

Biological Characterization of *Beauveria bassiana* (Clavicipitaceae:Hypocreales) from Overwintering Sites of Sunn Pest, *Eurygaster integriceps* (Scutelleridae: Heteroptera) in Iran

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ABSTRACT

Sunn pest is one of the most important cereal pests that affect both grain yield and quality of cereal in Iran. One potential control strategy is to use entomopathogenic fungi such as *Beauveria bassiana* Vuillemin as mycoinsecticide to control this pest. Naturally infested Sunn pests in Kermanshah province were collected and brought to laboratory. 10 isolates were selected for further analysis in the laboratory. Isolate characters such as colony growth, conidia production, virulence and dose response were studied under laboratory conditions. Colony growth increased linearly overtime at 27° C. Isolate IR-K-43, IR-K-23 and IR-K-10 had the largest growth rate of 3.6 mm/day. Isolate IR-K-36 with 4.6×10^8 conidia/cm² was the highest conidia producer. All of the selected isolates showed pathogenicity against *Eurygaster integriceps* Puton. Five Iranian isolates IR-K-40, IR-K-58, IR-K-10, IR-K-53 and IR-K-43, and one Turkish isolate SPT 22 were high virulent isolates causing mortality ranging between 88.7–94.4 %. Isolate IR-K-40 along with isolate SPT 22 from Turkey was selected for dose response study. The lethal concentration required to kill 50% of insects (LC₅₀) was 2.9×10^7 conidia/cm² for both isolates. The results of this study showed that *B. bassiana* can be isolated from Sunn pest overwintering sites in Kermanshah. In addition the selected isolates were pathogenic towards *Eurygaster integriceps* Puton under laboratory conditions. These results will help in selection and development of these fungi for Sunn pest, *E. integriceps* management in the future.

Keywords: *Beauveria bassiana*; Sunn pest; Biological control; Colony growth; LC₅₀; Pathogenicity test.

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INTRODUCTION

Sunn pest, *Eurygaster integriceps* Puton, belonging to Scutelleidae family of pests, is one of the dominant wheat and barley bugs in Europe, North Africa, the Middle East and Iran (Brown, 1962 and Rassipour *et al.*, 1996). Various genera of Scutelleidae or of Pentatomidae (including *Eurygaster integriceps*) are migratory, implying that they can even survive or hibernate in high altitudes and harsh wintering regions (Zwoller, 1930; Critchley, 1998). During spring and early summer, this pest spends annually approximately two and half to three months feeding on wheat and barley in the fields and then migrate to the foothills of mountains for the rest of the year which is called overwintering period (Brown, 1962). Although the migration to wintering areas also depends on other physiological factors such as food storage and avoiding flights (Critchley, 1998).

Chemical control is presently the only method used to prevent Sunn pest damage (Javahery, 1995). A broad spectrum insecticide; fenitrothion is used for chemical control of overwintered adults and new generation Sunn pest (Anonymous, 2009). Chemical control using fenitrothion and other chemical insecticides has not been satisfactory due to several reasons. First Sunn pest populations have been increasing and have resulted in periodic outbreaks over the past five decades. It was thought for many years that using new organophosphate compounds would end Sunn pest outbreaks. However, Sunn pest outbreaks have occurred three times in Iran during the period of 1963 to 1993 (Javahery, 1996). In addition other neighbor countries like Iraq had number of Sunn pest outbreaks from 2001-2004 (Hamma *et al.*, 2007). This shows the severity of Sunn pest problem.

Second, the intensive chemical control is not economically sustainable. For example, the total aerial and ground chemical treatment of about 1.8 million ha of wheat and barley in Turkey amounts to 42 million US dollars. The annual cost of chemical control in West Asia estimated at 40 million \$ US (Javahery, 1995).

Third, chemical control leaves adverse effects on the natural enemies of Sunn pest, and on the environment (Kinaci *et al.*, 1998). For instance egg parasitoids (e.g. *Trissolcus grandis* Thomson), which are one of the most important natural enemies of Sunn pest are affected by the chemical insecticides used for Sunn pest. Saber (Saber, 2007) reports the toxic effects of Fenitrothion on egg parasitoids of Sunn pest.

