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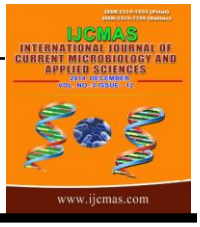

Original Research Article

Isolation and characterization of entomopathogenic fungi from hibernating sites of Sunn Pest (*Eurygaster integriceps*) on Ilam Mountains, Iran

Maryam NouriAiin[1], Hassan Askary[2]*, Sohrab Imani[1] and Rasoul Zare[3]

[1]Department of Entomology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Biological Control, Iranian Research Institute of Plant Protection, Tehran, Iran
[3]Department of Botany, Iranian Research Institute of Plant Protection, Tehran, Iran
**Corresponding author*

A B S T R A C T

Entomopathogenic fungi have shown high potential for biological control of insect pests. Natural habitats are the most important resources for these micro-organisms. The main goal of current research was to investigate occurrence and biodiversity of entomopathogenic fungi in hibernating sites of Sunn Peston, Ilam Mountains in West of Iran. Sampling was done both from Sunn Pest adults and soil using *Galleria mellonella* as bait trap. All isolates were studied for their growth rate, sporulation and germination rate on standard culture media. Pathogenicity of nine isolates of *B. bassiana* and an index isolate of *B. bassiana* (Atashgah) as a positive control, four isolates of *I. farinosa*, one isolate of *M. anisopliae*, one isolate of *I. fumosorosea* were studied on 3rd instar Sunn Pest nymphs using 10^6conidia/ml by estimating percent mortality, Lt_{25} and Lt_{50}. Results show that from 83 sites of habitats, 41 isolates were obtained from adult Sunn Pests and 66 isolates obtained from soils. Isolates consisted of 36.58% *B. bassiana* and 17.07% *Isariafarinosa* (from Sunn Pests), 71.24% *B. bassiana*, 21.21% *I. farinosa*, 1.51% *I. fumosorosea* and 1.51% *Metarhizium anisopliae* (from soils).The results of bioassays showed that the most virulent isolate, S5,belonged to *B. bassiana*. There were significant differences (p=0.01) between treatments for growth rate, sporulation, germination and pathogenicity of each isolate, from each fungus species. The highest level of pathogenicity was occurred by *B. bassiana*, isolate S5 (74%±3.7%). Evaluation of biodiversity and pathogenicity of these isolates can help to develop effective biological agents to control of Sunn Pest.

K e y w o r d s

Beauveria bassiana, Biological control, Distribution, *Isaria farinosa*, *Isaria fumosorosea*, *Metarhizium anisopliae*

Introduction

Sunn Pest, *Eurygaster integriceps* Puton (Het., Scutelleridae) is the most important pest of cereals in Central and West Asia and East Europe (Javahery, 1995).

Natural habitat of Sunn Pest is rangelands where, a high variation of hosts is available (Schuh and Salter, 1995). The main aestivation and hibernation sites of this insect are in the mountains under different vegetation such as *Astragalus* sp., *Artemisia*

sp., *Acantholimon bracteatum* (Girard) Boiss. (Plumbaginaceae), *Quercus* sp., *Amygdalus* sp. and *Crataegus aronia* (L.) Bosc *et* DC. *Eurygaster integriceps* attack cereals specially wheat by feeding on leaves, stems and grains, reducing yield and injecting a toxin into the grains which reduces the quality of flour, and substantially reduces baking quality of the dough (Hariri *et al.,* 2000). As little as 2–5% damaged grains can result in unacceptable dough. In the absence of control measures, infestations can lead to 100% crop loss (Javahery, 1995). 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 migrates to the foot hills of mountains for the rest of the year which is called overwintering period (Brown, 1965). *E. integriceps* are migratory, implying that they can even survive or hibernate in high altitudes and harsh wintering regions. Sunn Pest spends a dormant period of some 9 months at overwintering sites, hidden in some plants (e.g. *Acantholimon acerosum* (Willd.) and after hibernation adult feed, mate and lay eggs on cereal leaves and weeds (Amir-Maafi *et al.,* 2007).

