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Full Length Research Paper

Pathogenicity of some isolates of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin on 2nd and 4th larval instars of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae), under laboratory conditions

Jamshid Akbarian[1]*, Youbert Ghosta[1], Nouraddin Shayesteh[1,2] and Seyed Ali Safavi[1]

[1]Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran.
[2]Department of Plant Protection, Mahabad Branch, Islamic Azad University, Mahabad, Iran.

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The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is one of the major economically pests of potato throughout the world. In this study, the pathogenicity of six isolates of *Beauveria bassiana* (Bals.) Vuill. (four indigenous and two non indigenous isolates) and two indigenous isolates of *Metarhizium anisopliae* (Metsch.) were investigated. Five different concentrations from each isolate, 1×10^7, 5×10^7, 1×10^8, 5×10^8 and 1×10^9 conidia ml^{-1}, were applied in bioassays on 2nd and 4th instars larvae. Results showed that the percentage of mortality due to AKB isolate of *B. bassiana* at the highest concentration were 78.88 and 44.44, for 2nd and 4th instars larvae, respectively. Also there were significant differences in percentage of mortality between AKB and other treatments. Other isolates, LRC107, IRAN429C, LRC137, IRAN441C and Z-1 were scored in the subsequent classes, respectively. Both *M. anisopliae* isolates, DEMI001 and IRAN437C showed the lowest mortality rate on larvae in comparison with *B. bassiana* isolates. In all experiments, with increase in conidial concentration, the mortality rate was increased. Based on our results, the 2nd instar larvae were more susceptible than the 4th instar.

Key words: Biological control, *Beauveria bassiana*, *Metarhizium anisopliae*, *Leptinotarsa decemlineata.*

INTRODUCTION

The Colorado potato beetle (CPB) is a key pest in the potato-growing areas of Iran (Akbarian, 1995; Kazemi and Ardabili, 1999; Inglis et al., 2001). Outbreak of CPB populations occurs rapidly (Akbarian, 2008). Uncontrolled populations are capable of completely defoliating potato crop and causing total yield losses (Weber, 2008; Wraight and Ramos, 2002), therefore where CPB populations are present, control programs are necessary (Gullan and Cranston, 2005). Chemical control is still the predominant and effective method in pest control (Glazer and Nikaido, 1995), but continuous uses of chemical products cause problems such as environmental hazards (Kegley and Wise, 1998; Holloman, 1993), residue in tubers use as food and pest resistance to chemical insecticides (Janofsky, 2006; Georghiou, 1994). On the other hand, reliance on the use of insecticides has resulted in multiple resistances in CPB (Lacey et al., 1999; Whalon et al., 2003). Therefore researchers attempt to develop biological control methods as supplements or alternatives to chemical insecticides in integrated pest management (IPM) programs (Inglis et al., 2001).

In recent years, many researches were performed based on applications of biological control agents which called microbial control (Vincent et al., 2007). *Beauveria*

*Corresponding author. E-mail: jakbarian2010@gmail.com. Tel: +98-441-2779552. Fax: +98-441-2779558.

Table 1. Mean of Head capsule width in different larval instars of CPB (Boiteau et al., 1999; Khanjani, 2005).

Larval instars	Mean±SE
1st	0.690±0.008
2nd	1.130±0.012
3rd	1.724±0.015
4th	2.438±0.019

bassiana belongs to the class of Hyphomycetes, which belong to the Deuteromycota or fungi imperfecti (Steinhaus, 1949). A characteristic feature of the genus *Beaveria* is the zig-zag rachis bearing the conidia. The main differentiation between the two most common *Beaveria* species (*B. bassiana* and *Beaveria brongniartii*) is the shape of the conidia, which is globose in the first case and more or less oval in the second case (MacLeod, 1954).