An alternative to chemical pesticides is to use entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv)

Vuillemin, and the potential of this fungus to manage Sunn pest in their overwintering sites (Edgington *et al.*, 2007; Skinner *et al.*, 2007).

A significant proportion of the Sunn Pest population overwinters in highlands, sheltering under bushes and trees, therefore evaluation of the naturally fungal infested Sunn pests in overwintering sites could be the first step towards biological control of Sunn pest using insect-pathogenic fungus (Skinner *et al.*, 2007). Focus on just one element for example the isolate is not useful. The successful utilization of insect-pathogenic fungi to control Sunn pest will rely on a number of critical factors that need to be optimized. These factors include virulence, persistence and delivery of the fungus (Edgington *et al.*, 2006).

The aim of the current study was firstly to find naturally fungal infested Sunn pests from overwintering sites in Kermanshah province in Iran, and secondly to isolate and evaluate their biological characterization under laboratory conditions.

MATERIAL AND METHODS

Fungal collection and recovery

The search for infected Sunn pests was conducted in overwintering sites in Kermanshah province located in west of Iran close to the Iraq border for isolation of insect-pathogenic fungi.

Around 60 infected insects including bees and beetles were found in searching these sites, and brought to laboratory. To recover the specimens they were cultured several times to become pure.

Isolation and identification of insect-pathogenic fungi

Natural habitats of Sunn pest such as leaf litter under bushes and trees were searched for signs of fungal infection (A white mass covering the insect cuticle). Pieces of insect cuticle were collected by hand or forceps from randomly chosen sites. Each collected piece was stored in a plastic tube for further analysis. Each tube was opened in a sterile dish at the laboratory, and a small portion of the infected tissue was transferred to a sterile Sabouraud Dextrose Agar (SDA) plate. After isolation it is usually necessary to ensure that the isolated fungus is free from contaminant microorganisms (Goettel & Inglis, 1997), thus the isolates obtained in this study were sub cultured several times (depending on the specimen at least three times) to acquire pure cultures. The cultures were incubated at 27°C (all dark incubators).

Whole mount slide of each fungal isolate were inspected under 40X magnification for preliminary identification using diagnostic keys (Poinar & Thomas, 1978). If it was one of the known insect-pathogenic fungi, it was given a code and stored at 4°C for further research at Iranian Plant Protection Institute.

Selection of final isolates

Nine isolates from Kermanshah province (Iran) all identified as *B. bassiana* and one *B. bassiana* isolate collected from Turkey were selected. The isolate from Turkey was used as a positive control since it was used in the same experiments. In total ten isolates were used in all experiments (table 1).

Table1: Fungal strains selected for further studies in this thesis

Isolate code	Species	Collection data		vegetation
		Location	Host	
IR-K-10	<i>B.bassiana</i>	Tappeh Maran* (Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-23	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-31	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-36	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-37	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-40	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-43	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-53	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
IR-K-58	<i>B.bassiana</i>	Tappeh Maran(Iran)	<i>E. integriceps</i>	Astragalus sp
SPT 22	<i>B.bassiana</i>	Yavuzeli,(Turkey)	Adephaga	

* Tappeh Maran is the name of a hill in Kangavar region

Insect material

All the adult *E. integriceps* used in this study were collected from overwintering sites, mainly under *Astragalus spp.*, in Varamin in June 2007. At the laboratory the Sunn pests were maintained in boxes with wheat grains and water as food and water sources. The insect material was maintained under this condition as quarantine for two weeks to be checked for natural infestation. For each experiment alive healthy insects were used.

Pathogenicity study

The 10 selected isolates were cultured in sterile SDA Petri dishes (15 cm diameter) 10 days before the experiment. 55 individuals of *E. integriceps* were used for each fungi isolate. The insects were exposed to each isolate by placing them in a Petri dish of a well grown fungus for 45 minutes in order to make sure that all of them were in contact with the fungal conidia. After 45 minutes of exposure to fungi, the insects were taken out and placed in 15 cm Petri dishes. They were left with wet cotton inside a plastic cap and 20 wheat grains as sources of water and food and incubated at 27° C. As control samples, another set of 55 insects were placed into 3 Petri dishes without exposing them to the fungal conidia. From the next day on, the insects were checked daily

to see if they had enough water and also to collect the cadavers. The cadavers were taken out and subjected to superficial sterilization by placing them in Ethanol 50% for 5", in sodium hypochlorite for 30" and finally three times in sterile distilled water for 10". To encourage the growth of the fungal mycelia after the sterilization dried, the cadavers were placed in Petri dishes lined with pieces of damp filter. The Petri dishes containing the cadavers were sealed and incubated at 27°C to observe the out growth of fungi from cadavers.