Control of Sunn Pest is mainly based on chemical control which is widely used in countries with *E. integriceps* outbreaks. In total, approximately seven million hectares are treated chemically in affected regions each year (Popov, 1996). Use of chemical insecticides for control has caused the extinction of many beneficial insects, as well as poisoning waters, birds and reptiles that feed on poisoned insects, causing even more harm to humans and wildlife and resistance to the insecticides (Javahery, 2004). Entomopathogenic fungus has demonstrated good potential as biological agent to control insect sucking such Sunn Pest, when other biological agents do not, i.e. during diapause (El-Bouhssini *et al.,* 2004). Parker *et al.* (2000, 2003) collected entomopathogenic fungi of Sunn Pest from some parts of Syria, Turkey, Iran, Uzbekistan, Kazakhstan, Kyrgyz Republic and Russia. The most common species was *Beauveria bassiana* between other species such as *Verticillium* and *Paecilomyces*. Skinner *et al.* (2007) obtained more than 220 isolates of fungi from soil of hibernating sites of Sunn Pest in Russia, East and central part of Asia. Kazemi Yazdi *et al.* (2011) isolated more than 100 strains of fungi from overwintering sites of Sunn Pest. Soil is the main source and natural habitat for entomopathogenic fungi. Studies of Sun and Liu (2008) showed that from 425 soil specimens, six species of insect fungi were collected by Galleria bait method (GBM) among which the most important species were *Paeciloamyces farinosus* (19/6%), *B. bassiana* (14%), *M. anisopliae* var. *anisopliae* (10/6%), opportunistic pathogens (21%) and secondary colonizers (19%).

Iran is a vast country with high variation in geographical and ecological indices such as temperature, altitude, cover plants and etc. These factors lead to a rich fungal diversity. The main aim of the current investigation was to extend our knowledge on the occurrence of entomopathogenic fungi infecting Sunn Pests in hibernating and aestivating sites. Ilam (WIran) with its interesting plantation including oak, *Astragalus* spp. and *Amygdalus* spp. was particularly selected for this research as a case study.

Materials and methods

Insect and soil collection: Sunn Pest adults apparently attacked by fungi, were collected from overwintering sites under the litter around oak trees, *Astragalus* spp. and other major plant species from Ilam (W Iran), at different time periods in late winter and

spring (January and March, 2012). Eighty-three sites from four main regions were sampled (1–5 sites/region). Collected insects were transferred to the laboratory in clean vials for isolation of fungi.

Soil samples were also collected from different mountains of Ilam where Sunn Pest hibernates. The samples were placed into plastic bags and stored at 4–8°C. Eighty-three soil samples were collected from 83 sites of plant covering in mountains (Table 1).

Isolation and identification of fungi

Surface of dead insects were sterilized by 2% sodium hypochlorite solution for 3 minutes, rinsed in sterile distilled water, then dried using sterile filter paper. Sunn Pest cadavers or their particles were placed on potato dextrose agar (PDA medium was prepared following the manufacturer's instruction and the time required for the solidification of PDA also noted) or sterile filter paper in Petri dishes and incubated at 25±1°C. After emergence of fungal hyphae and sporulation, they were subcultured by transferring onto a new PDA plate and incubated at 25°C.

Insect-associated fungi were isolated from soil samples by using 'Galleria bait method' (Zimmermann, 1986). The wax moth larvae, *Galleria mellonella* L., were reared continuously in constant darkness at 28°C. The third or fourth instar larvae (approximately 30 days after hatching) were used as baits. Five larvae were placed on the soil samples in each tube and covered with a lid and incubated at 25±1°C for two weeks. The larvae were examined on days 7 and 14 days after inoculation. Surface of dead larvae were sterilized by 3% sodium hypochlorite for 3 min and then rinsed twice with sterile distilled water. After removing free water of the larvae surface, they were placed onto PDA plates. The fungi were identified using morphological characteristics of reproductive structures with the aid relevant taxonomic literature (De Hoog, 1972; Samson *et al.*, 1988).

Colony growth and sporulation rate of isolates: Seven-day-old cultures on PDA plates were used for colony growth rate. At the end of day 7, 5mm agar disc containing mycelium was placed in the middle of fresh PDA plates (in 3 replicates) and incubated at 22±1°C. Radial growth was measured every two days until day 15. Seven-day-old cultures of each fungal isolates on PDA plates were used for spore counting. 15 mm^2 PDA discs containing fungal hyphae and conidia were taken (using a cork-borer) and then suspended in 10 ml of distilled water containing 0.05% (v/v) Tween 80. Spore concentration was determined using a Neubauer Haemocytometer.

Conidial Germination rate: Conidial germination rate was determined by plating a 10 µl low concentration of each fungus isolates on PDA plates in 3 replicates. Conidial suspension was spread on the PDA medium and then incubated at 22±1°C. Germination was assessed only at 24 h by taking 3 samples of the culture media, followed by counting 100 spores. Conidial germination was considered when the length of the germ tube was longer than half size of conidium length.