B. bassiana is one of the entomopathogenic fungal agents which has drawn researchers attention in biological control programs and has a wider host range than the other fungal entomopathogenic species (Pendland and Boucias, 2004). This entomopathogenic fungus is a capable alternative control agent or complement in integrated pest management programs against the CPB (Anderson et al., 1989). *B. bassiana* has a high subsistence and geographical diversity (Leland et al., 2005). In addition, it is found naturally in many of the soils where CPB is problematic (Long et al., 1998) either occurs in soil as a ubiquitous saprophyte (Tanada and Kaya, 1993) or contaminates naturally populations of CPB (Todorova et al., 2000). Different isolates of *B. bassiana* and *Metarhizium anisopliae* have been specialized on different hosts (Florez, 2002). Allocated efficacy of different *M. anisopliae* isolates was studied via tegumental contamination on CPB, and it was demonstrated that the larvae were susceptible to *M. anisopliae* isolates (Fargues, 1976). *B. bassiana* has been used successfully in the USSR, Eastern Europe, USA (Anderson et al., 1989) and France (Farques et al., 1980). However, there is nearly no use of commercial formulated entomopathogenic fungi in less developed countries. In recent years, a lot of researches have been focused on the efficacy of *B. bassiana* isolates in the biological control of some important insect pests (Safavi et al., 2010). According to Todorova et al. (2000) from ten isolates of *B. bassiana* which were evaluated for their pathogenicity against the CPB under laboratory conditions eight days after treatments, six isolates were highly virulent. In another research on field-collected late larval instars of CPB that were treated with *B. bassiana* (Isolate of ARSEF252, formulated by Abbott Laboratories) at rate of 5×10^{12} and 5×10^{13} colony-forming units/ha, mycosis was observed 98 and 64%, respectively (Anderson et al., 1988). Pathogenicity of *B. bassiana* at a concentration of 3×10^{6} conidia ml^{-1} caused 4% mortality on fourth instar larvae of CPB (Fargues, 1972). The aims of this study were to evaluate the virulence of six isolates of *B. bassiana* and two isolates of *M. anisopliae* on second and fourth larval instars of CPB using dipping bioassay method under laboratory conditions and to determine the most effective isolate of used entomopathogenic fungi.

MATERIALS AND METHODS

Rearing of CPB

A pair of newly emerged adults of CPB which were collected from the Urmia contaminated potato farms was transferred to Urmia University's experimental station farm for laying eggs. The eggs were kept in an incubator at 25°C. After hatching the eggs, larvae were transferred onto foliage of Agria potato cultivar with no antibiosis effects for feeding of CPB (Ghassemi-Kahrizeh et al., 2010). Rearing and mass production of pest was continued during two farming seasons. Second and fourth instars larvae were collected by measuring head capsule width (Boiteau et al., 1999; Khanjani, 2005) (Table 1) and were used in all bioassay experiments.

Fungal isolates

Six isolates of *B. bassiana* and two isolates of *M. anisopliae* which had been isolated from different geographical areas were supplied and used in this study. Table 2 shows some characteristics of used fungal isolates. The isolates were grown on Potato Dextrose Agar (PDA®) medium in sterile 90 mm Petri dishes sealed with Parafilm®. PDA and Parafilm were purchased from Merck. Petri dishes were incubated at 25±1°C and a photoperiod of 16:8 h (L: D) for two weeks to be completed sporulation.

Preparation of conidial suspensions

Conidial suspensions were prepared by scrapping conidia from well sporulated 15 days old cultures into sterile distilled water containing 0.01% Tween-80®. Suspensions were agitated vigorously for 15 min, and then filtered through several layers of cheesecloth to remove mycelia and pieces of culture materials. Spore concentrations were determined by a haemocytometer and amounts of conidia and then adjusted to the required concentrations (Cherry et al., 2005; Butt et al., 1994). Conidial viability of different isolates was tested by spreading a droplet of 10^{7} conidia ml^{-1} concentration of each conidial suspension over PDA separately.

Cultures were kept at 25±1°C for 24 h, then; viability percentage was determined by random counting 100 spores in a microscope field (X400). Conidia with germ tubes longer than the conidia's width were considered to have germinated. Suspensions were kept at 4°C dark storage before use (Goettel and Inglis, 1997).

Bioassays

The concentration of each isolate was adjusted to 1×10^{7}, 5×10^{7}, 1×10^{8}, 5×10^{8} and 1×10^{9} conidia ml^{-1} based on a preliminary experiment. Second and fourth instar larvae of CPB were dipped for 30 s in 10 ml of different conidial suspensions. Then second instar larvae were transferred into plastic containers (d = 25 cm, h = 5 cm) containing fresh potato detached foliage. Potato stalks were placed

Table 2. Details of *B. bassiana* and *M. anisopliae* isolates used in bioassay.

