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Research

Entomopathogenic Fungi Collected from Sunn Pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), Overwintering Sites in Central Iran

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ABSTRACT

Sunn Pest, *Eurygaster integriceps* Puton, is the most important pest of wheat and barley in Iran and throughout the Middle East. In this study, different isolates of *Beauveria bassiana* from overwintering areas in the centre of Iran were collected. In this research, a total of 510 soil samples and cadavers were taken under plants harbouring overwintering Sunn Pest in Semnan, Qum, Kerman and Markazi provinces. The collections were done in late summer and winter 2015 and 2016. The pathogenic fungi were isolated from soil by insect bait method using larvae of wax moth (*Galleria mellonella*). Data on longitude, latitude, elevation, temperature, soil type and entomopathognic fungi present were recorded for each site. Results indicate that entomopathognic fungi were isolated from 257 of the 510 soil samples, with only *Beauveria bassiana* and two species *Isaria farinosa* and *Isaria fumosorosea* (50.4%). Environmental and geographical impact studies indicated the fungi preferred soil with lower soil temperatures. Significantly more isolates were found at 2300 meters elevation although some were present at 1900 to 2100 meters.

Keywords: Entomopathognic fungi, *Beauveria bassiana*, Sampling, Overwintering sites, Sunn Pest,*Eurygaster integriceps.*

INTRODUCTION

Throughout the world growers are struggling with their pest problems and what production strategies to use, There is one extremely significant reason for this struggle. The pests that are damaging their crops, whether they be insects or diseases, have over the past few years developed a high degree of resistance to the agriculture chemicals used for their management [1,2]. Growers are faced with two major options to use. They can apply pesticides or do nothing and suffer the losses.

For example in Lebanon when greenhouse vegetable growers apply pesticides many times they will dramatically increase the number of applications made in a given week and/or mix a series of pesticides for spraying with no regard for label directions [3]. What this does is to favour the pest by using a mixture of pesticides with very different modes of action. The pest subsequently in a single event starts to develop resistance to many different materials at the same time.

There is another option available and that is the use of Integrated Pest Management (IPM). This technique incorporates all available strategies into one complete package leaving the use of agricultural chemicals as a last resort [3,4]. Biological control thus becomes one of the major components. Keep in mind that the ultimate goal of IPM is to enable the grower to produce high quality crops and produce them in a way that is good for the environment and good for human consumption. Subsequently costs will be reduced and your produce whether it is wheat, barley or vegetables will be pesticide-free.

Biological control is, in many cases, only considered by many as using parasites and predators to maintain pest populations below economic injury levels. However, entomopathognic fungi are also natural enemies of pests and most recently have become the main focus within many management strategies. They are naturally occurring, easy to culture and inexpensive to formulate. However, the first step in any program involving their use is to search and isolate them. The research reported herein represents, for the first time, results of extensive explorations in soil environments of Sunn Pest overwintering sites in Central Iran.

MATERIALS AND METHOD

Insect and soil collection area

Fourteen areas from four provinces (Kerman, Semnan, Qum and Markazi) were selected (3-4 site/province) (**Figure 1**). In each province an Agricultural Extension Agent was contacted to assist in selecting areas where historically Sunn Pest (*Eurygaster integriceps*) overwintered.

Soil samples were collected in late summer (September and October) and winter (January and March) 2015 and 2016. In each site 10 soil samples were taken by randomly scraping approximately 20 grams of soil from the surface of the soil under *Astragalus spp*. and/or *Artemisia spp* harbouring live or old cadavers of Sunn Pest (**Figure 2**). Five hundred ten soil samples

