STUDY ON THE EFFECTS OF SOME IRANIAN ISOLATES OF THE FUNGUS *BEAUVERIA BASSIANA* (BALSOMO) VUILL. (DEUTEROMYCOTINA: HYPHOMYCETES) ON THE BIRD CHERRY-OAT APHID, *RHOPALOSIPHUM PADI* (LINNAEUS) (HEMIPTERA: APHIDIDAE), UNDER LABORATORY CONDITIONS

Aida Sedighi*, Mehran Ghazavi, Hana Haji Allahverdipour** and Ali Ahadiyat***

* Department of Entomology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, IRAN. E-mails: Aida.sedighi@gmail.com; Ali.ahadiyat@hotmail.com
** Iranian Research Institute of Plant Protection, Tehran, IRAN. E-mails: hana_agri@yahoo.com; Mehr729@yahoo.com

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ABSTRACT: According to the importance of non-chemical controlling methods based on integrated pest managment, *Beauveria bassiana* was considered as a pathogen of the bird cherry-oat aphid, *Rhopalosiphum padi*. In this study, the pathogenicity effects of some Iranian isolates of *B. bassiana* against adult aphids were evaluated using the spray bioassay method. Seven isolates, including DEBI 001, DEBI 002, DEBI 003, DEBI 006, DEBI 008, DEBI 010 and DEBI 015, were cultured on the SDA medium and incubated under dark conditions at 25°C for 15 days. The minimum and maximum dosages of each strain was determined and then 5 different conidial concetrations were prepared based on the logarithmic distances Experiments were carried out based on RCD, and repeated 4 times and for each replicate 20 adult aphids were treated. The control aphids were treated with distilled water_tween 80. Treated aphids put in plexiglass cups in an incubator at 25±2°C, and mortality was recorded daily for 5 day. Data were analyzed using SAS (6.2) and Curve Expert 1 4 to determine LC_{50} and LT_{50}. The lowest and highest LC_{50} were recorded 0 06 and 119 26 Spore/ml, and the lowest and highest LT_{50} were recorded 2 08 and 4 57 days, both respectively using DEBI 015 and DEBI 001 isolates.

KEY WORDS: Fungus, *Beauveria bassiana*, isolate, aphid, *Rhopalosiphum padi*, LC_{50}, LT_{50}.

The bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus), is one of the important aphid species related to gramineous plants. Species is widely distributed in most areas of grain-growing in Iran, and also has been observed on some rosaceous plants, including plum and wild plum (*Prunus* spp.) in north of the country (Rezwani, 2001 and 2004; Radjabi & Behrouzin, 2003). This aphid attacks to several gramineous species, especially wheat, barley, corn, rice and even herbs (Rezwani, 2001 and 2004), and produces toxic saliva and vectors plant viruses (Morrill, 1995), in which Barley Mosaic Virus, Barley Yellow Dwarf Virus, and some rice viruses can be mentioned (Rochow & Eastop, 1966; Rochow & Gill, 1978; Radjabi & Behrouzin, 2003). Using pathogens, as biological control agents of some insect's species, has been increased during the last few years (Hafez et al , 2001). There are over 500 fungi known to be associated with insect diseases.Among them, *Beauveria bassiana* (Balsomo) Vuill**.** is recognized as the first entomopthogenic microorganism (Romoer & Stoffolano, 1998). This fungus is one of the most important fungi associated with insects, and a wide range of

