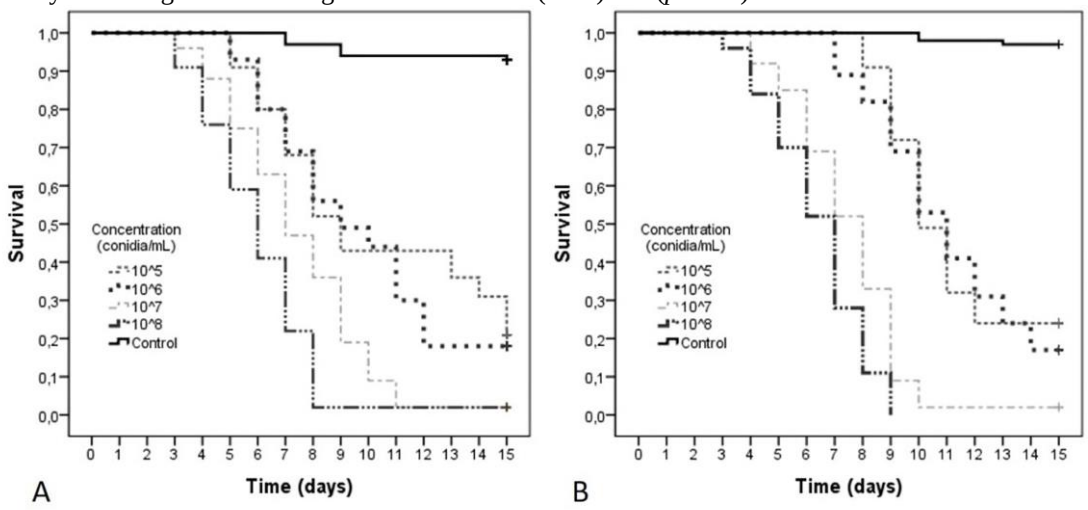

Figure 4. Survival graphs of *L. decemlineata* treated with different doses of *M. anisopliae* isolates Gg-12 against larvae (A) and adults (B). Data are combined from three experimental runs. Control: sterile 0.01% Tween 80.

high insecticidal activity on both the larvae and adults.

The effectiveness of three indigenous EPN isolates (*Hb*-ZET35, *Sf*-ZET31, and *Sw*-AS1) against 3rd instar larvae and adults of CPB were studied under laboratory conditions. The results indicated that the developmental stage of the CPB has significant influence on the activity of the EPNs. While *H. bacteriophora* ZET35 (*Hb*-ZET35), *S. feltiae* ZET31 (*Sf*-ZET31) and *S. websteri* AS1 (*Sw*-AS1) showed 55%, 83%, and, 92% mortality against the larvae of CPB with 1000 IJs mL^{-1} concentrations, respectively (Figure 5), the isolates did not show the insecticidal activity against the adults (data not shown). Previous studies about the control of adult chrysomelids with EPNs usually has not succeeded (Hongyi *et al*., 2000; Toepfer *et al*., 2005). On the other hand, some laboratory researches showed that adults of the CPB are also sensitive to EPNs (Stewart *et al*., 1998; Ebrahimi *et al*., 2011; Trdan *et al*., 2009). The reasons for

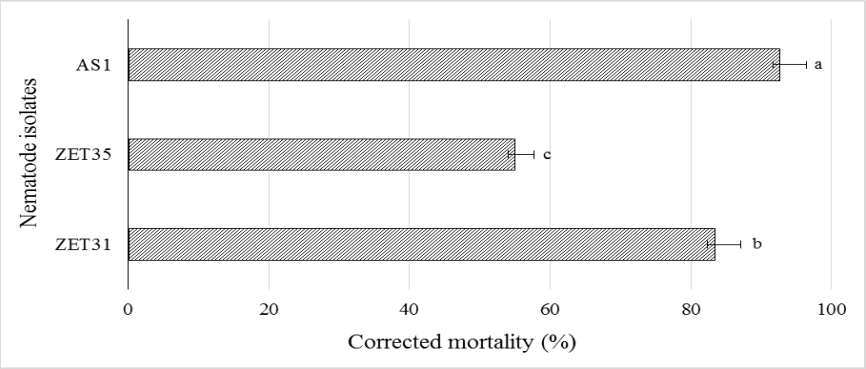

Figure 5. Screening test of nematodes against the larvae of CPB 7 days after application of 1000 IJs mL^{-1} nematode suspension. Different letters represent statistically significant differences between mortalities according to the least significant difference (LSD) test ($p<0.05$). Bars show standard deviation.

this difference may be biological and ecological differences among nematode isolates and species, and CPB populations. Also, application dose and bioassay conditions may be another reason for the difference. In addition, it is a known fact that EPNs can invade easily into the younger developmental stages of chrysomelids. In our study, while *Sw*-AS1 showed 92% mortality on 3rd instar larvae of CPB at 1000 IJs mL^{-1}, it had no effect on adults. However, when the application dose was reduced by half, there was a significant decrease in mortality ($p<0.05$), and when doubled, there was an increase in mortality, but it was not statistically significant ($p>0.05$) (Figure 6). LC_{50} value of *Sw*-AS1 on CPB was determined as 177 IJs mL^{-1} (Table 2). Adel and Hussein (2010) tested infectivity and biocontrol potential of *S. feltiae* strain PA and *H. bacteriophora* strain ALG12 on CPB under laboratory conditions and found that *S. feltiae* , which caused more than 70% larval mortality within 24 hours, was more effective and faster when compared with *H. bacteriophora*, which caused 40% mortality within 48-72 hours. In our study, LT_{50} was 2.97 days for *Sw*-AS-1 (1000 IJs mL^{-1}). While Berry *et al*. (1997) also reported that *Steinernema* spp. were more effective than *Heterorhabditis* spp. against CPB, *Steinernema* spp. have been found more effective in many studies, including our study.