Isolates	Insects of host	Location (City- Country)
B. bassiana		
AKB[a]	*Leptinotarsa decemlineata* (Col.: Chrysomelidae)	Urmia- Iran
LRC107[c]	*L. decemlineata*	Portugal
IRAN429C[b]	*Chilo suppressalis* (Lep.: Pyrlidae)	Rasht-Iran
LRC137[c]	*L. decemlineata*	Canada
IRAN441C[b]	*Rhynchophorus ferrugineus* (Col.: Curculionidae)	Saravan-Iran
Z-1[a]	*L. decemlineata*	Urmia-Iran
M. anisopliae		
DEMI001[b]	*R. ferrugineus*	Saravan-Iran
IRAN437C[b]	*C. suppressalis* (Lep.: Pyrlidae)	Rasht-Iran

a. Supplied isolates from Plant Protection Department of Urmia University, b. Supplied by Iranian Plant Protection Research Institute, Tehran, c. Supplied by Dr. M. S. Goettel, Lethbridge Research Center, Agriculture and Agric-Food Canada, Alberta, Canada.

into filled water bottles to prevent their wilting and dehydration. The lids of containers were covered by a net and kept in an incubator at 25 ± 1°C, 70 ± 5 RH and a photoperiod of 16: 8 h (L:D) for 15 days (Butt et al., 1994; Safavi et al., 2010). Three replicates each containing 15 larvae were prepared for each concentration. The containers were checked daily in order to cleaning the refuse and replacement of fresh food for a period of 15 days. Dead larvae were counted and placed in Petri dishes (90 mm) containing wet filter paper. The Petri dishes were sealed with Parafilm and incubated at 25 ± 1°C to observe fungal growth and mortality confirmation.

Fourth instar larvae were transferred into cultivated potato foliage grown according to natural conditions in black colored wide opening pots containing 4 litter permeable sandy-loam soil (3.6-5.5 pH) (Gaugler et al., 1989; Hiiesaar et al., 2006), then the lids of pots were covered by net. The containers were irrigated and incubated under up mentioned conditions for second instar larvae for 15 days. After this period without checking to end of pupation stage which is coinciding with the emergence of adult insects, the soil was poured out and larvae and pupae that showed signs of fungal infections were separated and counted. The emerged adult beetles were transferred onto fresh potato foliage and were kept in the incubator under mentioned conditions for one week to observe their probable fungal infections. All of experiments were repeated three times. Control insects were treated using sterile distilled water containing 0.01% Tween-80.

Statistical analysis

Analysis of variance (ANOVA) was conducted using a factorial experiment based on completely randomized design (CRD). In all experiments normality of mortality data was arcsine-transformed in SAS [9.1.3 P (G)] software (Soltani, 2008). The means were compared by Duncan's multiple range tests and diagrams designed using Excel 2007 software. The LT_{50} and LC_{50} values of fungal isolates were evaluated using Probit analysis in MINITAB program.

RESULTS

Germination of all *B. bassiana* and *M. anisopliae* isolates tested varied from 95 to 98%. Analysis of variance of larval mortality percentage data showed significant differences between treatments. In comparison of mortality percentages due to different fungal isolates and concentrations, there was statistically significant differences between different isolates and concentrations ($F = 194.03$ and 89.78, $df = 7$, $P<0.01$) (Table 3) and ($F = 252.82$ and 44.98, $df = 4$, $P<0.01$) (Table 4), respectively (Figure 1). Significant interactions were not observed between isolates and concentrations ($F = 5.64$ and 1.24, $df = 28$, $P<0.22$). Analysis of variance of data showed significant differences between treatments of *B. bassiana* and *M. anisopliae* fungi ($F = 247.90$, $df = 1$, $P<0.01$). Mortality rate between second and fourth instars larvae was significant differences ($F = 28.33$, $df = 1$, $P<0.01$) (Figure 1). Grouping of mean mortality percentages data was determined with Duncan's multiple range tests and showed in Table 3. The highest mortality rate due to AKB isolate at the highest concentration used (10^9 conidia ml^{-1}), reached to 78.88 ± 2.59 and 44.44 ± 3.39% for second and fourth instars larvae, respectively. Other isolates showed lower mortality rate. DEMI001 and IRAN437C isolates of *M. anisopliae* showed the lowest mortality rate in among other isolates on second and fourth instars larvae (there were no significant differences in comparison with control treatment)(Figure 1). LT_{50} and LC_{50} for different isolates are shown in Table 3. Based on the results it could be concluded that AKB was effective isolate among the others (especially on fourth larval instars) and it had the lowest rates of LT_{50} and LC_{50} values including 11.98 ± 1.01 days and 1.3×10^8 conidia ml^{-1}, respectively.