To inoculate the insects for the experiments in this work, we used the topical method, in which a specific volume of the conidia suspension is injected into the insect's joints. The use of this method usually depends on the size of the insect, the number of insects to be inoculated, the formulation, and the precision required (Goettel & Inglis, 1997). To study the virulence of the fungal strains, beside the isolate from Turkey, one isolate which made the insects die in shortest time in the pathogenicity study was selected. The isolate from Turkey was selected as the positive control.

For each concentration 60 individuals of *E. integriceps* were used. The two fungal isolates were grown and the conidia harvested as described below. After 10 days of incubation, the conidia of each isolate were harvested by washing with 10 ml tritinin X-100 and scrapping with a sterile spatula. The suspension was filtered through a single layer of linen or 8 layers of cheese cloth to remove culture debris and mycelia. The conidia were enumerated using a microscope and a hemacytometer, and their densities were adjusted to a countable concentration by dilution.

Details of the conidia enumeration and the density dilution are as follows: In an experimental tube 9 ml distilled water was poured. 1 ml of the filtered conidia suspension was added to this tube to obtain the first concentration. After shaking the first concentration 1 ml of it was added to 9 milliliter of distilled water to obtain the second concentration. This will be continued until the concentration was measureable.

Conidia were enumerated using a hemacytometer and the highest density for each isolate was diluted into four concentrations. The concentrations for IR-K-40 were 23×10^7 , 23×10^6 , 23×10^5 , 23×10^4 conidia/ml while for isolate SPT 22 they were 8×10^7 , 8×10^6 , 8×10^5 , 8×10^4 conidia/ml.

For each concentration the topical method was used where 0.5 µl of each concentration were insulated to every insect with a sampler. 240 insects used as control were insulated with 0.5µl of tritinin X-100. Thereafter the procedure was as in the pathogenicity study.

Statistical methods

A comparison between isolates in their conidial production with corrections for multiple testing was made.

The collected data on pathogenicity study was analyzed using the proportional Odds model for cumulative mortality. The Proportional odds models are fitted using polr (in R version 2.6.0 (2007-10-03)). The duration of experiment (14 days) was divided to 3-day test groups (1-6, 7-11 and 12-14, the groups within which the mortality of the insects were tested for different isolates. The day groups were chosen because it was easier to interpret the result of the statistical analysis compare to the individual days. The effect of isolate on the mortality of insects was tested using the likelihood ratio test of ordinal regression model (Chisq). Pair wise t was made to compare the isolates concerning insect mortality with corrections for multiple testing. The Mean Survival Time (MST) defined as the number of days needed to achieve an accumulated 50% mortality was calculated using the life test in SAS.

Data of the virulence test for the Isolates SPT 22 and IR-K-40 were analyzed using the probit model in SAS. The data was corrected with the control mortality. The concentrations converted to logarithms scale for better homogeneity. Table 2 summarizes the models and the mathematical formula used for each data set.

Table 2; Percent corrected cumulative mortality of *E. integriceps* treated with the 10 *B. bassiana* strains and percent mortality of non-treated control

