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Effect of Some *Beauveria bassiana* (Bals.) Viull. Isolates on Common Pistachio Psylla *Agonoscena pistaciae* Burck. and Laut

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ABSTRACT

The common pistachio psylla, *Agonoscena pistaciae* is one of the most important pests of pistachio trees distributed in all pistachio producing regions of Iran. In this investigation, pathogenicity of seven isolates of *Beauveria bassiana* (Bals.) Viull. with 2.5×10^7 spore/mL suspension, were studied on 5th instar nymph of *A. pistaciae* by topical application. Results indicated pathogenicity of all isolates on test insects and among them, DEBI007، DEBI008، LRC107 and KCF107 caused more than 50% mortality. By using logarithmic doses of isolates, cumulative mortality, LD_{50} and LD_{90} of each isolate was recorded during one week. Results showed that DEBI008 isolate with 3.91×10^2 spore/Insect has the lowest and DEBI007 isolate with 3.63×10^4 spore/Insect has the highest LD_{50}. To study the LT_{50} of isolates, only 2.5×10^7 spore/mL suspension of each isolate, which resulted in more than 50% mortality was used and isolates were compared based on LT_{50}. The maximum and minimum LT_{50} with 5.6 and 3.4 day were due to DEBI008 and DEBI007 isolate, respectively. The results indicated that DEBI008 isolate with the lowest LD_{50} and LT_{50} is the most appropriate isolate.

Key Words: Pistachio psylla; Entomopathogenic fungi; *Beauveria bassiana; Agonoscena pistaciae*

INTRODUCTION

The common pistachio psylla (CPP), *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.: Aphalaridae) is one of the most important pests of pistachio trees distributed in all pistachio producing regions of Iran (Samih *et al.*, 2005). The CPP occurs in many pistachio growing regions around Iran's borders, including Armenia, Iraq, Turkmenia and Turkey, as well as Mediterranean regions such as Greece and Syria (Burkhardt & Lauterer, 1989; Mart *et al*., 1995; Lauterer *et al*., 1998). Regarding growing resistance of CPP against pesticides and repetitive invasion of other pests after sprayings and intense reaction of pests for compensating lost population after sprayings, it seems that chemical control is not an appropriate approach for controlling the population of this pest. Therefore, we have to exert biocontrol approaches against this pest. One of the fundamental and effective approaches is the use of fungi. There are 100 genera and 1000 fungal species that are entomopathogenic (Jenkins & Goettel, 1997; Bidochka *et al.*, 2000). Many of entomopathogenics fungi are important factors in regulating the populations of insects and caused epizootics among these populations (Butt & Goettel, 2000). Well-known entomopathogenic fungi include: *Beauveria bassiana*، *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* are used for pest control (Feng *et al.*, 1990; Wraight *et al.*, 1998). *B. bassiana,* was used for many sucking pests and showed satisfactory results. Wraight *et al*. (1998) reviewing entomopathogenics of *P. fumosoroseus* ، *P. farinosous* and *B. bassiana* isolates on silver leaf whitefly showed that all of them have pathogenicity on this pest. Hatting *et al*. (2004) showed that *B. bassiana* could control up to 65% of *Duraphis noxia* in field condition. Lababidi 2002, tested *B. bassiana* on *A. targionii* and showed that were highly effective especially on the 1st and 2nd nymph instars in the April. In current research, seven isolates of *B. bassiana* were tested on 5th instar nymph of CPP *A. pistaciae.*

MATERIALS AND METHODS

Collecting, rearing and preserving psylla. In order to collect samples, the pistachio orchards of Boein Zahra region were tested. For this purpose, the leaves infested to egg, psylla nymph were collected and brought to laboratory and the adult insects were exposed to pistachio trees. For preserving colonies, light plastic cylindrical pots (50 × 16.5 cm) were prepared. Rearing and total examining of psylla were conducted under 26 ± .2ºC, 55 ± 5% RH and 16: 8 (L: D) photoperiod on *pistacia vera* (*var*. Ghazvini).

Laboratory Investigations

Making psylla peer. The leaves infested to nymph, were cut from petiole then put in water. To do this the petiole was located into the hole created at the top of ependorph, which contained water. Ependorph, was put in the cylindrical pots (11 × 8 cm) until the emergence of adults. After 24 h, 50 male and female insects collected by aspirator then were exposed to pistachio plants. After 24 h, these insects went out of the cage then, 100 - 200 eggs were remained for laboratorial tests.

Cultivation of fungus isolates. Seven isolates from *B. bassiana,* were evaluated in this study (Table I). In order to culture isolates after infesting insects to fungus, pathogenicity of isolates was established and the spores that sporulate on the corpse of insect were used for culturing in SDAY. Monosporulating of spores, was carried out.