Pathogenicity test: Colony of Sunn Pest was mass reared in the laboratories of Biological Control Department of IRIPP. The initial colony of Sunn Pest was derived from Pakdasht (South of Alborz Mountains) where Sunn Pesthibernates annually. Adults and nymphs reared on wheat seed germ under optimal condition. In all experiments third nymphal instar of Sunn Pest were used for the bioassays. Sixteen isolates (nine of *B. bassiana*, four of *I. farinosa*, one of *M.*

anisopliae, one of *I. fumosorosea*) were selected based on growth characteristics and origin of each isolates for pathogenicity test. Conidia were harvested from 3-week-old cultures and suspended in 20 ml sterile distilled water containing Tween 80 (0.01% v/v) adjusting to 1×10^6 spores ml^{-1}. The viability of conidia was determined by spread-plating 0.1 ml of conidial suspension after 20 h.

Thirty of 3rdnymphal instars of Sunn Pest were immersed in conidial concentration of each isolate for 5 seconds. Controls were sterile distilled water containing Tween 80 (0.01% v/v) as a negative control and an index isolate of *B. bassiana* (Atashgah) as a positive control. Each treatment consisted of three replicates.

Treated third nymphal instars were maintained in growth chamber (22±1°C, RH 85±5 %) and 16:8 (L:D). Daily mortality rates were measured over two weeks by macroscopic observation of specimens and their reflex to hair brush.

Statistical analyses: Cumulative mortality was corrected for natural mortality using Abbott's formula (Abbott, 1925). Data were analyzed using a one-way analysis of variance (ANOVA Proc. mixed; SAS, (Ver. 9.2). In all tests, $p<0.05$ was considered significant. LT_{50} were estimated by Life Test software (SAS, Ver. 9.2). Excel software was used to draw graphs.

Results and Discussion

Isolation and identification of fungi: Based on our observations, the most abundant Sunn Pest distribution sites were under oak trees, *Astragalus* sp., *Artemisia* sp. and *Acantholimon bracteatum*. For this reason, the most infected cadavers of Sunn Pest to fungi were also obtained from these habitats.

Incubation of dead Sunn Pest adults in humid condition revealed that infection to entomopathogenic fungi occur naturally in hibernating sites. All fungal isolates were single-spored and identified using relevant literature and were deposited at the Iranian Fungal Culture Collection (IRIPP), Tehran.

We sampled from 83 overwintering sites distributed over a range of climatic and geographical areas. One hundred and seven fungal isolates were obtained from Sunn Pest cadavers and soils (41 from Sunn Pest and 66 from soil of overwintering sites). Fungal infection in Sunn Pest cadavers (from 83 sites) were 34.93% among which *Beauveria bassiana* (36.58%) and *Isaria farinose* (17.7%) were the most frequent. The other 46.34% included *Aspergillus niger* Tiegh. *Fusarium* sp., *Trichoderma* sp., *Trichoderma harzianum* Rifai, *Aspergillus fumigatus* Fresen., *Aspergillus melleus* Yukawa (46.34%). The most important entomopathogenic fungi isolated from soils were *B. bassiana* (71.24%), *I. farinosa* (21.21%), *M. anisopliae* (1.51%), *Isaria fumosorosea* Wize (1.51%) and the other4.53% were*T. harizanum*, *A.flavus* Link and *Fusarium sambucinum* Fuckel. In general, *B. bassiana* and *I. farinosa* were the most abundant fungi in all sites and samples (57.94% and 19.62%, respectively). Based on the regional distribution, two entomopathogenic species (*B. bassiana* and *I. farinosa*) were obtained from soils of each four regions and these were the most abundant species in regions, localities, sites and oak forest soil (Tables 1, 2 and 3).

Conidial germination, sporulation and colony growth rate of isolates

***Beauveria bassiana*:** Conidial germination was observed for all isolates of *B. bassiana*. Germination rate was significantly different ($F = 13.5$; df = 37,113; P-value = 0.0001) between *B. bassiana* isolates. Isolate S11

and S9 had the highest and lowest germination rates, respectively.

Sporulation rate of *B. bassiana* isolates showed a significant difference between each treatment (F = 18.07; df = 37,113; P-value = 0.0001). Isolates I12 and S9 had the highest and lowest sporulation rates, respectively (Table 4).

Mycelial growth rates were also significantly different between *B. bassiana* isolates (F = 4.9; df = 37,113; P-value = 0.0001). Isolates I1 and S7 had the highest and lowest rate of mycelial growth rate, respectively (Table 4).