Isolate	Species	Mortality		
		Day6	Day 11	Day15
Control	-	0.1(0-0.3)%	2.5(0.8-7.8)%	5.6(1.8-16.2)%
IR-K-40 ¹	<i>B. bassiana</i>	22.5(7.1-52.3) %	88.0(65.9-96.5)%	94.4(81.7-98.4)%*abce
IR-K-58 ¹	<i>B. bassiana</i>	17.6(5.3-45.1) %	84.3(58.3-95.4) %	92.5(76.4-97.9) %*abce
SPT 22 ¹	<i>B. bassiana</i>	16.0(4.8-41.7) %	82.8(56.1-94.7) %	91.7(74.7-97.6) %*abce
IR-K-10 ¹	<i>B. bassiana</i>	14.6(4.3-39.5) %	81.2(53.1-94.3) %	90.9(72.3-97.4) %*abcde
IR-K-53 ¹	<i>B. bassiana</i>	13.1(3.8-35.6) %	79.1(50.0-93.5) %	89.7(69.8-97.1) %*abcde
IR-K-43 ¹	<i>B. bassiana</i>	11.9(3.5-33.7) %	77.3(47.5-92.7) %	88.7(67.6-95.9) %*abcdef
IR-K-31 ²	<i>B. bassiana</i>	7.3(2.1-22.6) %	66.5(34.9-88.0) %	82.1(55.3-94.4) %*bcdefg
IR-K-37 ²	<i>B. bassiana</i>	5.(1.4-16.2)%	56.9(26.4-82.6)%	75.3(45.7-91.8)%* defghij
IR-K-36 ³	<i>B. bassiana</i>	2.6(0.7-8.9) %	40.3(15.7-71.0) %	61.0(30.1-85) %*ij
IR-K-23 ³	<i>B. bassiana</i>	2.0(0.6-7.1) %	34.5(12.0-65.7) %	54.9(25.0-81.6) %*j

A * indicates a significant difference of the isolates from the control

Isolates followed by the same letter were not significantly different for each column

1: high virulent

2: medium virulent

3: low virulent

RESULTS AND DISCUSSION

Fungal collection and recovery

The population and species of the Sunn pests in each of the inspected climatic regions was different. From the collections of infected cadavers from Kermanshah province, around 100 fungal isolates were recovered. According to the colleagues in Iranian Research Institute of Plant Protection, *Beauveria*. Spp and *Paecilomyces*. Spp. were the most commonly genera recovered. All of the 9 selected isolate for further analysis were identified as *B. bassiana* (Table.1). Identification of fungal isolates was based on the observation of slides and the keys (Poinar & Thomas, 1978). The formation of the spore balls, zigzag shape of the phialids and the swollen conidiophores are some of the characteristics used to identify the isolates (Table1).

Pathogenicity studies

In these studies all isolates tested showed pathogenicity to *E. integriceps*. Differences between the isolates need to be confirmed by repetition of this study with the same isolates. However under the particular conditions of this work there were significant differences between isolates in causing mortality to *E. integriceps* ($p=0$, $df=10$, $LR=235.4881$). Mortality in the control group was low at about 5.6% 14 days post infection. Isolate IR-K-23 and IR-K-43 had the highest growth rate of 3.6 mm per day within 10 days. There was a significant difference between the isolates in their growth rate and conidial production and there was no correlation between the colony size and the conidial production. Isolate IR-K-36 had the highest conidial production of 4.6×10^8 conidia/ml after 10 days.

None of the isolates reached to 50% mortality at the 6th day after infection and the highest percentage belonged to isolate IR-K-40 with 22.5% mortality of *E. integriceps*. The mortality of *E. integriceps* recorded on the 11th days post infection ranged from 34.51% and 87.97% and the control mortality was 2.49% . The most efficient isolate was IR-K-40 with 94.4% mortality 14 days post infection and the least efficient one was IR-K-23 with 54.9% mortality 14 days post infection (Table.3).

Table 3: The mean survival time of *E. integriceps* subjected to 10 isolates of *B. bassiana*

Isolate	species	mean survival time	Standard Error
IR-K-10	<i>B. bassiana</i>	9.0 a*	0.39
IR-K-23	<i>B. bassiana</i>	12.0 b	0.35
IR-K-31	<i>B. bassiana</i>	10.3 cb	0.34
IR-K-36	<i>B. bassiana</i>	11.9db	0.37
IR-K-37	<i>B. bassiana</i>	10.5 ecd ⁺	0.43
IR-K-40	<i>B. bassiana</i>	8.1 a ⁺ f ⁺	0.28
IR-K-43	<i>B. bassiana</i>	9.4 ac ⁺ g	0.36
IR-K-53	<i>B. bassiana</i>	9.2 ac ⁺ gh	0.37
IR-K-58	<i>B. bassiana</i>	9.3 ac ⁺ ghi	0.31
SPT 22	<i>B. bassiana</i>	8.7afghi	0.32

*Numbers followed by the same lower case letter are not significantly different (Log-rank and Wilcoxon test were used to do pair wise comparison between the isolates p<0.05).