Providing the inoculums of fungus. In order to provide inoculums after putting isolates in SDAY for 10 - 14 days by disinfecting material and tools, the harvesting plate was scratched using a rod and poured into plate then, 10 mL of sterile distilled water (Tween 80 0.02%) was pipetted onto each sporulating plate in order to have homogeneous and spores were collected into a sterile pipette. Afterwards, the suspension obtained was passed through a two-folded mesh cloth to separate mycelium. In order to determine the number of spores, Heamocytometer was applied. In order to determine the survival of spores, viability test was performed. For this purpose, a little amount of suspension was cultured on SDAY after 24 h, the percentage of germination of spores was calculated and suspension with more than 90% spore germination for bioassay tests was used.

Selecting virulent isolates. Pathogenicity test was performed for selecting better isolates. The 5th nymph was used at all tests, because in this stage the psylla nymphs were bigger and could be transferred and identified simply. 2.5×10^7 spore/mL suspension of each isolate was provided. Then, 12 fifth nymphs in four repetitions accompanied with control were used for each isolate. The nymphs relevant to control were treated using sterile distilled water (Tween 80 0.02%). In order to treat nymphs, they were stimulated using peer nymphs under artificial light and picking them with a miniature paintbrush, 12 nymphs were laid on each leaf. Bioassay tests were conducted by putting 0.25 µL from each isolate on insect by micro-applicator using topical application method. Having treated insects, they are put on the leaves in the light cylindrical pots at 11 × 8 cm randomly. The cylindrical pots were located under 26 ± .2ºC, 65 ± 5% RH and 16: 8 (L: D) photoperiod. The mortalities were recorded for seven days daily.

Pathogenicity of fungus. The corpses of insects were collected and disinfected using sodium hypochlorite 1% suspension. First, the corpses were held for 2 - 3 min in the suspension then, they were put in sterile distilled water in two turns for 3 min. The sterile samples were transferred to disinfected Petri dishes in which humid filter paper were laid in advance. The Petri dish was conserved in laboratory condition to sporulate.

Bioassay Tests

Bracketing tests. The bracketing tests were performed with four isolates, which were selected in advance. These tests were carried out in several turns until the doses, which cause mortalities 5 - 95% were obtained.

Main tests. Using logarithmic doses of cumulative mortality, according to which, 5 - 6 suspensions were used and the main tests were carried out. Cumulative mortality was achieved for 7 - 8 days then; LD_{50} and LD_{90} of each isolate were obtained.

Analysis. The analysis of data using SPSS software and the comparison of means by Dunkan test, designing of diagrams using Excel software and determining LD_{50} and LD_{90} using Polo-PC were performed. Median lethal time was determined by probit analysis.

RESULTS AND DISCUSSION

Selection of virulent isolate of fungus. Due to the pathogenicity of isolates, with 2.5×10^7 spore/mL the mortality between 23% - 79.8% was inflicted. The analysis results of variance showed that there is a significant difference in the mortality of nymphs treated by *B. bassiana* isolates ($F_{7, 24} = 42.831$, $P < 0.0001$). Among seven isolates, the DEBI007، DEBI008، LRC107 and KCF107 could inflict 50% mortality in 5th instar nymph. The maximum mortality was of DEBI008 isolate and them minimum effect was pertinent to St.II p.c isolate. The LD_{50} and LT_{50} tests of above-mentioned isolates were conducted.

Using Duncan's test the grouping and the similarity degree of isolates were carried out. Having a general consideration to these grouping it is revealed that DEBI008 and KCF107 isolates in group (1) had the maximum mortality in psylla nymphs. Talaei Hasanloui (2000, 2005) reported that there are different effects of mortality for an isolate on various pests as well. The results showed that all of isolates could cause virulence in pistachio psylla. Talaei Hasanloui (2005) also investigated the effects of DEBI007 ،KCF107، LRC107, LRC137 and DEBI008 isolates on *plutella xylostella* and *Leptinotarsa decemlineata* and showed that KCF107 isolate has more effect rather than other isolates but in the performed investigation, it was disclosed that DEBI008 isolate had the most effect. The difference in the effect of these isolates on these three pests in these two investigations is almost certainly pertinent to the difference in the insect species of the test and the way of bioassay conduction and test conditions. Todorova *et al*. (2002) maintained that pathogenicity of isolates related to the host and source country is different as well.

Comparison of LD_{50}. Results showed that DEBI008 isolate with 3.91×10^2 spore/Insect has the minimum and DEBI007 isolate with 3.63×10^4 spore/Insect has the maximum LD_{50} (Table II).