***Isaria farinosa*:** Conidial germination was observed for all *Isaria farinosa* isolates. Germination rates were significantly different (F = 20.16; df = 11, 35; P-value = 0.0001) between *I. farinosa* isolates. IsolateS12had the highest and isolates I9, I16, S14 had the lowest germination rates (Table 5). There was significant difference between sporulation rate of *I. farinosa* isolates (F = 16.35; df = 11, 35; P-value = 0.0001). Isolate SI8 and S14 had the highest rate of sporulation (Table 5). There was also significant difference between growth rates of *I. farinosa* isolates (F = 25.43; df = 37,113; P-value = 0.0001). Isolates I8, I9, I4had the highest and S12had the lowest rate of growth rates (Table 5).

Metarhizium anisopliae: The means of sporulation rates, conidial germination and colony radial growth rate were estimated for *M. anisopliae* isolate 4.1±0.15 ($\times 10^9$conidia/ml.), 82.81%±1.36% and 2.9±0.05cm, respectively.

***Isaria fumosorosea*:** The means of sporulation rates, conidial germination and colony radial growth rate were estimated for *I. fumosorosea* isolate 2.3±0.15 ($\times 10^9$conidia/ml.), 89.62%±0.65% and 2.4±0.05 cm, respectively.

Pathogenicity test

***Beauveria bassiana*:** Mortality rate of 3thnymphal instar of Sunn Pest by selected isolates of *B. bassiana* (10^6conidia/ml.) were different from 74.07±3.7% (maximum rate) to 25.9±3.7 (minimum rate) for S5 and I11 isolates, respectively. Whereas, moderate mortality rate belonged to control isolate, S4 (Atashgah) with 40.7±3.7% (Table 6).

The estimated Lt_{25} values varied from 6 to 12 days by concentration10^6conidia/ml for selected *B. bassiana* isolates (Table 6). The minimum and maximum Lt_{25} belonged to isolates S5 with 6 (4–7) and S8 6 (2–8) and S16 with 12 (7-*) and I5 12 (7 = *), respectively. Between selected isolates of *B. bassiana*, S5 was more virulent to Sunn Pest nymphs with estimated 8 (7-9) days for Lt_{50}. Estimated Lt_{25} and Lt_{50} for isolate S4 (Atashgah) were 7 (3-10) and * (8-*) days, respectively (Table 6).

***Isaria farinosa*:** Mortality due to *I. farinosa* isolates on 3th instar nymphal stage of Sunn Pest varied between 25.9±3.7 % to 37.03±7.4%. However, there was no difference between treatments ($F_{11,3}$ = 0.74; P-value = 0.55) (Table 7).

The minimum and maximum Lt_{25} were 8 (3–11) and 11 (7-*) days belonged to isolates I3, and I9 and I13, respectively (Table 7). Lt_{50} was in calculatable for all of *I. farinosa* isolates.

***Metarhizium anisopliae, Isaria fumosorosea*:** Mortality rate was 40.74±3.7% for *M. anisopliae* and 44.44±6.41% for *I. fumosorosea* isolates. Estimated Lt_{25} was 8 (4–12) days for *M. anisopliae* and 7 (4–12) days for *I. fumosorosea* (Table 8).

This study was conducted for the first time on entomopathogenic fungi associated with Sunn Pest from overwintering sites in Ilam region (W Iran). On the other hand, there are only a few attempts in order to isolate entomogenous fungi of Sunn Pests in central Asia (Parker *et al.,* 2003). Occurrence of other fungal species such as *B. bassiana* and *I. farinose* is similar to other parts of Sunn Pest habitats that were studied such as Syria, Turkey, Iran, Uzbekistan, Kazakhstan, The Kyrgyz Republic, Russia and North of Iraq (Meyling and Eilenberg, 2006a, b; Parker *et al.,* 2000, 2003; Jordan and Pascoe, 1996).

Table.1 Entomopathogenic fungi isolated from Sunn Pest and soils based on regions and locals of Ilam Province