DISCUSSION

In present study susceptibility of second instar larvae to all *B. bassiana* and *M. anisopliae* isolates was significantly higher than fourth larval instar of CPB. It was

Table 3. Grouping of mean mortality percentage and evaluation of LT_{50} for 2nd instar and LC_{50} for 2nd and 4th larval instars of CPB, 15 days after immersion in conidial suspensions of *B. bassiana* and *M. anisopliae* isolates.

Fungal isolates	Mean mortality		LT_{50} (Days) *	LC_{50} (conidia ml^{-1})	
	2nd instar	4th instar	2nd instar	2nd instar	4th instar
			B. bassiana		
AKB	48.44±5.70^{A}	32.00±2.18^{a}	11.83	1.3×10^{8}	7.2×10^{9}
LRC107	47.55±5.04^{AB}	28.44±2.52^{b}	12.75	1.5×10^{8}	9.6×10^{9}
IRAN429C	45.35±5.56^{BC}	25.77±2.23^{b}	11.98	1.8×10^{8}	1.5×10^{10}
LRC137	43.10±5.32^{CD}	27.55±1.83^{b}	12.82	2.2×10^{8}	3.6×10^{10}
IRAN441C	40.44±4.60^{D}	25.33±2.02^{bc}	13.05	3.1×10^{8}	7.6×10^{9}
Z-1	29.70±2.37^{E}	21.33±1.96^{c}	15.33	6.2×10^{9}	3.5×10^{10}
			M. anisopliae		
DEMI001	12.44±2.29^{F}	7.55±1.5^{d}	Uncountable	2×10^{10}	2×10^{11}
IRAN437C	4.88±1.39^{G}	3.75±0.72^{e}	Uncountable	2.6×10^{10}	Uncountable

*4th instar larvae treatments were investigated to end of the pupation stage and the larvae after migration into the soil were not observed daily; therefore evaluation of LT_{50} was impossible.

Table 4. Significance of mean mortality percent on second and fourth instars larvae due to different concentrations of fungal isolates using Duncan's multiple range tests.

Fungi	Instars	Mean mortality in concentrations				
		10^{7}	5×10^{7}	10^{8}	5×10^{8}	10^{9}
B. bassiana	2nd	14.18±1.77^{A}	30.18±2.90^{B}	39.25±3.05^{C}	58.14±2.55^{D}	64.99±2.85^{E}
	4th	16.86±2.33^{A}	21.10±2.34^{B}	25.55±1.77^{BC}	29.25±2.48^{C}	36.29±2.68^{D}
M. anisopliae	2nd	2.22±1.11^{A}	4.44±1.11^{AB}	5.55±2.39^{B}	11.11±1.69^{C}	19.99±1.13^{D}
	4th	2.22±1.69^{A}	2.22±1.11^{A}	5.55±2.8^{B}	5.55±2.33^{B}	9.99±0.64^{C}

noted that although larvae of CPB are susceptible to *B. bassiana* infection, but mortality varies between larval instars (Fernandez et al., 2001). Mortality of CPB due to infection with *B. bassiana* in late instars larvae may be low because of the hardiness of insect cuticle (Charnley, 2003). According to study of Wraight and Ramos (2002), *B. bassiana* is more virulent against early versus late-instar larvae of CPB. Chemical constituents of the larval cuticle vary with age, leading to progressive hardening of the cuticle and increased humoral defense mechanisms to the microbial infection (Boman, 1981). Penetration through the external integument of CPB is the most common invasion route of *B. bassiana* (Vey and Fargues, 1977). Differences in susceptibility of different instars larvae could be attributed to the differences in hemocyte volume or immune responses between instars (Fernandez et al., 2001). On the other hand, in this study the research of fourth instar larvae was continued to end of pupation stage, therefore it is well known that ecdysis of the mentioned larvae in the soil can remove fungal conidia from the surface of body and decrease susceptibility to the pathogen (Furlong and Groden, 2003).

In our study *B. bassiana* isolates were more virulent than *M. anisopliae* isolates. Chabchoul and Taborsky (1991) noted that direct action of *M. anisopliae* on CPB larvae was poor. In studies of Gaugler et al. (1989) *B. bassiana* was highly virulent to the CPB. The insect cuticle is an important barrier to the invasion of fungal pathogens (Klinger, 2003) and prerequisite of the beginning stages of infection is spore adhesion on integument of susceptible pest (Safavi, 2007). In some cases, spore adhesion is correlated with the aggressiveness or host specificity of a fungal species, such as with *M. anisopliae* on Scarabaeids (Tanada and Kaya, 1993; Fargues, 1976). Furthermore, certain strains may show no pathogenicity to one host, but cause high mortality on the other insects (Todorova et al., 1994).