insect orders, including Coleoptera, Hemiptera, Lepidoptera, Orthoptera and Thysanoptera are considered as its hosts (Hajek, 2004). It has several characters which help it to be more effective against pests. The growth rate is moderately rapid. It possesses many strains that exhibit considerable variation in virulence, pathogenicity and host range (Herington, 2006). Several studied have been carried out on fungal pathogens against cereal aphids. For example, Ganassi et al. (2001) studied the effect of some entomopathogens against the greenbug, *Schizaphis graminum* (Rond), and found that many fungal species, such as *B. bassiana*, might be good candidates as biocontrol agents of the aphid. Virulence of *Verticillium lecanii* (Zimm) Viegas and an aphid-derived isolate of *B. bassiana* for six species of cereal-infesting aphids were evaluated by Feng et al. (1990). They found that *B. bassiana* kills aphids more rapidly. This fungus species has been examined on other aphid species. For example, during Van Hanh et al. (2007) studies, it was evaluated on Green peach aphid *Myzus persicae* (Sulz) and it could be one of the most effective fungi against the pest, with other entomopathogenic fungi. The effect of entomopathogenic fungi in Iran has extremely been studied, but their roles against aphid pests have been evaluated more or less. For example, Savafi et al. (2003) investigated the virulence of *V. lecanii.* against pea aphid *Acyrthosiphom pisum* (Harris). In the other research, Derakhshan Shadmehri (2009) tested the effect of 25 isolates of pathogenic fungi on mealy cabbage aphid *Brevicoryne brassicae* (L.).

Because of the problems related to using chemical insecticides against pests, such as environmental contamination, adverse effects on non-target organisms, residues in food and water, and accumulation of chemicals in food chain, there has been renewed interest in using microbial insecticides for controlling crop pests (Powell et al , 2005). This study evaluated seven isolates of the entomopathogenic fungus for their virulence to the bird cherry-oat aphid, in order to select a suitable isolate with the greatest potential for controlling the pest in a successful integrated management program. According to the importance of this aphid in Iran, it is necessary to achieve safe control ways for environments. So this study has been done for recognizing the entomopathogens for controlling aphids.

MATERIALS AND METHODS

Collecting, rearing, and preserving aphids

Aphids were collected in autumn with cutting the stem of wheat from, Karaj. This region is cold and one of the most important areas of *R. padi* in Iran. Collected insect were identified by Dr. Rezwani in Iranian Research Institute of Plant Protection. After separating aphids, they were transferred to the plastic little vases (8 diameters) that were planted 15 days before. Every week vases were changed until aphids were fed on fresh wheat. They were maintained in the laboratory of the Department of EntomologyIranian Research Institute of Plant Protection, Tehran, Iran under controlled conditions (23±2 ˚C, 60±10 RH and 16L: 8D).

Fungal culture

Seven isolates of *Beauveria bassiana* were obtained from a collection at the Iranian Research Institute of Plant Protection. Table1 shows a list of the isolates, their hosts, in which the isolate was collected, and their reigns. The fungus was culture on Sabouraud Dextrose Agar (SDA) in 90 mm petridishes and covered with parafilm. Fungal culture were kept in an incubator at 25±1˚C and photoperiod of 16:8 hours (L: D) for 15 days.

Spore germination

Fungal suspensions were prepared by scrapping conidia from the surface of cultures using a sterile loop and added to tween 80 (0 5 sterile distilled water) Tween 80 were sterilized with membranous filter before. Suspensions were shaken fast. Resulting suspension were filtered through three layer sheer to separate mycelium. Resulting suspensions were kept in Mc cartney bottle with some sterile tiny balls in order to break hypha chain. Viability was determined by using Agar-agar (granulated) medium. Suspensions were sprayed all over medium. After 18 hours the germinated spores were counted in 100 random spores in a microscope field. The viability of all isolates was confirmed before using in experiments. By haemoctometere lam, the concentration series were determined.

Pathogenicity assay

Repeated cultures in artificial medium were decreased viability of each isolate. So, each suspension was used against *Galleria mellonella* (L.) larva. Each larva was dipped through water for 10-20 seconds. Larvae were kept in sterile petri in incubator for 7-10 days. After appearance of spores through larva bodies, new culture of each isolate were culture in SDA medium. All aphids used in this study were as adult stage. The minimum and maximum dosages of each strain were determined and 5 different conidial concentrations were prepared based on the logarithmic distance. Experiments were carried out based on RCD and repeated 4 times and for each replicate 20 adult aphids were treated. The control aphids were treated with distilled water-tween 80. Each 20 adult aphids were put in Buchner funnel that was covered with whatman paper (9cm diameters/Whatman No.1). The funnel was connected with vacuum flask. Aphids were sprayed for 5 seconds from 25 centimeter distance. Treated aphids were put into sterile petridish that contained with wet cotton and fresh wheat leaves. They were kept in incubator at 23±2°C, 60±10 humidity and 16:8 (L: D). Petridishea were observed daily for ten days and dead aphids were counted and transferred to another petridishes to observation of fungal growth.