Regions	Locations	From soil	No. of Isolates	From Sunn Pest	No. of Isolates
Eyvan	Veneyt	*B. bassiana* (8) *I. farinosa* (2) *I. fumosorosea*(1)	11	*B. bassiana* (2) *A. melleus*(1) *T. harizanum* (1*)*	4
	Renou Heights	*B. bassiana* (8) *I. farinosa* (1) *A. flavus*(1)	10	*B. bassiana* (1) *Trichoderma* sp.(3) *I. farinosa* (2) *Aspergillus* sp. (1)	7
Ilam	Shirezool	*I. farinosa* (3) *F. sambucinum*(1)	4	*Fusarium* sp.(1)	1
	Sheshdar	*B. bassiana* (5) *I. farinosa* (1)	6	*Trichoderma* sp. (2)	2
	Dalab	*B. bassiana* (2) *I. farinosa* (1)	3	*Fusarium* sp.(1)	1
DarehShahr	Poshteh	*B. bassiana* (5) *I. farinosa* (1)	6	*B. bassiana* (2) *I. farinosa* (1)	3
Sarableh	Manesht	*B. bassiana* (3)	3	*Fusarium* sp. (1) *B. bassiana* (1)	2
	Karezan	*B. bassiana* (3) *I. farinosa* (1)	4	*B. bassiana* (3) *I. farinosa* (1) *A. niger*(1) *Fusarium* sp. (1)	6
	Ghalajeh	*B. bassina* (10) *M. anisopliae* (1) *I. farinosa* (4) *T. harizanum* (1)	16	*B. bassiana* (3) *I. farinosa (2)* *T. harizanum* (1) *Trichoderma* sp.(2)	8
	Asaman Abad	*B. bassiana* (3)	3	*B. bassiana* (3) *I. farinosa* (1) *A. fumigatus*(1) *Fusarium* sp.(2)	7
			66	Total	41

Table.2 Distribution of entomopathogenic fungi isolated from Sunn Pest and soils based on altitude range (%)

	From Sunn Pest		From soils			
Altitude range	*B. bassiana*	*I. farinosa*	*B. bassiana*	*I. farinosa*	*I. fumosorosea*	*M. anisopliae*
1300−1100	43.7%	6.2%	75%	12.5%	−	−
1500−1301	−	−	20%	40%	11.1%	−
1700−1501	−	−	50%	16.6%	−	−
1900−1701	15.3%	15.3%	61.5%	7.6%	−	4%
2100−1901	12%	8%	53.8%	19.2%	−	−

Table.3 Distribution of entomopathogenic fungi isolated from Sunn Pest and soil based on plant species (%)

Host plant	From Sunn Pest		From soils			
	B. bassiana	*I. farinosa*	*B. bassiana*	*I. farinosa*	*I. fumosorosea*	*M. anisopliae*
Astragalus sp.	14.6%	7.3%	53.6%	21.9%		−
Oak trees	23.7%	11.1%	53.8%	15.3%	3.8%	3.8%
Grasses	18.7%	6.2%	68.7%	6.2%		-

This study also confirmed the wide distribution and high occurrence of *B. bassiana* and *I. farinose* in soils and on Sunn Pest cadavers in overwintering sites. Results of this study correspond well with earlier investigations by Kazemi Yazdi (2011) in Kermanshah province and Parker *et al.* (2003) in Central Asia. Parker *et al.* (2000, 2003) collected entomopathogenic fungi from Sunn Pest in Syria, Turkey, Iran, Uzbekistan, Kazakhstan, Kyrgyz Republic and Russia. The most common entomopathogenic fungi of Sunn Pest in overwintering populations were recognized *Beauveria bassiana*, *Lecanicillium* (*Verticillium*) *lecanii* and *Paecilomyces farinosa*.

Klingen and Haukeland (2006) and Klingen *et al.*, (2002) indicated that tolerance to a wide range of climatic conditions is an important factor affecting the distribution of both *B. bassiana* and *I. farinosa*. High climatic variation between overwintering sites of Sunn Pest in Ilam province suggests that there might be high genetic variation among entomopathogenic fungi found in this study. Although all fungal isolates were pathogenic to Sunn Pest nymphs, there were considerable variations in their pathogenic ability. Bioassays by a few selected isolates of *B. bassiana* indicated a high variation in their pathogenicity on Sunn Pest. We showed that isolate S5 from *B. bassiana* was the most virulent compared with the rest of isolates.

Soil is considered as a good habitat and genetic resource of insect pathogenic fungi and other microorganisms, protected of fungal particle against environmental different stress such as UV radiation, lower humidity and providing food elements (Keller and Zimmerman, 1989; Ignoffo and Hostetter, 1997). Insect pathogenic species of the genera *Beauveria*, *Conidiobolus*, *Metarhizium* and *Paecilomyces* are commonly found in the soil. For this reason, fungal epizootics may happen for soil-inhabiting insects in various regions of the world (Samson *et al.*, 1988; Keller and Zimmerman, 1989; Klingen and Haukeland, 2006).