Different isolates of entomopathogenic fungi showed a wide range of genetic variety, so it could be expected that only certain isolates will be virulent towards any given insect species (Charnly, 2003). Results of these experiments indicated that the fungal isolates caused a different mortality on CPB larvae. Ferron et al. (1991) reported that, *B. bassiana* has differences in host specificity and virulence among isolates. Evidently, the observed variation in virulence of different isolates highlights the importance of selecting appropriate isolates

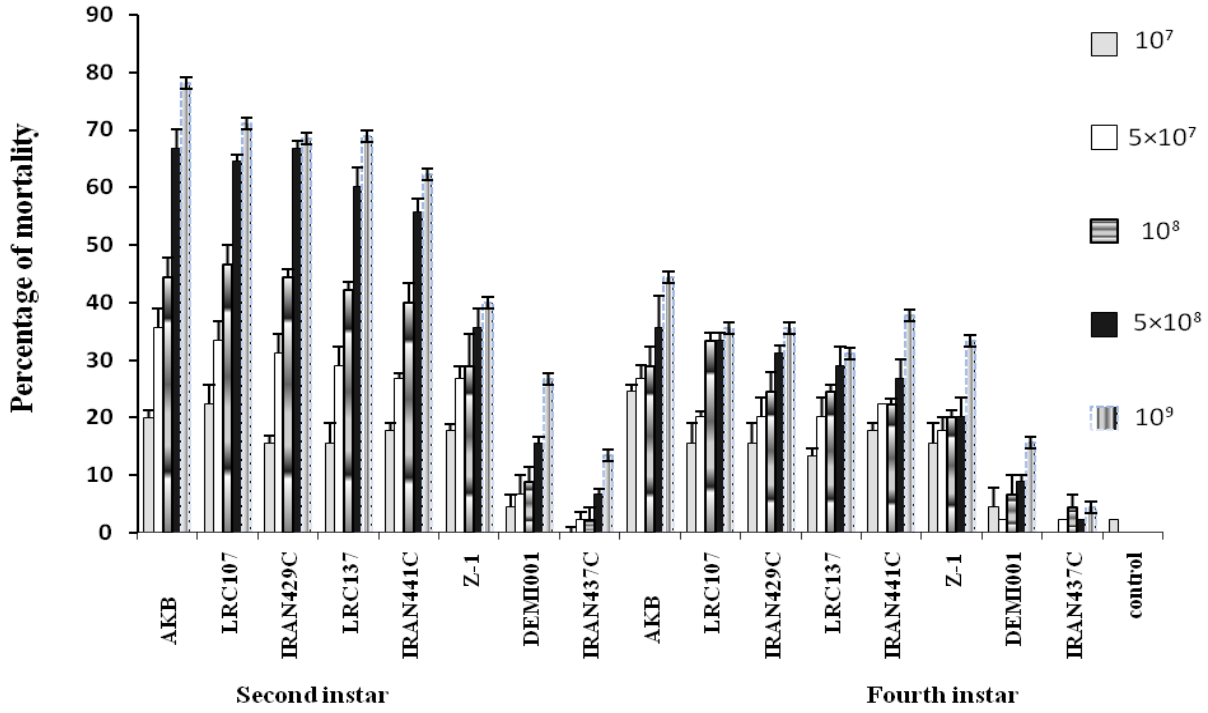

Figure 1. Percentage of mortality caused by *B. bassiana* and *M. anisopliae* isolates at five concentrations on second and fourth instars larvae of CPB.

for further studies. In present study, the AKB isolate was more effective than other isolates and mortality of second and fourth instars larvae were 78.88 and 44.44%, respectively in the highest concentration. Whereas according to experiment results of Chkubianishvili et al. (2010) mean mortality of different larval instar of CPB due to isolates of T3, T4 and T5 belonging to *B. bassiana* was 70%. Goettel et al. (1990) noted that the isolates most virulent to a host are those that isolated from the same or related host species. In our experiments, four isolates that were derived from CPB, showed different mortality, and Z-1 had the lowest mortality against CPB. So, genetic variety and other factors such as the amount of enzymes produced by different isolates should be considered. All fungi use mechanical force and a combination of enzymes to penetrate the host cuticle (Butt, 2002). Since proteins constitute a major component of the insect's cuticle, it follows that proteases must play an important role in the penetration process (Gupta et al., 1994; Butt, 2002). Relationship between enzyme activities and the virulence of *B. bassiana* has been demonstrated (Gupta et al., 1994). Pendland and Boucias (2004) have demonstrated that different strains of *B. bassiana* produce different amounts of cuticle-degrading enzymes.

