**Effect of Infection by *Beauveria bassiana* and *Metarhizium anisopliae* on the Feeding of *Uvarovistia zebra***

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**ABSTRACT.** To identify the susceptibility of long-horned grasshoppers to entomopathogenic fungi, the effect of infection with the fungi *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) on food consumption by *Uvarovistia zebra* (Uvarov) (Orthoptera: Tettigoniidae) was investigated. Preliminary results showed that both fungi had a negative effect on food consumption of the insects. For both fungi a significant reduction of food consumption and faeces production by insects were observed between the highest spore concentration (5 x 10⁶ spores/ml) and other treatments. Compared with control insects, the insects treated with 5 x 10⁶ spores/ml of *B. bassiana* and *M. anisopliae* showed 60 and 63% reduction in mean food consumption/insect, respectively. The corrected cumulative percent mortality of the insects treated with the highest concentration of *B. bassiana* and *M. anisopliae* were 57.7 and 55.5%, respectively. This was the first account of these entomopathogenic fungi being used against a species from this family, therefore based on the results obtained from this research, it could be said that the fungi have pathogenicity effect on *U. zebra* as a long-horned grasshopper.

**Key Words:** entomopathogen, fungi, feeding, grasshopper

The length of time that entomopathogenic fungi take to kill the target insect is one of their perceived disadvantages. Insect pathogens unlike chemical insecticides do not have a rapid impact on pest insects and need several days to kill their hosts; during this period the insects may continue feeding and cause damage to crops (Hajek 1989, Fargues et al. 1994).

The inability of mycoinsecticides to kill the insect host rapidly is one of the major factors limiting the utility of these biological control agents for control of pests including locusts and grasshoppers (Bateman and Thomas 1996). However, infection with entomopathogenic fungi can control damage resulting from reduced food consumption (Thomas et al. 1997). Therefore, reduction in food consumption by locusts infected with entomopathogenic fungi reduces the progressive damage and a locust that has stopped feeding could not be considered a significant pest (Moore et al. 1992).

*Uvarovistia zebra* (Uvarov) (Orthoptera: Tettigoniidae) is a native univoltine grasshopper, distributed on the southern slopes of the Alborz Mountains in the north of Qazvin province in Iran (36°33'45.3601" N, 50°51'12.0117" E). In some years, following good conditions for population growth, they can invade and damage field crops, and rangeland grass. The nymphs of the first instar appear in early April.

These insects inhabit rangelands which must be protected as natural environments. To prevent the adverse impacts of chemical insecticides use, entomopathogenic fungi are considered as a potential alternative for control of this pest. Orthoptera constitute 10% of the insects targeted by the entomopathogenic fungal products presented by Faria and Wraight (2007). However, evaluations of entomopathogenic fungi for control of grasshoppers and locusts generally have focused on Acrididae (short-horned grasshopper) species.

Significant reduction of feeding by the Brown locust *Locusta pardalina*, *Schistocerca piceifrons* piceifrons, and Desert locust *Schistocerca gregaria* have been demonstrated following infection by *M. anisopliae* var *acridum* (Moore et al. 1992, Seyoum et al. 1994, Arthurs and Thomas 2000, Hernandez-Velazquez et al. 2007).

Reduction of feeding by the *Rhammatocerus schistocercoides*, Variegated grasshopper *Zonocerus variegatus*, and African rice grasshopper *Hieroglyphus daganensis* infected with *Metarhizium flavoviride* was observed 2–3 d after inoculation (Thomas et al. 1997, 1998; Faria et al. 1999).

Apart from locusts and grasshoppers, reduction in feeding by insects infected with the fungi *B. bassiana* and *M. anisopliae* has also been reported from other insects species such as *Leptinotarsa decemlineata*, *Megalurothrips sjostedi*, *Chilo partellus*, *Ocinarana varians*, and *Liriomyza huidobrensis* (Fargues et al. 1994, Ekesi et al. 2000, Tefera and Pringle 2003, Hussain et al. 2009, Migiro et al. 2011).

In contrast, Cheung and Grula (1982) reported that no reduction in feeding by larvae of Corn earworm *Helicoverpa zea* infected with *B. bassiana* was observed before death. Food consumption by infected insects is one of several factors, such as host mortality, which indicates the virulence of a fungal pathogen and needs to be evaluated to measure the extent of pathogenicity. (Fargues et al. 1991, Moore et al. 1992, Seyoum et al. 1994, Thomas et al. 1997, Ekesi and Maniania 2000, Ondiaka et al. 2008).