Table.4 Means of conidium germination (after 24 h), conidium production (after 7 days) and mycelial radial growth (after 15 days) for *B. bassiana* isolates

Isolates	Colony radius (Mean±SE)	Means level	Conidiumgermination (Mean±SE)	Means level	Conidium production (Mean±SE)	Means level
I1	0.26±3.8	a	4.17±63.9	m	0.12±1.66	o
I2	0.62±2.56	defgh	3.24±74.86	ijkl	0.08±3.33	def
I4	0.35±2.9	bcdefgh	3.72±80.93	efghijk	0.14±2.36	ijklm
I5	0.08±3.23	abcde	3.72±80.93	efghijk	0.14±2.36	ijklm
I6	0.2±3.2	abcde	2.96±75.18	hijkl	0.15±3.3	def
I7	0.03±3.13	abc	2.5±79.78	ghijk	0.08±4.16	ab
I10	0.11±3.5	abcdef	0.19±72.94	Jklm	0.23±3.7	bcd
I11	0.1±3.6	ab	4.31±71.93	klm	0.15±4	abc
I12	0±3	abcdefg	0.62±79.64	ghijk	0.18±4.46	a
I13	0.14±3.23	abcde	5.8±80.65	fghijk	0.08±3.46	cde
I15	0.05±2.4	efgh	1.29±71.34	klm	0.18±3.6	defgh
I17	0.05±3.6	ab	1.69±68.94	lm	0.4±3.66	bcd
I18	0.25±3.3	efgh	2.03±83.54	cdefghi	0.12±2.63	ghijk
I19	0.05±3.1	defgh	2.29±72.49	klm	0.08±2.93	efghi
I20	0.62±2.56	abcdef	1.05±80.48	ghijk	0.08±2.63	ghijk
I21	0.33±2.86	bcdefgh	4.39±66.76	lm	0.06±2.83	fghij
I22	0.62±2.56	defgh	3.36±83.4	cdefghi	0.14±2.36	ijklm
S3	0.17±2.2	ghi	1.26±90.16	abcdef	0.12±2.76	fghij
S4	0.05±3.5	abc	2.08±91.7	abcd	0.11±2.6	hijk
S5	0.41±3.1	abcdef	9.07±84.04	bcdefghi	0.11±3.9	bc
S6	0.18±3.23	abcde	0.56±91.98	abc	0.2±3.2	defg
S7	0.08±1.43	i	1.31±82.14	defghij	0.34±2.46	ijkl
S8	0.29±2.56	defgh	1.08±86.32	abcdefg	0.14±3.33	def
S9	0.14±2.86	bcdefgh	2.25±44.01	n	0.03±1.03	p
S10	0.08±2.16	ghi	0.55±91.51	abcd	0.08±2.43	ijklm
S11	0.08±2.13	hi	0.66±94	a	0.45±3.3	def
S15	0.12±2.3	fgh	4.25±72.01	jkl	0.17±2.22	ijklm
S16	0.05±3	abcdefg	0.75±91.89	abc	0.12±3.33	def
S17	0.3±2.3	fgh	0.63±90.14	abcdef	0.26±2.66	ghijk
S18	0.05±2.4	efgh	1.1±84.75	abcdefgh	0.14±2.33	jklm
S20	0.08±2.33	gh	1.07±87.54	abcdefg	0.11±2.1	klmno
S21	0.08±22.33	fgh	0.46±90.42	abcde	0.15±2.7	ghij
S22	0.11±2.2	ghi	0.3±90.5	abcde	0.03±1.86	mno
S23	0.1±2.2	ghi	1.31±91.35	abcd	0.05±2.3	jklmn
S24	0.11±2.3	fgh	0.36±93.47	ab	0.08±2.43	ijklm
S25	0.05±2.1	hi	0.08±79.96	ghijk	0.08±1.76	no
S27	0.05±2.5	defgh	0.37±91.85	abcd	0.08±2.76	fghij
S29	0.17±2.76	cdefgh	0.74±91.29	abcd	0.1±2	lmno

In each column, means followed by different letters are significantly different (F-LSD, P <0.05)

Table.5 Means of conidium germination (after 24 h), conidium production (after 7 days) and mycelial radial growth (after 15 days) for *I. farinosa* isolates