CONCLUSION

The effectiveness of indigenous entomopathogenic microorganisms that had significant insecticidal activity on different insects was evaluated on CPB. *B. thuringiensis* strain Xd3, *M. anisopliae* isolate Gg-12, and *S. websteri* AS1 showed significant insecticidal effect on the pest. These entomopathogens appear to be

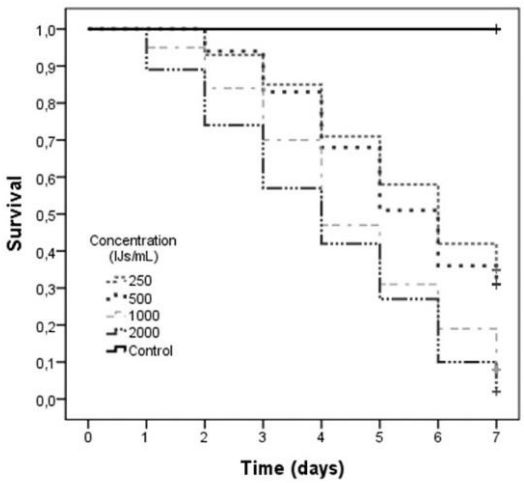

Figure 6. Survival graphs of *L. decemlineata* treated with different doses of *S. websteri* isolate AS1 against larvae (A) and adults (B). Data are combined from three experimental runs. Control: sterile 0.01% Tween 80.

Table 2. Lethal concentrations of entomopathogens against larvae and adult of CPB.

Isolates	Stage	LC_{50}	LC_{95}	$X^{2\,a}$	df^{b}	*p*-value
Bacillus thuringiensis Xd3	Larvae	1.73×10^{6} CFU mL^{-1}	6.15×10^{9} CFU mL^{-1}	5.15	2	≤0.05
	Adult	1.69×10^{7} CFU mL^{-1}	3.72×10^{10} CFU mL^{-1}	6.31	2	≤0.05
Metarhizium anisopliae Gg-12	Larvae	1.18×10^{4} conidia mL^{-1}	4.74×10^{7} conidia mL^{-1}	5.32	2	≤0.05
	Adult	6.2×10^{3} conidia mL^{-1}	7.0×10^{6} conidia mL^{-1}	10.3	2	≤0.05
Steinernema websteri AS1	Larvae	177 IJs mL^{-1}	1685 IJs mL^{-1}	6.05	2	≤0.05

[a] X^2: Chi square. [b] *df*: Degree of freedom,

promising candidates for microbial control of the pest. In subsequent studies, the combination effects of these entomopathogens against *L. decemlineata* can be studied. Also, biopesticide formulations from these pathogens should be developed and its effectiveness be tested in the field.

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ارزیابی موثر بودن بیمارگران حشرات در کنترل سوسک کلرادو سیب زمینی(*Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae

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چکیده

سوسک کلرادو سیب‌زمینی (*Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae به سیب زمینی، گوجه فرنگی، و بادمجان خسارت میزند و یکی از مهمترین آفت های کشاورزی در سراسر دنیاست. به علت مقاومت این حشره در برابر حشره کش‌های شیمیایی و برخی حشره‌کش‌های زیستی، برای برنامه های مدیریت یکپارچه این آفت، عوامل کنترل زیستی جدیدی که با شرایط زیست بومی مختلف سازگار باشد به فوریت مورد نیاز است. به این منظور، اثرات حشره‌کشی 13 جدایه میکروبی بومی و بیمار گر حشرات شامل باکتری ها، قارچ‌ها، و نماتدها که از کلکسیون کشت شده بر علیه *L. decemlineata* تهیه شده بود با غربالگری و آزمون‌های دوز- پاسخ در شرایط آزمایشگاه مورد بررسی قرارگرفت. سویه *Xd3* از *Bacillus thuringiensis* در غظت 10^9 CFU mL^{-1} طی 10 روز منجر به مرگ ومیر 83٪ و73٪ به ترتیب لاروها و بالغین آفت شد. در حالی که جدایه قارچی *Metarhizium anisopliae Gg-12* با غلظت 10^7 کنیدیا در میلی لیتر در 15 روز موجب 98٪ مرگ و میر لاروها شد، *Gg-12* درهمان غلظت و مدت باعث مرگ ومیر 100٪ بالغین شد. بر پایه نتایج، *Steinernema websteri AS1* موثرترین نماتد بیمارگرحشره تشخیص داده شد و طی 7 روز باعث مرگ ومیر 92٪ لاروها گردید. بر اساس تجزیه و تحلیل پروبیت (*probit*) مقادیر LC_{50} از *B. thuringiensis Xd3* علیه لاروها و بالغین برابر $10^6 x 1/73$ CFU mL^{-1} و $1/69 x\ 10^7$ و در مورد *M. anisopliae Gg12* برابر $1/18\ x10^4$ و $6/2\ x\ 10^3$ کنیدیا در میلی لیتر و د رمورد *S. websteri AS1* برابر 117 *IJs* mL^{-1} محاسبه شد. با توجه به این نتایج، آفتکش‌های زیستی از این جدایه‌ها را میتوان در مدیریت برنامه کنترل آفت سوسک کلرادو سیب‌زمینی با ایمنی وموفقیت مصرف کرد.