Evaluation of entomopathogenic fungi for control of grasshoppers and locusts generally have focused on Acrididae (short-horned grasshopper) species. This is the first account of these entomopathogenic fungi being used against a species from the long-horned grasshoppers. The current work was done to identify the effect of fungi on the feeding of third instar nymphs of the long-horned grasshopper, *U. zebra* by both direct (food consumption) and indirect (faecal production) investigation.

**Materials and Methods**

**Source of Insects and Fungal Isolates.** Third instar field collected nymphs of *U. zebra* were used for the bioassay. The nymphs were collected from mountainous rangeland of the Alamout, Ghazvin, Iran. This insect is susceptible to mechanical damage and also shows cannibalistic behavior during transferring to the laboratory, particularly when many individuals were transferred together in a confined space.
In order to minimize this, a smaller numbers of locusts (4-7) were placed into plastic bags and the bags were then placed on ice. The low temperature helped decrease insect activity and in this way, locusts were transferred to the laboratory unharmed.

The insects were kept in groups of 15 in aluminum cages (45 by 38 by 38 cm, L by W by H) with wire No. 20 mesh sides and placed in the laboratory. They were fed fresh lettuce for 5 d in order to acclimate to a lettuce diet and laboratory conditions before beginning the experiments. The isolates B. bassiana DEBI 001, and M. anisopliae 715 C, obtained from the Iranian “Plant Pests and Diseases Research Institute” were used in the experiments.

**Culturing of the Fungi.** Both isolates were maintained on standard potato dextrose agar (PDA) (Merck). Spores were harvested from 14 to 18-d-old surface culture for each fungus by scraping and suspending the inoculum in 1 liter liquid medium potato-dextrose-broth (PDB) which had been autoclaved for 20 min at a temperature of 121°C and pressure of one atmosphere. The inoculated solution was shaken (110 rotations a minute) for 5 d at 25°C to produce conidia. The suspensions were stirred and filtered through a single layer of linen to remove culture debris and mycelia. After this time the conidia concentrations were determined using a haemocytometer and were calibrated to 5 × 10^6 spore/ml for each fungus. These suspensions represented the primary stock suspensions of conidia. Four different spore concentrations (5 × 10^5, 5 × 10^4, 5 × 10^3, 5 × 10^2 spore/ml) were then diluted from 5 × 10^6 spore/ml to 5 × 10^6 spore/ml for each fungus. These suspensions represented the primary stock suspensions of conidia. Four different spore concentrations (5 × 10^6, 5 × 10^5, 5 × 10^4, 5 × 10^3 spore/ml) were then diluted from the primary stock suspensions.

**Bioassay and Experimental Methods.** The experiments were arranged as a completely randomized design with five treatments (four conidial concentrations 5 × 10^6, 5 × 10^5, 5 × 10^4, 5 × 10^3 spores/ml and untreated control treatment of only water). Each treatment involved forty-five third instar nymphs of the locust divided in three replicates (three cages). The experiments were performed under laboratory conditions at 25–30°C with 75–80% relative humidity.

The conidial suspensions and control (distilled water) treatments were sprayed on the insects using a hand held sprayer with a flow rate of 70 ml/min adjusted by appropriate nozzles (Kassa et al. 2004). The sprayer was calibrated so that 160 ml of each suspension was used for three replicates (45 insects). Each insect was directly and individually sprayed with ~3.5 ml of the appropriate concentration. After 15 min the treated insects were transferred to the cages. Control groups received the same rate of water without conidia. The insects in each replicate were fed on lettuce (35 g every 48 h).

Feeding was assessed by measuring food consumption and monitoring faecal production.

At the beginning of the experiment, a sample of lettuce was dried to determine its dry weight percentage. For this purpose, some different parts of mature and young leaf (20 g) of the lettuce were broken into small pieces and mixed with each other. Fresh weight (7 g) from the mixed lettuce was taken and placed into an oven (95°C) for ~17 h (the sample was left in the oven until it reached a constant weight). Food consumption of insects for a 48 h period was measured before the insects were inoculated. Afterwards, every 48 h the uneaten lettuce from each cage was replaced with fresh lettuce and dried at 95°C until it reached a constant weight to get the dry weight percentage of the uneaten lettuce. The faeces produced from each cage were also collected every 48 h and dried as described earlier.

Mortality caused by each treatment was recorded every 48 h for 12 d. The data obtained were analyzed by analysis of variance (ANOVA). Multiple comparisons were used to determine significant differences between means of infected and control locusts at P < 0.05 (Tukey test), while comparisons between the two fungi were made using the t-test.