Allahverdipour (2004) by studying DEBI008 isolate on wheat bug showed that DEBI008 isolate with the minimum LD_{50} had the maximum effect rather than other isolates. Ugine *et al*. (2005) in a study showed that GHA isolate has a great deal of effect on 2nd instar nymph of Trips flower and LD_{50} was $0.33 - 0.66 \times 10^2$ spore/insect for this isolate. In current investigation, the LD_{50} for DEBI008 isolate confirm that the LD_{50} in our test was an appropriate quantity for an isolate of high effect.

Table I. Name, place and the host (origin) of fungus isolates of *B. bassiana* used in the research.

Name of isolate	Region of collecting	Host or origin
DEBI008	Iran (Tehran)	*Chorthippus brunneus*(Ort: Acaridae)
DEBI007	Iran (Tehran)	Soil
St.II p.c.	Iran (Sari)	Soil
LRC137	Canada	Leptinotarsa (Col: Chrysomelidae) desemlineata
KCF107	Iran (karaj)	Soil
LRC107	Portugal	*L. desemlineata*
SARI p.a.	Iran (Sari)	Soil

Table II. The log of LD_{50} and LD_{90} of population, certainty range 95% and slope

Isolate	LD50	Limits 95%	LD90	Limits 95%	Slop(±SE)
DEBI007	4.66	4.94-5.71	5.18	4.03-4.63	1.68(±0.413)
DEBI008	2.59	3.84-4.82	4.22	2.2-2.9	0.785(±0.113)
LRC107	4.31	5.91-7.21	6.34	3.68-4.76	0.6(±0.099)
KCF107	2.76	3.75-4.69	4.11	2.3-3.1	0.953(±0.163)

Table III. LT_{50} of four isolates in 2.5×10^7 spore/insect

Isolates	LT_{50} (day)
DEBI007	5.6
DEBI008	3.4
LRC107	5.01
KCF107	4.57

The comparison of LT_{50}. In order to calculate LT50 for used isolates, only 2.5×10^7 spore/insect was selected and compared with each other is which caused mortalities more than 50% (Fig. 1). The results showed that DEBI008 and DEBI007 had the maximum and minimum LT_{50}, respectively (Table III).

Miranpuri and Khachatourians (1995) showed that LT_{50} of different isolates on *Sitobion avenae* was 3 - 5.4 days. Cherry *et al.* (2005) investigated the effects of *B. bassiana* on *Callosobruchus maculates* and using 1×10^8 spore/insect obtained 3.11 - 6.13 days for LT_{50}. In this study, LT_{50} was in the above-mentioned range and the difference observed about LT_{50} probably is due to difference in physiological combinations. Also according to Todorove *et al.* (2002) LT_{50} depends on the spore suspension and with increase in spore suspension; there is a decrease in its quantity proportionately.

In bioassay tests of pathogens, LT_{50} and LT_{50} are the indicative of their pathogenicity intensity. Nevertheless the pathogenicity and its intensity are influenced by various factors. The difference in virulence may be due to the fungal genetic material and other internal physiological factors within the host (Miranpuri & Khachatourians, 1995). The results of the study indicated that DEBI008 isolate with the lowest LD_{50} and LT_{50} is the most appropriate isolate. It is emphatically suggested to tested this isolate in natural conditions to control CPP.

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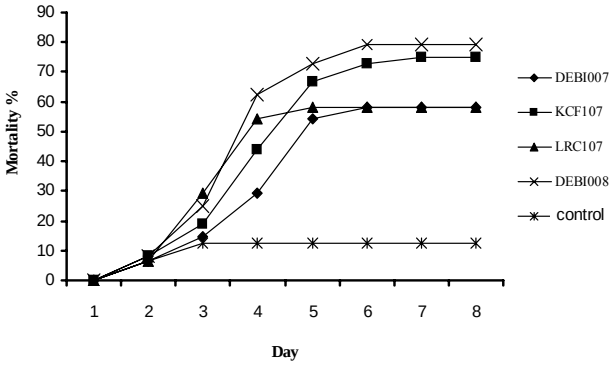
Fig. 1. Diagram of 5th instar nymph's daily mortality through the different isolates of *B. bassiana*



REFERENCES

Allahverdipour, H., A. Kharrazi-Pakdel, M. Ghazavi and P. Azmayeshfard, 2004. The assay of pathogenicity of the Iranian isolates *Beauveria bassiana* (Bals.) Vuill. On sunnpest (*Eurygaster integriceps* Put.) and investigation of mass culture conditions of one isolate. *M.Sc. Thesis*, P: 122. Department of Entomology, School of Agriculture, Tehran University