In our studies each isolate at the highest concentration imposed the highest mortality to the larvae of CPB. Higher concentrations of fungal suspensions demonstrated higher virulence and significant differences in mortality of larvae. It may be mentioned that suspensions consisting higher conidial concentrations could increase the chance of bringing conidia into contact with host integument. Kuepper (2003) noted that mortality rate of treated insects depends on the number of spores contacting the insect body.

LT_{50} and LC_{50} estimates of AKB isolate was the least among the isolates used in this study, therefore it was identified as the most effective isolate in our study. But in a study Samsinakova et al. (1981) used a commercial formulation of *B. bassiana* against second larval instar of CPB and the estimated LT_{50} was 3.5 days. High virulence obtained in mentioned study could be attributed to the commercial formulation quality. Butt and Goettel (2000) noted that continuous culture and a long-term preservation of fungal isolates under laboratory conditions even in the best situations impress negatively on their virulence. The isolates used in our study have been obtained from the fungal collections that had been preserved for a long period.

In spite of the fact that susceptibility of CPB larvae to fungal isolates of present study was demonstrated clearly, it seems that the efforts to select the most effective isolates of *B. bassiana* and or *M. anisopliae* should be continued. The most effective isolates of *B. bassiana* with elevated larvicidal activity can be a promising candidate as a complement and an alternative in integrated pest management (IPM). Moreover such periodical introduction of new *B. bassiana* isolates can

potentially reduce the incidence of insect resistance.

REFERENCES

Akbarian J (1995). Study of natural enemies of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) in Ardebil. In Persian, Master of Science Thesis in the University of Tarbiat Modarress, Iran, pp. 1-27.

Akbarian J (2008). Fundamentals of insect's classification, pests and biological agents. 1st ed. In Persian. Urmia University, Iran, pp. 372-374.

Anderson TE, Hajek AE, Roberts DW, Preisler HK, Robertson JL (1989). Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae): Effects of combinations of *Beauveria bassiana* with insecticides. J. Econ. Entomol. 82(1):83-89.

Anderson TE, Roberts DW, Soper RS (1988). Use of *Beauveria bassiana* for suppression of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae) population in New York state. Environ. Entomol. 17(1):140-145.

Boiteau G, Jean-Pierre R, Blanc LE (1999). Colorado potato beetle life stages. Otawa Agriculture, Canada Publication, K1AOC7, Available in: http://res 2 agr. ca/fredricton/home/texts/staff/studies/3500/cpb.htm.

Boman HG (1981). Insect responses to microbial infections. In: "Microbial control of pests and plant diseases, 1970- 1980". Burges HD (ed.) Academic Press, London, pp. 769-784.

Butt TM (2002). Use of entomogenous fungi for the control of insect, pests, in: "The Mycota: Agricultural applications". Kempken F (ed.). Springer-Verlag, Berlin, Heidelberg. pp. 111-134.

Butt TM, Goettel MS (2000). Bioassays of entomogenous fungi. In: Navon A, Ascher KRS (eds.) Bioassays of entomopathogenic microbes and nematodes. CABI Publishing, Wallingford, UK, pp. 141–195.

Butt TM, Ibrahim L, Ball BV, Clarck SJ (1999). Pathogenicity of the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana* against crucifer pests and the honey bee. Biocontrol Sci. Technol. 4: 207–214.

Chabchoul H, Taborsky V (1991). Use of *Metarhizium anisopliae* (Metsch.) Sorokin against Colorado potato beetles (*Leptinotarsa decemlineata*). Agricultura Tropica et Subtropica 24:31-38.

Charnley AK (2003). Fungal pathogens of insects: Cuticle degrading enzymes and toxins. Adv. Bot. Res. 40:241-321.

Cherry AJ, Abalo P, Hell K (2005). A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin to control of *Callosobruchus maculates* F. (Col.: Bruchidae) in stored cowpea. J. Stored Prod. Res. 41:295 – 309.