**Results**

Food consumption, and faecal production by the insects treated with different concentrations of the fungi were analyzed. In both B. bassiana and M. anisopliae, the greatest reduction in feeding was observed by insects infected with the highest spore concentration (5 × 10^6 spores/ml) (Table 1). Reduction in feeding of the infected insects started 2 d after treatment, but a significant reduction in feeding was not observed until 3–4 d after treatment (Fig. 1). A significant reduction of food consumption [F(4, 10) = 16.22, P < 0.05] and faeces production [F(4, 10) = 14.57, P < 0.05] were observed between the insects treated with the highest concentration (5 × 10^6 spores/ml) of B. bassiana, and other concentrations. Significant differences in feeding [F(4, 10) = 52.65, P < 0.05] and faeces production [F(4, 10) = 25.83, P < 0.05] were observed between the insects treated with the highest concentration of M. anisopliae and other concentrations were observed. Faeces production is also reduced when the insects were treated with

Table 1. Mean food consumption and faeces production (mg dry weight) measured every 48 h over 12 d, by third instar Uvarovistia zebra treated with different concentrations of Beauveria bassiana and Metarhizium Anisopliae

| Treatment (spores/ml) | Food/insect (mg dry weight) | Faeces/insect (mg dry weight) |
|----------------------|-----------------------------|------------------------------|
|                      | Means ± SE                  | Means ± SE                   |
| Control              | 83.41 ± 5.73^a              | 71.73 ± 4.02^d               |
| 5 × 10^3             | 87.57 ± 3.95^a              | 68.82 ± 6.55^d               |
| 5 × 10^4             | 59.36 ± 5.53^b              | 47.78 ± 4.68^d               |
| 5 × 10^5             | 69.04 ± 8.08^ab             | 55.40 ± 6.68^d               |
| 5 × 10^6             | 33.09 ± 1.56^b              | 22.32 ± 3.04^*               |
| 5 × 10^7             | 63.56 ± 2.88^d              | 5.71ab 71.73                 |
| 5 × 10^8             | 10^6 65.90 ± 3.34^a         | 30.73 ± 4.20^f               |

Means followed by a different letter is significantly different. For each fungus: N = 90 (number of samples), n = 15 (number of insects in each replicate).

Fig. 1. Mean (± SE) food consumption/insect (n = 45 nymphs within three replicates) by insects treated with 5 × 10^6 spores/ml of Beauveria bassiana and Metarhizium anisopliae. Mean followed by the same letter not significantly different.
M. anisopliae, but a significant difference \[F(4, 10) = 32.52, P < 0.05\] was observed between the highest concentration, and three other concentrations as shown in Table 1. Non significant differences in reduction of food consumption \[t(4) = 1.75, P = 0.1541\], faeces production \[t(4) = 0.93, P = 0.4044\], and mortality \[t(4) = 0.70, P = 0.5185\] were observed between B. bassiana and M. anisopliae at concentration of \(5 \times 10^6\) spores/ml.

In comparison with control insects, the insects treated with \(5 \times 10^6\) spores/ml of B. bassiana and M. anisopliae showed 60 and 63% reduction in mean food/insect, respectively.

However, there was nonsignificant difference between food consumption in the control and treatment with lowest concentration \((5 \times 10^5\) spores/ml) of both fungi was observed.

Mean food consumption (every 48 h) by insects treated with the highest concentration \((5 \times 10^8\) spores/ml) is shown in Fig. 1. The maximum feeding reductions by insects were observed at 6–8 d after treatment when they were treated with M. anisopliae and B. bassiana, respectively. Also, in comparison with the first 48 h, after 8 d the mean food consumption by insects was reduced by 52 and 56% at 8 days after treatment using B. bassiana and M. anisopliae, respectively.

Table 2 shows the percent mortality recorded every 48 h. The maximum mortality by insects infected with the concentration \(5 \times 10^6\) spores/ml of B. bassiana and M. anisopliae were recorded at day 8 and 12, respectively. The results showed that the cumulative percent mortality of the locust treated with \(5 \times 10^6\) spores/ml of B. bassiana and M. anisopliae reached 58 and 56%, respectively. Significant differences in mortality were found between the highest concentration \((5 \times 10^6\) spores/ml) and the other treatments of each fungus \([B. bassiana: F(4, 10) = 129.80, P < 0.05; M. anisopliae: F(4, 10) = 108.00, P < 0.05] (Table 3).