Log-rank test shows how different are the two isolate in the beginning of the experiment and the

Wilcoxon test shows how different are the two isolates at the end of the experiment

+ indicates that the two tests do not show the same result (one is significant and one is not)

The pair wise comparison between the isolates indicated that all of the isolates are significantly different from the control at 14 days post infection . Although isolate IR-K-40 has the highest cumulative mortality 14 days post infection, it was still not significantly different from the other highly virulent isolates.(Table.3).

The cumulative mortality increased more rapidly in day group (7-11) except for the control, IR-K-36 and IR-K-23. The Mean Survival Time (MST) ranged between 8.1 and 12.0 days. Isolate IR-K-40 caused the lowest MST and isolate IR-K-23 caused the highest MST (Table4).

Table 4: Percent corrected cumulative mortality of *E. integriceps* treated with four concentrations of isolates IR-K-40 and SPT 22 and percent mortality of non-treated control and percent mortality of non-treated control

Isolate	Species	Mortality		
		Day6	Day 11	Day15
Control	-	2.7(1.5-4.3) %	7.1(4.6-10.8) %	9.7(6.4-14.7) %
SPT 22(8×10 ^{4a})	<i>B. bassiana</i>	2.3(0.8-6.1) %	6.1(2.2-15.4) %	8.3(3.1-20.4) %
SPT 22(8×10 ⁵)	<i>B. bassiana</i>	4.6(2-10.1) %	11.8(5.4-24.0) %	15.9(7.5-30.7) %
SPT 22(8×10 ⁶)	<i>B. bassiana</i>	2.1(6.3-21.9) %	27.7(15.8-43.9) %	35.0(18.9-49.4) % *
SPT 22(8×10 ⁷)	<i>B. bassiana</i>	35.8(22.9-51.2) %	60.9(45.3-74.5) %	68.7(50.8-78.5) % *
IR-K-40(23×10 ⁴)	<i>B. bassiana</i>	4.4(1.9-9.7) %	11.4(5.2-23.1) %	15.32(7.2-29.7) %
IR-K-40(23×10 ⁵)	<i>B. bassiana</i>	2.2(0.9-6.0) %	6.0(2.4-15.2) %	8.2(3.0-20.2) %
IR-K-40(23×10 ⁶)	<i>B. bassiana</i>	12.8(7.0-22.2) %	29.1(17.4-44.3) %	36.6(20.9-49.9) % *
IR-K-40(23×10 ⁷)	<i>B. bassiana</i>	51.7(35.9-67.2) %	75.0(61.0-85.1) %	80.8(66.2-87.7) % *

A * indicates a significant difference of the isolates from the control. If the confidence interval contains zero then the isolates are not significantly different from control.

^a The conidia concentration (conidia/ml)

Some of the insects also turned they color to red a few days after fungal infection. The reason why all of the treated insects didn't turned they color to red is unknown.

Isolate SPT 22 produced the highest percentage of sporulating cadavers, 67.9%, whereas isolate IR-K-10 had the lowest percentage of sporulating cadavers, 12.6%. Observation of cadavers indicated that the mycelium was first seen on the abdominal joints of *E. integriceps*.

Virulence study

One purpose of the virulence study was to select a virulent isolate for further study.

The maximum mortality, 80.80% and 68.70% achieved at the highest concentration for both isolates [IR-K-40 (23×10⁷) and SPT 22 (8×10⁷) conidia/ml] 14 days post infection respectively (Table. 5).

Table 5: The statistical values to calculate the LC₅₀

Estimate value	Std. Error	Pr >Chisq
Intercept -23.2	3.4	<0.0001
Slope 1.3	0.2	<0.0001
Natural ^a 0.10	0.015	

^a Estimated probability that insects die even if they receive dose (0)