Chkubianishvili T, Kakhadze M, Malania I, Goettel MS (2010). Prespectives of the Colorado potato beetle fungi pathology in Georgia. 10th International Colloquium on Invertebrate, 11 – 15 july 2010, Karadeniz Technical University, Trabzon, Turkey, p. 140.

Fargues J (1972). Study of the condition of infection of larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), with *Beauveria bassiana* (Bals.) Vuill. (Fungi imperfect). J. Entomophaga 17(3):319-337.

Fargues J (1976). Specificity champignons entomopathogenes imparfaits (Hyphomycetes) pour les larves de coleopters (Scarabaeidae et Chrysomelidae). Entomophaga 21:313-323.

Fargues J, Delmas JC, LeBrun RA (1994). Leaf consumption by larvae of the Colorado potato beetle (Col.: Chrysomelidae) infected with the entomopathogen, *Beauveria bassiana*. J. Econ. Entomol. 87:67-71.

Farques J, Cugier JP, Van de Weghe P (1980). Experimentation enparcelles du champignon *Beauveria bassiana* (Hyphomycete) contre *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae). Acta Ecologica 1:49-61.

Fernandez S, Groden E, Vandenberg JD, Furlong MJ (2001). The effect of mode of exposure to *Beauveria bassiana* on conidia acquisition and host mortality of Colorado potato beetle, *Leptinotarsa decemlineata*. J. Invert. Pathol., 77:217-226.

Ferron P, Fargues J, Riba G (1991). Fungi as microbial insecticides against pests. In: Handbook of applied mycology: Humans, animals and insects, eds. Arora DK, Mukherji KG, Drouhet E (1991). New York, Marcel Dekker, ISBN 0-82478435 2:665-705.

Florez FJP (2002). Fungi for coffee berry borer control, Colombia. The 35th annual meeting of the Society of Invertebrate Pathology, Foz Do Iguassu, Brazil.

Furlong MJ, Groden E (2003). Starvation induced stress and the susceptibility of the Colorado potato beetle, *Leptinotarsa decemlineata*, to infection by *Beauveria bassiana*. J. Invert. Pathol. 83:127-138.

Gaugler R, Costa SD, Lashomb J (1989). Stability and efficacy of *Beauveria bassiana* soil inoculations. Environ. Entomol. 18 (3):412-417.

Georghiou GP (1994). Principles of insecticide resistance management. Phytoprotection 75:51-59.

Ghassemi-Kahrizeh A, Nouri- Ganbalani G, Shayesteh N, Bernousi I (2010). Antibiosis effects of 20 potato cultivars to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae). Int. J. Food Agric. Environ. 8(2):795-799.

Glazer AN, Nikaido H (1995). Microbial Insecticides. In: Freeman WH (ed.) Microbial Biotechnology Fundamentals of Applied Microbiology. CITED Inc. New York. pp. 209-229.

Goettel MS, Inglis DG (1997). Fungi: Hyphomycetes. In: Lacey LA (ed.) Manual of techniques in insect pathology. Academic Press, London, UK. pp. 213–249.

Goettel MS, Poprawski TJ, Vandenberg JD, Li Z, Roberts DW (1990). Safety to nontarget invertebrates of fungal biocontrol agents. In: "Safety of microbial insecticides", Laird M, Lacey LA, Davidson EW (eds.). Boca Raton, FL: CRC Press, pp. 209-231.

Gullan PJ, Cranston PS (2005). The Insects: an outline of entomology. 3rd ed. Blackwell Science, Oxford, USA, pp. 418-419.

Gupta SC, Leathers TD, El-Sayed GN, Ignoffo CM (1994). Relationships among enzyme activities and virulence parameters in *Beauveria bassiana* infections of *Galleria mellonella* and *Trichoplusia ni*. J. Invert. Pathol. 64:13-17.

Holloman DW (1993). Pesticide resistance. Chem. Ind. 15:822-895.

Inglis GD, Goettel MS, Butt TM, Strasser AH (2001). Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N (eds.) Fungi as biocontrol agents. CAB International. Wallingford, Oxon, pp. 23-69.

Janofsky M (2006). "E.P.A. recommends limits on thousands of uses of pesticides" New York Times, Retrieved, pp. 8-24.

Kazemi MH, Ardabili J (1999). Studies on bioecological status of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae) during 1984-90 in Ardabil region. Scientific-Research Journal of Agriculture Faculty. In Persian, University of Tabriz, Iran, 9(1): 41-53.