Table 2. Percent mortality of insects treated with highest concentration \((5 \times 10^6\) spores/ml) recorded every 48 h after treatment.

| Days | N1  | N2  | B. bassiana | N1  | N2  | M. anisopliae |
|------|-----|-----|-------------|-----|-----|-------------|
| 2    | 45  | 45  | 0*          | 45  | 45  | 0*          |
| 4    | 45  | 45  | 6.66ab      | 45  | 44  | 2.22a       |
| 6    | 42  | 46  | 6.66ab      | 44  | 42  | 4.44        |
| 8    | 39  | 30  | 0.00c       | 42  | 36  | 13.33ab     |
| 10   | 30  | 30  | 15.55bc     | 36  | 30  | 13.33ab     |
| 12   | 23  | 19  | 8.88ab      | 30  | 20  | 22.22ab     |
| Cm   | 57.7% | 55.5% |             |      |     |             |

N1 = number of surviving insects at the beginning of the 48 h period.
N2 = number of surviving insects at the end of that 48 h period.
Cm = cumulative mortality. Mean followed by a different letter is significantly different.

Table 3. Mean mortality caused by each fungal concentration at the end of experiment

| Treatment (spores/ml) | B. bassiana | M. anisopliae |
|-----------------------|-------------|---------------|
| Control               | 0*          | 0.33ab        |
| 5 \times 10^5         | 1.33ab      | 1.67         |
| 5 \times 10^4         | 2.00b       | 1.67         |
| 5 \times 10^3         | 7.00c       | 6.33b        |
| 5 \times 10^2         | 8.67d       | 8.33d        |

N = 45 insects within three replicates for each treatment.

Discussion

Entomopathogenic fungi-like B. bassiana and M. anisopliae unlike chemical insecticides do not have a speedy effect, while the reduction in food consumption and activity due to the application of fungi by the locusts can be considered as an advantage in pest control.

In this study, a large volume of spore suspensions were sprayed to the grasshoppers; however, this volume of spray was not needed for the bioassays. The bioassays could be done using very low volume of spore suspension pipetted beneath the dorsal pronotum (Prior et al. 1995) or immersion bioassay method which insects are immersed into the spore suspension. However, the spray method using high volume of spore suspensions were used to assay the isolates because (1) this was the first time that the efficacy of entomopathogenic fungi had been evaluated on a long-horned grasshopper, so it was necessary to ensure that the sufficient fungal spores have been received by insects (2) as this study will be continued in the field conditions, spraying bioassay method is similar to the use of fungi when they will be subsequently sprayed against the grasshoppers in the field.

The present study showed that a reduction of feeding in nymphs of U. zebra infected with the highest concentration \((5 \times 10^8\) spores/ml) started 2 d after inoculation (Table 1), but a significant reduction in feeding comparing the same conidial concentration with control was observed 3–4 d after treatment for both fungi. The results obtained from the present study showed a significant feeding reduction by insects 3 d after treatment, it agrees with findings by Moore et al. (1992) and Hernández-Velázquez et al. (2007) demonstrating a significant reduction in the S. gregaria and S. piceifrons feeding 3 d after application of M. anisopliae var. acridum, respectively.

Significant mortality in locusts was observed 6 and 8 d after infection with B. bassiana and M. anisopliae, respectively. It demonstrates that food consumption of third instar nymphs of U. zebra infected with B. bassiana and M. anisopliae was reduced before dying caused by the fungi.

Nonstatistical difference was observed in mortality of insects treated with the fungi at the same concentration \((5 \times 10^6\) spores/ml), but B. bassiana caused more mortality than M. anisopliae. In contrast, at the same concentration, reduction in feeding by the insects treated with M. anisopliae was more than those treated with B. bassiana, but again with no statistical difference.

The treatments containing less than \(5 \times 10^6\) spores/ml did not have a notable effect on the food consumption by the insects. Reduction in feeding due to fungal infection may affect body fat accumulation at sexual maturity and consequently reproductive potential of infected, but surviving insects. Effect of infection by B. bassiana and M. anisopliae on the oviposition of locust and other insect hosts have been investigated in some studies (Arthurs and Thomas 2000, Ondiaka et al. 2008, Migiro et al. 2011). Accordingly, reduction in feeding of infected insects can affect their population density in the next generation.

In conclusion, reduction in feeding can be observed in U. zebra infected by the fungi; consequently, the insect damage decrease. However, in the case of very high population, reduction of food consumption resulting by the fungi may not be consequent to reduce the damage caused by locusts.

Achieving high population of the grasshopper R. schistocercoides, the reduction of food following application of M. flavoviride would not be sufficient to prevent economic damage (Faria et al. 1999).

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