Statistical analysis

Mortality data were corrected by Abbott s formula (Goettel and Inglis,1997). LC_{50} and LC_{99} estimates were carried out using SAS (6.2) program. Also analysis of variance was carried out by SAS program.LT_{50} of each isolates was recorded by Curve Expert 1.4. Data were analyzed using SAS (6 2) and Curve Expert 1 4 to determine LC_{50} and LT_{50}

RESULTS

The observations of the developing of the fungal hypha through insect bodies were made sure that the death of aphids cussed by fungi attack no other agents Germination of all isolates ranged from 85 to 95 Lethal time of every isolates was calculated (table 2) Between the Lethal times of every isolates were not significant differences (Figure 1).

Probit used for calculating LC_{50} The lowest lc_{50} on the adult aphids were pertained to DEBI 015 and the highest LC_{50} was recorded for DEBI 002 (Table 3).

According to the amount of P_value, there were significant differences among various isolates (Table 4) Comparing LC_{50} of each isolate with one Index isolate (the index was DEBI 002 with the highest LC_{50}) indicated that there were not significant differences between DEBI 003, DEBI 001, and DEBI 021 with DEBI 002 And also there were significant differences between DEBI015, DEBI004 and

DEBI 021 with DEBI 002. These analyses were obtained with ɵ analysis (Robertson et al , 2007).

DISCCUSSION

Result showed that DEBI 002 isolate with maximum LC_{50} had a lowest virulence and DEBI015 with the minimum LC_{50} had highest virulence again the *Rhopalosiphum padi* (Table 3) DEBI002 was an isolate derived from soil. Although some soil derived isolates imposed higher mortality in comparison with other isolates of coleopteran and orthopteran (Goettel et al., 1990), DEBI002 showed the lowest impact in our tests. That needs more revision. The investigation on Russian aphid indicated that DEBI 002 isolate with the lowest LC_{50} had the maximum virulence in comparison to other isolates (Mohamadipor, 2006). In bioassay tests on *Eurygaster integriceps,* the DEBI 002 $3\,78\times10^{3}$ (spore insect) was the best native isolate (Rastegar, 2007). In study on the common pistachio psylla, *Agonoscena brunneus*, results showed that DEBI008 the native isolate that extract from a grasshopper, *Chorthippus brunnes* (Thunberg) with $3\,91 \times 10^{2}$ spore insect has minimum LD50 (Alizadeh et al , 2007). The comparison of the LT50 showed that there were not significant diffrences between various isolate in 10 days (Table 2). The study on the LT50 of different isolates against *Sitobion aveane* showed that their LT50 were 3 – 5 4 days (Miranpuri & Khachatourians, 1995). The lowest LT50 that was recorded for DEBI 002 isolate against Russian aphid was 2 4 day (Mohamadipor, 2006) in bioassay tests of pathogens, LT50 and LC50 are indicative of their pathogenicity intensity (Miranpuri & Khachatourians 1995). The high virulence of the isolates DEBI004 and DEBI 015 against *Rhopalosiphum padi* indicates that these isolate are appropriate agents for biological control against cereals aphids. However, there is nearly no use of formulated entomopathogenic fungi in less developed countries, such as Iran (Safavi, et al., 2010). According to the importance of this aphid in Iran, It is necessary to acchive safe control ways for environment. It seems that the usage of entomopathogense in framework of IPM is promising and encouraging in Iran.

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Table 1. Details of the isolates of entomopathogenic fungi used in tests.