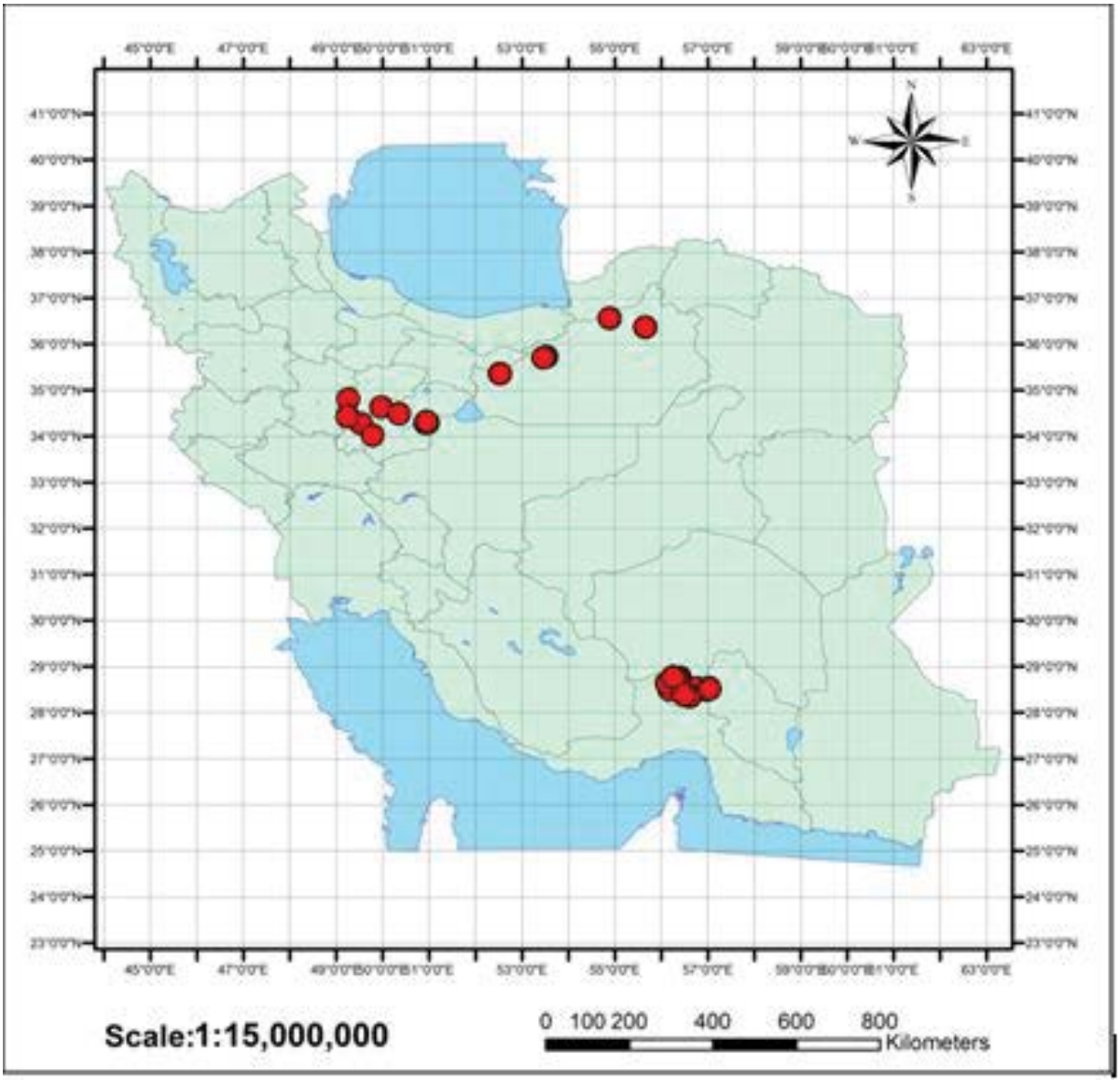

Figure 1: Map of research sites at Kerman, Markazi, Qum, and Semnan Provinces in Iran, using the GCS-WGS-1984.

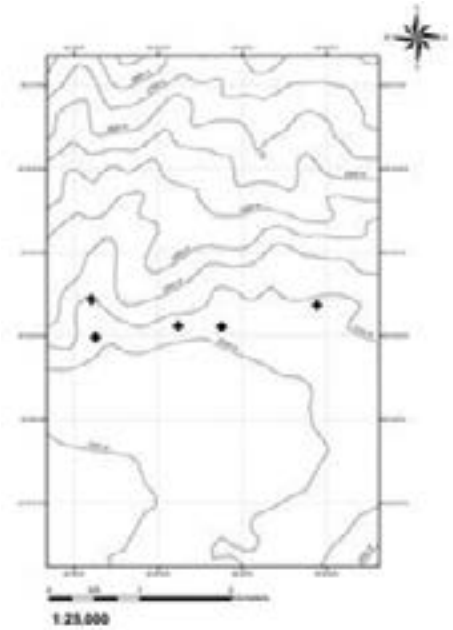

Figure 2: Contour map of research sample plots at Ashin, Kerman Province, using the GCS-WGS-1984; elevation, 1900-2200 m.

were transferred to laboratory in plastic bags for isolation of fungi (Table 1).