Bidochka, M.J., M. Kkamp Andrena and J.N. Amriha De Croos, 2000. Insect pathogenic fungi: from genes to population, 171-193, *In*: Kronstad, J.W. (ed.), *Fungal Pathology*, Kluwer Academic publishes Netherlands

Burckardt, D. and P. Lauterer, 1989. Systmatics and biology of the Rhinocolinae (Homoptera: Psylloidea). *J. Natural History,* 23: 643–12

Butt, T.M. and M.S. Goettel, 2000. Bioassys of entomogenous fungi. *In*: Navon, A. and K.R.S. Ascher (eds.), *Bioassys of Entomopathogenic Microbes and Nematodes,* Pp: 141–96. CABI publishing Wallingford

Cherry, A.J., P. Abalo and K. Hell, 2005. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Basamo) Vuillemin and *Metarhizium anisopeliae* (Metschnikoff) to control *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae) in stored cowpea. *J. Stored Products Res*., 41: 295–309

Feng, M.G., J.B. Johnson and L.P. Kish, 1990a. Virulence of *Verticillium lecanii* and an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) for six species of cereal aphids (Homoptera: Aphididae). *Environ. Entomol.,* 19: 815–20

Hatting, J.L., S.P. Wraight and R.M. Miller, 2004. Efficacy of *Beauveria bassiana* (Hyphomycetes) for control of Russian wheat aphid (Homoptera: Aphididae) on resistant wheat under field conditions. *Biocontrol Sci. Technol*., 14: 459–73

Jenkins, N.E. and M.S. Goettel, 1997. Methods for mass-production of microbial control agents of grasshoppers and locusts. *Memo is of the Entomological Society of Canada,* 171: 37–48

Lababidi, M.S., 2002. Effects of Neem Azal T/S and other insecticides against the pistachio psyllid *Agonoscena targionii* (Licht.) (Homoptera, Psyllidae) under field conditions in Syria. *J. Pest Sci.,* 75: 84–8

Lauterer, P., T. Broumas, S. Drosopoulos, C. Souliotis and A. Tsourgianni, 1998. Species of the genus *Agonoscena targionii*, pests on *pistacia* and first record of *A. pistaciae* in Greece. *An. Insect. Phytopathol*., 18: 123–8

Mart, C., L. Erkilic, N. Uygun and M. Altin, 1995. Species and pest control method used in pistachio orchards of Turkey. *Acta Horticul*., 419: 379–68

Miranpuri, G.S. and G.G. Khachatourians, 1995. Entomopathogenicity of *Beauveria bassiana* (Balsamo) Vuillemen and *Verticillium lecani* (Zimmerman) toward English Grain Aphid, *Sitobion avenae* (Fab.) (Homoptera: Aphididae). *J. Insect Sci.,* 8: 34–9

Samih, M.A., A. Alizadeh and R. Saberi Riseh, 2005. *Pistachio Pests and Diseases in Iran and their IPM,* P: 301. Jahad Daneshgahi-Tehran

Talaei Hasanloui, R., A. Kharrazi-Pakdel and G. Hejaroud, 2000. Laboratory investigation on pathogencity of the foungus *Beauvaria bassiana* (Bals.) Vuill. On sunnpest (*Eurygaster integriceps* Put.). *MSc. Thesis*, P: 94. Department of Entomology, School of Agriculture, Tehran University

Talaei Hasanloui, R., A. Kharrazi-Pakdel and M.S. Goettel, 2005. The investigation of genetical diversity of the foungus *Beauvaria bassiana* (Bals.) Vuill. and their pathogencity on *plutella xylostella* and *Leptinotarsa decemlineata. Ph.D. Thesis*, P: 170. Department of Entomology, School of Agriculture, Tehran University

Todorova, S.I., C. Cloutier, J.C. Cote and D. Coderre, 2002. Pathogenicity of Six Isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deutetomycotina), (Hyphomycetes) to *perillus bioculatus* (F) (Hem, Pentatomidae). *J. Appl. Entomol*., 126: 182–5

Ugine, T.A., S.P. Wraight, M. Brownbridge and J.P. Sanderson, 2005. Development of a novel bioassay for estimation of median lethal concentrayions (LC_{50}) and doses (LD_{50}) of the entomopathogenic fungus *Beauveria bassiana*, against western flower thrips *Frankliniella occidentalis*. *J. Invertebr. Pathol*., 89: 210–8

Wraight, S.P., R.I. Carruthers, C.A. Bradleg, S.T. Jaronski, L.A. Lacey, P. Wood and S. Galaini–Wroigh, 1998. Pathogenicity of the entomopathogenic fungi *paecilomyces* spp. and *Beauveria bassiana* against the Silverleaf whitefly, *Bemesia argentifolii*. *J. Invertebr. Pathol*., 71: 217–26

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