Kegley SE, Wise LJ (1998). Pesticides in fruits and vegetables. Sausalito, CA: University Science Books.

Khanjani M (2005). Vegetable pests in Iran. 1st ed. In Persian. Bu-Ali Sina University, Hamedan, Iran, pp. 467-468.

Klinger E (2003). Susceptibility of adult Colorado potato beetle (*Leptinotarsa decemlineata)* to the fungal entomopathogen *Beauveria bassiana*. Master of Science Thesis in the University of Main. pp. 1-13.

Kuepper G (2003). Colorado potato beetle: Organic control options. http:/attar.ncat.org/attar-pub/ 23#23.

Lacey LA, Horton DR, Chauvin RL, Stocker JM (1999). Comparative efficacy of *Beauvaria bassiana*, *Bacillus thuringiensis* and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. Entomologia Experimentalis et Applicata 93:189-200.

Leland JE, Mc Guire MR, Grace JA, Jaronski ST, Ulloa M, Park Y-H, Plattner RD (2005). Strain selection of a fungal entomopathogen, *Beauveria bassiana* for control of plant bugs (*Lugus* spp.)(Heteroptera: Miridae). Biol. Control 35:104-114.

Long DW, Drammond FA, Groden E (1998). Susceptibility of Colorado potato beetle (*Leptinotarsa decemlineata*) eggs to *Beauveria bassiana*. J. Invert. Pathol., 71:182-188.

MacLeod DM (1954). Investigation on the genera *Beauveria* Vuill. and *Tritirachium* Limber. Can. J. Bot. 32: 818–890.

Pendland JC, Boucias DG (2004). *Beauveria*. Springer Science, Encyclopedia of Entomology, University of Florida, Gainesville

Florida, USA, pp. 1-4.
Safavi SA (2007). Comparative investigation of the virulence of some isolates of the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin toward European corn borer, *Ostrinia nubilalis* (Hübner) larvae and its relationship with production of an important cuticle-degrading protease. In Persian. A Degree of PhD Thesis in the University of Tehran, Iran pp. 1-30.
Safavi SA, Kharrazi A, Rasoulian GhR, Bandani AR (2010). Virulence of some isolates of entomopathogenic fungus, *Beauveria bassiana* on *Ostrinia nubilalis* (Lepidoptera:Pyralidae) larvae. J. Agric. Sci. Technol. 12:13-21.
Samsinakova A, Kalalova S, Vlcek V, Kybal J (1981). Mass production *Beauveria bassiana* for regulation of *Leptinotarsa decemlineata* populations. J. Invert. Pathol. 38(2):168-174.
Soltani A (2008). Application of SAS in Statistical Analysis. 3rd ed. JDM Press, Mashhad, Iran p.182
Steinhaus EA (1949). Principles of insect pathology. McGraw-Hill Book. New York.
Tanada Y, Kaya HK (1993). Insect Pathology. Academic Press, Inc. San Diego pp. 322-358.
Todorova SI, Coderre D, Côté JC (2000). Pathogenicity of *Beauveria bassiana* isolates toward *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae), *Myzus persicae* (Hom.: Aphididae) and their predator *Coleomegilla maculata lengi* (Col.: Coccinellidae). Phytoprotection 81 (1):15-22.
Todorova SI, Côté J-C, Martel P, Coderre D (1994). Heterogeneity of two *Beauveria bassiana* Strains revealed by biochemical tests, protein profiles and bioassays on *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae) and *Coleomegila maculata lengi* Timberlake (Col.: Coccinelidae) larvae. Entomophaga 39:159-169.
Vey A, Fargues J (1977). Histological and ultrastructural studies of *Beauveria bassiana* infection in *Leptinotarsa decemlineata* larvae during ecdysis. J. Invertebr. Pathol. 30:207-215.
Vincent C, Goettel MS, Lazarovits G (2007). Biological Control: A global perspective. CAB International. Wallingford, UK pp. 457.
Weber DC (2008). Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae) pest on the move. Pest outlook. Encyclopedia of Entomology, USDA Agricultural Research Service, Springer Science, Beltsville USA. pp. 1-6.
Whalon ME, Winged BA (2003). *Bt*: mode of action and use. Arch. Insect Biochem. Physiol. 54:200-211.
Wraight SP, Ramos ME (2002). Application factors affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle, *Leptinotarsa decemlineata*. Biol. Control 23(2):164-178.