Soil temperatures and humidity were recorded with an Electronic Thermo-Hygrometer (Oregan Scientific THG-912). Data on longitude, latitude and elevation were determined with a Garmin GPS (Garmin Ltd, Olathe, Kansas, USA).

Soil types were analyzed for each site by Soil Classification map of Iran in ArcGIS 10.6.1 (Ref. Soil & Water Research Institute (SWRI), Department of Soil Genesis, Classification and Cartography, Soil Classification map of Iran).

Isolation and identification of fungi

Entomopathogenic fungi were isolated from soil samples by using the insect baiting system with larvae of the wax moth, *Galleria mellonella* [5]. For identification and long-term storage single spore fungal cultures were prepared using methods described in detail by Goettel, et al. and Ho, et al. [6,7].

RESULTS AND DISCUSSION

Two hundred fifty seven fungal isolates(Table 1) were discovered from soil samples collected from 14 sites in 4 provinces in Central Iran. The sites were located between 1700 and 2300 meters elevation.

Using the keys of Humber [8] and assistance from the Botany Research Department, IRIPP, and 42 isolates were identified and 16.6% were *Beauveria bassiana* (**Table 2**). Other species found were *Isaria farinosa*, *Isaria fumosorosea, Clonostachys rosea (Ghocladium roseum)*, *Penicillium rugulosum, Aspergillus ochroceus,* and *Penicillium implicatum*.

Beauveria bassiana has been extensively documented as having great potential as part of an effective control strategy for Sunn Pest [9].

Most of the isolates were taken from soil samples located underneath *Artemisia sp*. and *Astragalus sp*. bushes (**Figure 3**) These bushes are very commonly found in areas favoured as sites for Sunn Pest overwintering. The soil where these bushes normally grow was Lithosols of semi-arid soils (58.4%), Desert Solis (19.4%) and Brown Steppe Solis and Saline Soils (11.1%). Sunn Pest was found overwintering at the interphase between the plant debris and the soil surface which is the same location where the soil samples were taken [10] **(Table 3).**

Relative humidity beneath the bushes was recorded at each site at each sampling period in the spring and fall. In general RH was very low (7% to 33%).

Sunn Pest populations in all sites were also mostly very low. The highest populations were found in Lithosols of semi-arid soils (**Figure 4**), the lowest in Non-Saline Alluvial soils. Soil temperatures beneath the plants at the fall sampling period were 6.5°C to 15.3°C (**Table2**).

Table 1: Soil sampling and number of isolates based on provinces and locals in Central Iran.

Province	Site	No. of sample	No. of isolates
Kerman	Orzuiyeh-Bagh borg	50	34
	Orzuiyeh-Ashin	50	
	National Park Khabr	37	
Markazi	Khondab	35	76
	Arak-Anjirak	30	
	Chehreghan	28	
	Navazan	25	
Qum	Kahak-Veshnaveh	50	53
	Kahak-Virich	40	
	Salafchegan-Zavarian	10	
Semnan	Aradan-Shamshir Kooh	50	94
	Semnan-Pighambaran	50	
	Shahrood-Kalat Eghbalieh	30	
	Miamei	25	
Total	14 sites	510	257

Table 2: Distribution of entomopathognic fungi isolates from soils based on soil temperature (°C), soil humidity (RH%) and host plants (%).

Isolates	Soil temperature°C		Soil humidity RH%		Host plant	
	Summer	Winter	Summer	Winter	*Artemisia sp.*	*Astragalus sp.*
B. bassiana	18.8-30.1	6.5-15.5	7-17	13-22	46.50%	50%
Isaria farinosa	19.7-24.1	7.5	14-28	19	22.20%	77.80%
Isaria fumosorosea						