Letter name	Uppercase	Lowercase
DEBI 001	Soil	Fashan,Iran
DEBI 002	Soil	Karaj,Iran
DEBI 003	*Rhynchophorous ferrugineus*	Saravan,Iran
DEBI 004	*Hyper postica*	Ghazvin,Iran
DEBI 010	*Eurygaster integriceps*	Varamin ,Iran
DEBI 015	*Shingonotus sp*	Garmsar,Iran
DEBI 021	*Ceroplastes sinensis*	Sari,Iran

Table2. LT50 of entomopathogenic fungi against *R. padi*.

Fungal Isolate	LT50
DEBI 001	2 08
DEBI 002	2 53
DEBI 003	2 29
DEBI 004	2 41
DEBI 010	2 38
DEBI 015	2 41
DEBI021	2 96

Table 3. The log of LC 50 and LC 99 of population and slop

Fungal	LC50	Limits 50	LD99	Limit 99	Slop
DEBI 001	1 71	(1 16-2 02)	4 04	(3 58-4 95)	1 00
DEBI 002	2 21	(1 57-2 63)	6 9	(6 1-8 27)	0 44
DEBI 003	1 17	(0 21-1 87)	6 65	(5 69-8 62)	0 42
DEBI 004	0,22	(0 5-0 75)	5 17	(4 17-8 91)	0 58
DEBI 010	2 16	(1 44-2 62)	7 27	(6 36-7 86)	0 45
DEBI 015	0 23	(o 43-0 99)	3 73	(3 08-6 57)	0 58
DEBI 021	2 08	(2 71-2 91)	5 26	(0 91-4 07)	0 72

Table 4. Analysis of variance.

Source	DF	Sum of Square	Mean Square	F-value	P-value
Isolates	6	83.48	13 91	6.39	0.0006*
Error	21	45.70	2 17		
Total Error	27	129.18			

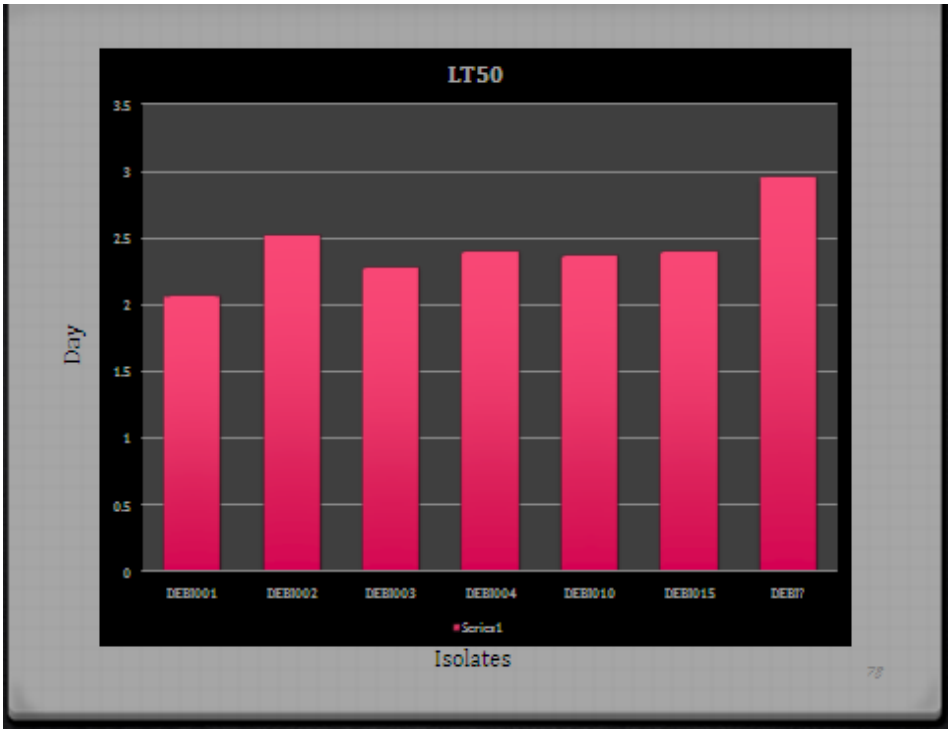

Figure 1. Lethal time of different isolates of *B. bassiana*.