The earliest deaths occurred on day1 for the two higher concentrations of isolate SPT 22. In all of the concentration of isolate IR-K-40 the earliest deaths occurred on the third day. The earliest deaths occurred on day 4 and 5 for 8×10^5 and 8×10^4 concentrations of SPT 22 respectively. The Mean Survival Time was only calculated for the two highest concentrations for both isolates, since the mortality was very low for the two lower concentrations. The lowest MST belonged to isolate IR-K-40 at highest concentration with 7.7 days and the highest MST belonged to the same isolate at 2.3×10^7 conidia/ml with 11.4 days. There was an increase in days to 50% mortality with decreasing concentration for both isolates. To analyze the concentration data for the number of conidia required to kill 50% of insects (LC50) value of the two selected isolates the probit model was used. The statistical analysis of the concentration data showed that there is not an interaction between the isolates and the different concentrations ($p=0.09$, $\text{Chisq}=2.9$) meaning that at a fixed concentration there was no difference between the two isolate. The statistical analysis also indicated that there was no effect of isolate ($p=0.23$, $\text{Chisq}=1.4$). This means that we can have one LC50 for the two isolates. The LC50 value calculated for the two isolates using the following formula: $\text{LC50} = \exp [(-\text{Intercept}/\text{slope})]$ was $2.9 \times 10^7 (2.1-3.9 \times 10^7)$ conidia/ml (table 6).

Table 6: Percentage of sporulating insects of the dead *E. integriceps* in the virulence study

Isolate	species	Infected insects
IR-K-10	B.bassiana	12.6%
IR-K-23	B.bassiana	66.9%
IR-K-31	B.bassiana	56.8%
IR-K-36	B.bassiana	31.4%
IR-K-37	B.bassiana	40%
IR-K-40	B.bassiana	41.8%
IR-K-43	B.bassiana	65.3%
IR-K-53	B.bassiana	25%
IR-K-58	B.bassiana	41.2%
SPT 22	B.bassiana	67.9%
Control		33.2%

The results of the infected dead insects in concentration study showed that there is not any relation between the two isolates and different concentrations. The percentage of sporulating cadavers treated with isolate SPT 22 ranged between 44.4% and 80% for different concentrations. This percentage ranged between 27.6% and 100% for isolate IR-K-40. 4.5% of the cadavers in the control group were sporulated.

To the extent of this research it was possible to find insect pathogenic fungi in Sunn pest overwintering sites in Kermanshah province, Iran. All nine Iranian isolates studied in this study for further analysis were identified as *B. bassiana* and were isolated from *E. integriceps*.

The growth rate of the 10 selected *B. bassiana* isolates were (9 Iranian and one from Turkey) similar to the previous studies before (Parker *et al.*, 2003; Tefera & Pringle, 2003). The pink color which has been observed on some of the isolates could be related to the production of oosporein (El Basyouni *et al.*, 1968). This characteristic should be investigated more profoundly in the future studies to see if there is a relationship between the production of oosporein and other characteristics of insect-pathogenic fungi such as its ability to grow out from the cadavers.

The conidia concentration after 10 days had large variability among the 10 isolates. El Damir (2006) proposed that such variability is an important factor that should be considered in selecting isolates for mass production and large scale application purposes. Temperature can have an important effect on conidial concentration and radial growth rate (Tefera & Pringle, 2003; El

Damir, 2006). this was not investigated in this study, but it seems highly relevant to study the effect of temperature both on radial growth and conidia production in order to find the optimum temperature (Tefera & Pringle, 2003; El Damir, 2006).

For the virulence study the insects were exposed to dry conidia and the conidia concentration of the isolates was not measured, while Adan *et al.* (1996) used the suspension of conidia with known concentration. One of the reasons for exposing the insects to the dry conidia was that under the conditions of my experiment the insect were dying when suspended in the fungal conidia.

In my work, all selected isolates of *B. bassiana* from Kermanshah province were pathogenic towards adults of *E. integriceps*. However, they showed large differences in virulence. Since the insect material for this study was obtained from nature and was not reared under laboratory conditions, 5.56% mortality in the control group of virulence study seems to be low. On the other hand Parker *et al.* (2003) had around 16% mortality in the control group treated with Tween 80 which is almost more than three times of the mortality in my studies.

Among the selected isolates, IR-K-40 caused 94.4% mortality in *E. integriceps* in 14 days. Isolate SPT 22, the only isolate collected from Turkey which has been tested before for pathogenicity against *E. integriceps*, caused 91.7% mortality after 14 days of experiment. By looking at the statistical result of the virulence study it can be recommended to view the isolates as low, intermediate and high virulent. According to Tanada and Kaya (1993), strains of fungi within a species isolated from a specific host are more virulent for that host than those isolated from another host, but in my experiment the isolate SPT 22 which was isolated from *Adephaga* was among the highly virulent isolates. Medium virulent isolates also caused relatively mortality of more than 70% in *E. integriceps*.