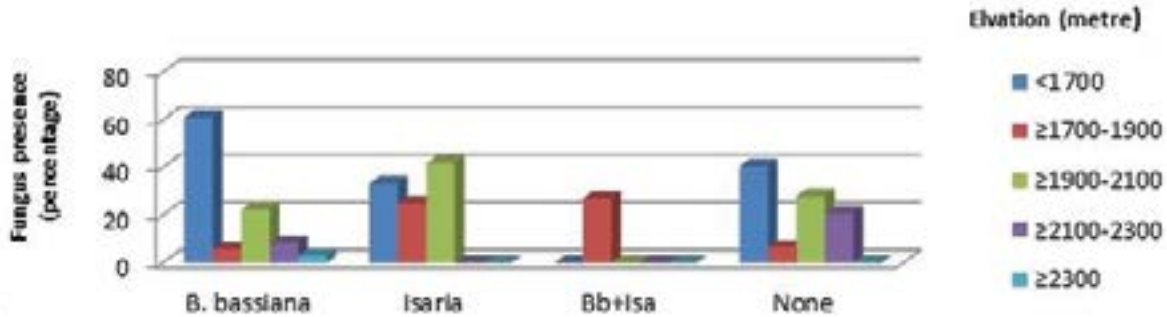

Figure 3: Distribution of entomopathognic fungi isolates from Sunn Pest and soils based on elevation (%).

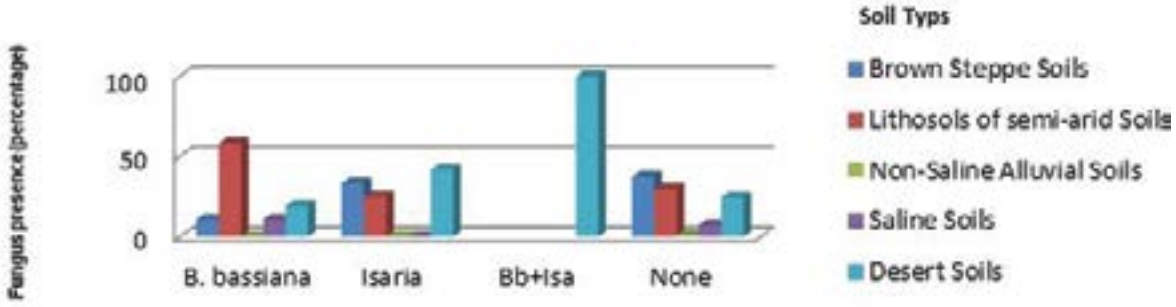

Figure 4: Distribution of entomopathognic fungi isolates in different soil types.

Table 3: A listing of sites in Central Iran where *Beauveria bassiana, Isaria fumosorosea* and *Isaria farinose* were extracted from soil samples.