Isolates	Colony radius (Mean±SE)	Means level	Conidium germination (Mean±SE)	Means level	Conidium production (Mean±SE)	Means level
I3	0.31±2.56	bcd	3.16±84.62	bc	0.12±2.76	bc
I8	0±3.3	a	3.05±76.39	d	0.24±4.46	a
I9	0.08±3.53	a	3.56±65.91	e	0.06±2.83	b
I14	0.08±3.46	a	2.66±80.89	cd	0.14±2.36	bcd
I16	0.06±2.86	b	4.33±65.7	e	0.26±2.66	bcd
S1	0.03±2.86	b	1.76±74.45	d	0.2±2.2	de
S12	0.08±1.46	e	0.44±94.91	a	0.2±2.43	bcd
S13	0.05±2.25	d	0.68±89.13	ab	0.15±2.75	b
S14	0.08±2.33	cd	8.84±74.52	e	0.41±2.26	e
S19	0.11±2.3	d	1.44±87.46	abc	0.05±2.8	b
S28	0.08±2.76	b	0.77±90.31	ab	0.14±2.56	bcd
S30	0.05±2.7	bc	0.42±90.51	ab	0.08±2.26	cde

In each column, means followed by different letters are significantly different (F-LSD, P <0.05).

Table.6 Means of mortality by 10^6 spores/ml. of selected isolates of *B. bassiana* with estimated Lt_{50}, 12 days after inoculating 3th nymphal stages of Sunn Pest ($F_{29,9}$ = 10.48; P-value = 0.0001)

Isolates	Mortality (%)±SE	Lt_{25} (Limits)(day)	Lt_{50} (Limits)(day)
S5	74.07±3.7 a**	6 (4-7)	8 (7-9)
S16	25.8±3.1 d	12 (7-*)	* (*-*)
I11	25.9±3.7 d	8 (3-12)	* (11-*)
I5	29.6±3.7 cd	12 (7-*)	* (*-*)
S23	29.6±3.7 cd	10 (6-10)	* (10-*)
I20	37±3.7bcd	10 (3-12)	* (11-*)
S3	33.3±3.7bcd	8 (6-11)	* (9-*)
S8	44.4±6.4 b	6 (2-8)	10 (6-*)
I6	40.7±7.4 bc	9 (4-*)	* (11-*)
Atashgah (S4)	40.7±3.7bc	7 (3-10)	* (8-*)

*Unable to estimate

**Means followed by different letters are significantly different (F-LSD, P <0.05)

Table.7 Means of mortality by 10^6conidia/ml of selected isolates of *I. farinosa* with estimated Lt_{50}, 12 days after inoculating 3th nymphal stages of Sunn Pest

Isolates	Mortality (%)±SE	Lt_{25} (Limits) (day)	Lt_{50} (Limits) (day)
S19	33.3±6.4	9 (6-12)	* (11-*)
I9	25.9±3.7	11 (7-*)	* (*-*)
I3	29.6±3.7	8 (3-11)	* (9-*)
I13	37.03±7.4	11 (7-*)	* (*-*)

Table.8 Means of mortality by 10^6conidia/ml. of selected isolates of *I. fumosorosea* and *M. anisopliae* with estimated Lt_{50}, 12 days after inoculating 3th nymphal stages of Sunn Pest

Isolates	Mortality (%)±SE	Lt_{25} (Limits) (day)	Lt_{50} (Limits) (day)
I. fumosorosea (S26)	40.74±3.7	8 (4-12)	* (9-*)
M. anisopliae (S2)	44.44±6.41	7 (4-12)	* (10-*)

Adults of Sunn Pest spend a major part of their life in soil under plants. It seems that this behavior provides suitable condition for fungal epizootics. Further research lead us to have more understanding from relationship between fungal epizootics and natural control of Sunn Pest population in overwintering sites as an important steps in management of this major cereal pest.

This study demonstrated the virulence under controlled conditions of several isolates of *B. bassiana*, *I. farinosa*, *M. anisopliae* and for the first time*I. fumosorosea* from the soils of Ilam province against *E. integriceps*. This is an essential first step in the development of a bio-pesticide for biological management strategy which integrates entomopathogenic fungi.

References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265–267.

Amir-Maafi, M., Majdabadi, M., Aghdasi, F., Parker, B.L., Parsi, F. 2007. Degree days for forcasting migration of Sunn Pest. In: B.L. Parker, M. Skinner, M. El-Bouhssini, S.G. Kumari, (Eds.), Sunn Pest Management, A Decade of progress 1994–2004. Arab Society for Plant Protection. Pp. 139–145.

Brown, E.S. 1965. Notes on the migration and directions of flight of *Eurygaster* and *Aelia*species (Hemiptera, Pentatomoidea) and their possible bearing of the invasion of cereal crops. *J. Animal Ecol.*, 34(1): 93–107.

De Hoog, G.S. 1972. The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov. *Stud. Mycol.*, 1: 1–41.