The Mean Survival Time and the Standard Errors for all of the insects treated with selected isolates were underestimated because of the censored data except IR-K-40 in which all of the insects died before the end of the experiment. The censored data includes the insects surviving the whole experiment.

The Mean Survival Time of the insects for 10 isolates in virulence study was correlated with results of the cumulative mortality in the same study, meaning that the isolates with higher mortality had lower MST. The MST in the virulence study was relatively high (8.1-12 days) compare to study of Adane *et al.*(1996), where *B. bassiana* was assayed against *Sitophilus zeamais* (2.7-8.7 days).

External white mycelial growth from some of the cadavers held under high humidity on Petri dishes containing damp filter paper was evident. The reason why all of the cadavers didn't sporulate is unclear. Adane *et al.* (1996) demonstrated that the white mycelial growth from all cadavers was evident 24-48 hours after death. He also indicated that the exact length of time was isolate dependant. Although the exact day of mycelial growth of the cadavers has not been recorded in this thesis and therefore the dependence of isolate on the time required for infection sign was not clear, but since the cadavers were stored in damp conditions for more than two weeks, the number of insects with mycelial growth is reliable. Since the mycelial growth was analyzed for the dead insects, in some cases where the number of cadavers was very low there was a large standard error in the statistical analysis. Although there is a significant effect of isolate on mycelial growth in cadavers, but no clear relationship can not be detected. The only observed relationship was that isolates with the pink color had higher percentage of infected cadavers after the virulence and concentration study except isolate from Turkey which didn't have a pink color colony. The red color of some of the treated insects in my studies has been also described by Wraight *et al.* (1998). Since oosporein possess antibiotic activity against some of the bacteria (Vining *et al.*, 1962) it might assisted the outgrowth of *B. bassiana* in the dead insects.

In view of the fact that the *E. integriceps* used in this experiment were collected from the nature, it is possible that some of them had other infections or diseases before this experiment, although these insects were reserved in quarantine for two weeks before the experiment and only healthy ones were chosen for the experiment. This also could be a possible reason for having an infected dead insect in the control group in the virulence study.

Different concentrations for the two isolates were used in the dose-response study while Lecuona *et al.* (2001) used same concentrations for different isolates. This of course made the comparison between the two isolates to some extent difficult in my studies. On the other hand the statistical analysis of this data showed that the difference between the two isolates (IR-K-40, SPT 22) was not significant regarding the value of LC50 ($p=0.23$, $\text{Chisq}=1.4$). Quesada-Moraga *et al.* (2006) found that the LC50 of the nine *B. bassiana* isolates assayed against white flies was ranged between 6.5×10^5 – 3.5×10^8 conidia/ml. In my studies the LC50 of the two isolates was 2.9×10^7 conidia/ml which is in the range of LC50 in their study. In addition Quesada-Moraga *et al.* (2006) suggests that the virulence of a given fungal isolate is not only indicated by LC50 but also by the time in days required to achieve 50% mortality. The Mean Survival Time of treated insects was only calculated for the two higher concentrations for both isolates, since there were many insects surviving the whole experiments for the lower concentrations. Alves *et al.* (2005) indicated that MST decreased with an increase in conidia concentration in their study of using *B. bassiana* against citrus rust mite. In my study also, MST of insects treated with the highest concentration for isolate IR-K-40 was lower than MST of insects treated with the highest concentration of isolate SPT 22. The two lower concentrations in both isolates caused mortality close to the control and the third concentration also caused less than 50 % mortality among the treated insects. Therefore it can be assumed that only the highest concentration had effective results. In the meantime it is not clear why the mortality was higher when exposing the insects to the dry conidia in virulence study than when the known dose was injected to each insect. One of the reasons could be that the insect were in contact with more conidia in the virulence study while in the dose-response study a small dose of each isolate is insulated to the insect. In the dose-response study there was higher percentage of sporulating cadavers, but since the number of dead insects was very low this results should be under estimated.

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