ID	Latitude	Longitude	Elevation/meter	Date of collection	Province	Site	Entomo-Fungi	Vegetation
1	28/495267	56/968401	2077	09/29/15	Kerman	Ashin	*B.bassiana*	*Artemisia sp.*
2	28/493940	56/967606	2045	09/29/15	Kerman	Ashin	*B.bassiana*	*Artemisia sp.*
3	28/494185	56/990459	2059	01/20/16	Kerman	Ashin	*B.bassiana*	*Artemisia sp.*
4	28/491547	56/968782	2017	01/20/16	Kerman	Ashin	*B.bassiana*	*Grasses/Gramine*
5	28/515258	57/031295	2423	09/29/15	Kerman	Bagh borj	*B.bassiana*	*Artemisia sp.*
6	28/515409	57/033590	2383	09/29/15	Kerman	Bagh borj	*B.bassiana*	*Artemisia sp.*
7	28/514983	57/042431	2390	09/29/15	Kerman	Bagh borj	*B.bassiana*	*Artemisia sp.*
8	28/514979	57/043233	2331	02/22/15	Kerman	Bagh borj	*B.bassiana*	*Artemisia sp.*
9	28/514613	57/042529	2337	02/22/15	Kerman	Bagh borj	*B.bassiana*	*Artemisia sp.*
10	28/511427	57/024579	2465	01/19/16	Kerman	Bagh borj	*B.bassiana*	*Artemisia sp.*
11	28/637967	56/115815	1398	01/18/16	Kerman	Ghadamgah	*B.bassiana*	*Artemisia sp.*
12	28/759339	56/397800	2371	01/18/16	Kerman	Khabr	*B.bassiana*	*Artemisia sp.*
13	28/731166	56/354034	2068	01/18/16	Kerman	Khabr	*B.bassiana*	*Astragalus sp.*
14	28/730702	56/353202	2078	01/18/16	Kerman	Khabr	*B.bassiana*	*Astragalus sp.*
15	28/731220	56/353098	2072	01/18/16	Kerman	Khabr	*B.bassiana*	*Astragalus sp.*
16	34/806947	49/262513	2202	08/25/15	Markazi	Chehreghan	*B.bassiana*	*Artemisia sp.*
17	34/807064	49/262742	2205	08/25/15	Markazi	Chehreghan	*B.bassiana*	*Artemisia sp.*
18	34/425682	49/253157	2137	03/08/15	Markazi	Khondab	*Isaria sp.*	*Astragalus sp.*
19	34/425620	49/253111	2142	03/08/15	Markazi	Khondab	*Isaria sp.*	*Astragalus sp.*
20	34/425636	49/252454	2170	03/08/15	Markazi	Khondab	*Isaria fumosorosea*	*Astragalus sp.*
21	34/424584	49/253571	2226	03/08/15	Markazi	Khondab	*B.bassiana*	*Astragalus sp.*
22	34/424717	49/253671	2223	03/08/15	Markazi	Khondab	*B.bassiana*	*Astragalus sp.*
23	34/275770	49/538600	2012	08/26/15	Markazi	Navazen	*B.bassiana*	*Artemisia sp.*
24	34/275790	49/538490	1998	08/26/15	Markazi	Navazen	*B.bassiana*	*Artemisia sp.*
25	34/296017	50/972558	1967	10/26/15	Qum	Veshnaveh	*B.bassiana*	*Artemisia sp.*
26	34/285654	50/918506	1970	10/26/15	Qum	Virich	*Isaria farinosa*	*Artemisia sp.*
27	34/289575	50/919639	1918	10/26/15	Qum	Virich	*Isaria sp.*	*Astragalus sp.*
28	34/289761	50/91964	1917	10/26/15	Qum	Virich	*Isaria sp.*	*Astragalus sp.*
29	34/289895	50/919462	1918	10/26/15	Qum	Virich	*Isaria sp.*	*Astragalus sp.*
30	34/283501	50/929177	1947	02/15/16	Qum	Virich	*Isaria sp.*	*Ephorbia sp.*
31	34/486792	50/35346	1761	02/15/16	Qum	Zavarian	*B.bassiana*	*Artemisia sp.*
32	34/486868	50/353656	1747	02/15/16	Qum	Zavarian	*Isaria sp.*	*Artemisia sp.*
33	35/361738	52/524522	2050	10/27/15	Semnan	Dogosh	*Isaria fumosorosea*	*Astragalus sp.*
34	35/361728	52/520099	2112	10/27/15	Semnan	Dogosh	*B.bassiana*	*Artemisia sp.*
35	35/361738	52/519919	2115	10/27/15	Semnan	Dogosh	*Isaria sp.*	*Astragalus sp.*
36	35/355852	52/533307	1993	10/27/15	Semnan	Dogosh	*B.bassiana*	*Astragalus sp.*
37	35/355516	52/533948	1985	10/27/15	Semnan	Dogosh	*B.bassiana*	*Astragalus sp.*
38	35/355488	52/533633	1986	10/27/15	Semnan	Dogosh	*B.bassiana*	*Astragalus sp.*
39	36/367964	55/648164	1316	03-08-2016	Semnan	Maiamei	*B.bassiana*	*Artemisia sp.*
40	36/368172	55/648037	1341	03-08-2016	Semnan	Maiamei	*B.bassiana*	*Artemisia sp.*
41	36/368688	55/658193	1441	03-08-2016	Semnan	Maiamei	*B.bassiana*	*Astragalus sp.*
42	35/704719	53/456648	1694	10/29/15	Semnan	Pigambaraneh	*B.bassiana*	*Astragalus sp.*

CONCLUSION

In conclusion, in the summer temparatures were 18.8°C to 30.1°C. Mountainous snow cover could effectively provide extensive insulation for fungal survival in Sunn Pest overwintering locations. All isolates have been assigned documentation labels and placed in long-term storage at -80°C at the Sunn Pest Department at IRIPP, Tehran. They are available for scientific r esearch upon request. Contact: parsi@iripp.ir.

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