El Bouhssini, M., Parker, B.L., Skinner, M., Reid, B., Canhilal, R., Aw-Hassan, A., Nachit, M., Valcun, Y.,

Ketata, H., Moore, D., Amir-Maafi, M., Kutuk, H., Abdel Hay, M., El-Haramein, J. 2004. Integrated pest management of Sunn Pest in west and central Asia. World Entomological Congress, Brisbane, Australia. Australian Entomological Society.

Hariri, G., Williams, P.C., El-Haramain, F.J. 2000. Influence of Pentatomid insect on the physical and dough properties and two-layered flat bread baking quality of Syrian wheat. *J. Cereal Sci.,* 31(2): 111–118.

Ignoffo, C.M., Hostetter, D.L. 1997. Summary of environmental stability of microbial insecticide. *Miscellan. Publ. Entomol. Soc. Am.,* 10: 117–119.

Javahery, M. 1995. A technical review of Sunn Pests (Heteroptera: Pentatomidae) With special reference to *Eurygaster integriceps* Puton. FAO/RNE, 80 Pp.

Javahery, M. 2004. Sustainable management of cereal Sunn Pests in the 21st century. World Entomological Congress, Brisbane, Australia. Australian Entomological Society.

Jordan, M., Pascoe, R. 1996. Sunn Pest control program: 1995–1996 the three northern governorates of Iraq. Phase 2, Report, Overseas Development Administration, UK.

Kazemi Yazdi, F., Eilenberg, J., Mohammadipour, A. 2011. Biological characterization of *Beauveria bassiana* (Clavicipitaceae: Hypocreales) from overwintering sites of Sunn Pest, *Eurygaster integriceps* (Scutelleridae: Heteroptera) in Iran. *Int. J. Agric. Sci. Res.,* 2(2): 7–16**.**

Keller, S., Zimmerman, G. 1989. Mycopathogens of soil insects. In: Wilding, N., Collins, N.M., Hammond, P.M., Webber, J.F. (Eds.), Insect-fungus interactions. Academic Press, London. Pp. 240–270.

Klingen, I., Elinberg, J., Meadow, R. 2002. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soil. *Agricult. Ecosys. Environ.,* 91: 191–198.

Klingen, I., Haukeland, S. 2006. The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes. In: J. Eilenberg and H.M.T. Hokkanen (Eds.), An ecological and societal approach to biological control. Series: Progress in biological control, Vol. 2, Springer. Pp. 145–211.

Meyling, N.V., Elinberg, J. 2006a. Isolation and characterization of *Beauveria bassiana* isolates from phylloplanes of hedgerow vegetation. *Mycol. Res.,* 110: 188–195.

Meyling, N.V., Elinberg, J. 2006b. Occurrence and distribution of soil born entomopathogenic fungi within a single organic agroecosystem. *Agricult. Ecosys. Environ.,* 113: 336–341.

Parker, B.L., Skinner, M., Brownbridge, M., El-Bohssini, M. 2000. Control of insect pests with entomopathogenic fungi. *Arab. J. Plant Protect.,* 18: 133–138.

Parker, B.L., Skinner, M., Costa, S.D., Gouli, S., Reid, W., El-Bouhssini, M. 2003. Entomopathogenic fungi of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae): collection end characterization for development. *Biol. Control,* 27: 260–272.

Popov, C., Barbulescu, A., Vonica, I. 1996. Population dynamics and management of Sunn Pest in Romania. In: R.H. Miller and J. G. Morse (Eds.), Sunn Pests and their control in the Near East. Food and Agriculture Organization of the United Nations, Rome. Pp. 47–59.

Samson, R.A., Evans, H.C., Latge, J.P. 1988. Atlas of entomopathogenic fungi. Sproner-Verlag, Berlin, Heidelberge, NewYork, 187 Pp.

Schuh, R.T., Slater, J.A. 1995. True bugs of the world (Hemiptera: Heteroptera). Cornell University Press, New York.

Skinner, M., Parker, B. L., Gouli, S., Reid, W., El Bouhssini, M., Amir-Maafi, M., Sayyasi, Z. 2007. Entomopathogenic fungi for Sunn Pest management: Efficacy trials in overwintering sites. In: B. L Parker, M. Skinner, M El-Bouhssini and S. G. Kumari (Eds.), Sunn Pest Management, A Decade of progress 1994–2004. Arab Society for Plant Protection. Pp. 319–328.

Sun, B.D., Liu X.Z. 2008. Occurrence and diversity of insect- associated fungi in natural soils in China. *Appl. Soil Ecol.*, 39: 100–108.

Zimmermann, G. 1986. The *Galleria* bait method for detection of entomopathogenic fungi in soil. J. Appl. Entomol., 102: 213–215.