Impact of Entomopathogenic Fungi on the Desert Locust, *Schistocerca gregaria* (Forskal)

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ABSTRACT

Commercially formulated entomopathogenic fungi, Bioranza (*Metarhizium anisopliae*) and Biovar (*Beauveria bassiana*) were evaluated against the desert locust, *Schistocerca gregaria* (Forskal). Results clarified that *M. anisopliae* proved significant higher rapid effects than *B. bassiana*, on the pest, either applied by direct spray on young nymphs or indirect, through soil treatment. In case of indirect treatment, mortality percentages among hatching nymphs 4 days post contamination with *M. anisopliae*, were almost significantly higher than those contaminated with *B. bassiana*. LT_{50} values for *B. bassiana* were about 1.25, 1.53 and 1.38 times higher than those for *M. anisopliae*, at 4, 2 and 1g/L concentrations, respectively, which indicates that the infectivity and virulence of *M. anisopliae* against locust nymphs was much faster than that with *B. bassiana*. Similar results were also obtained in case of direct treatment of nymphs, when observations of nymphs mortality extended till either death of nymphs or adult formation. More than 95% mortality was achieved after 10, 12 and 18 days for *M. anisopliae* and after 10, 18 and 22 days for *B. bassiana* at 4, 2 and 1g/L concentrations, respectively.

Key Words: Entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana*, desert locust, *Schistocerca gregaria*

INTRODUCTION

Locusts and grasshoppers are among major pests of many tropical and subtropical countries, causing serious damage to the foliar part of many plants. Alternatives to hard chemicals for their control have been extremely limited.

Entomophthogenic fungi are often important in the natural control of some insect pests under warm humid conditions. Research has focussed on the relatively easily produced asexual spores (conidia) of the hyphomycete genera *Metarhizium*, *Beauveria*, *Verticillium* and *Paecilomyces*. These fungi often have a wide host range although there is a considerable genetic diversity within species and some varieties showed a high degree of specificity (Driver *et al.*, 2000).

Unlike other potential biocontrol entomopathogens, fungi do not have to be ingested to infect their hosts but they invade directly through the cuticle, and so can, potentially, be used for control of all insects including sucking insects (Onsager *et al.*, 1992). Mycoinsecticides are usually formulated products with live conidia as the active ingredient. The conidia germinate on contact with the cuticle of the insect, particularly the intersegmental portions, produce a penetrating germ tube and establish a systemic infection which kills the host in 7 to 21 days depending on conditions, especially temperature and dose. At death, the host insect shows hard cadaver full of mycelium which grows out through the cuticle to form a new generation of conidia on the outside of the cadaver (Onsager *et al.*, 1992).

Metarhizium is a genus composed of three species divided into ten varieties (Driver *et al.*, 2000). The most common form is the genetically highly diverse *Metarhizium anisopliae* var. *anisopliae* (Metsch.) Sorokin. The soil forms its normal habitat, although it does not grow saprophytically in soil but exists as dormant conidia which infect susceptible hosts on contact. The soil-inhabiting larvae of scarab beetles are typical hosts and co-evolution has led to some isolates being specific to one or two genera of Scarab. Thus, the most virulent strains are usually those which cause natural epizootics in that particular host. This host specificity is dose-dependent: a high dose will infect a very wide range of hosts. Most isolates grow well between 15 and 30°C, although some develop at temperatures as low as 5-10°C and others grow even at 35-40°C. Some isolates produce one or more members of a family of toxins called destruxins and while production of destruxin does sometimes correlate with virulence (Kershaw *et al.*, 1999), their role in pathogenicity is controversial. Certainly these compounds are toxic to some non-target hosts when injected directly into the body cavity and one, destruxin E, is toxic *per os* for other insects such as Diptera, leading to speculation that destruxins could be used as insecticides. They can also be antifeedants (Amiri *et al.*, 1999).

Many trials carried out all over the world to control desert locust and grasshoppers using the

entomopathogenic fungi, especially those of *Metarhizium* spp. and *Beauveria* spp. (Bateman *et al.* 1993, 1996; Lomer, *et al.*, 1997; Neethling and Dent, 1998).

The objective of this study is to evaluate the efficacy of the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* formulated commercially as Bioranza and Biovar, respectively, against the desert locust, *Schistocerca gregaria* (Forskal) under laboratory conditions.

MATERIALS AND METHODES

Pathogens

Commercial formulations Bioranza and Biovar were used in this investigation; they were manufactured and produced by The Kingdom of Bahrain, Ministry of Municipalities Affairs and Agriculture Wealth Directorate. The active ingredient of Bioranza is *M. anisopliae* (10%) formulated as WP, while the inert ingredient represented 90%; the recommended application dose is 200g/100L water. Biovar active ingredient is *B. bassiana* formulated as WP; the active ingredient concentration is 32 x 10^6 viable spore/mg; the recommended application dose is 200g/100L water. Three concentrations of each fungus were used (4, 2 and 1g/L water).

Insect host

Newly hatched hoppers of *S. gregaria* were kept in wooden cages with wire-gauze sides (40x40x60 cm) with a small door in the upper side to permit daily feeding and routine cleaning. The bottom was coated with 20cm layer of sterilized sand. Egyptian clover, *Trifolium alexandrinum* was daily provided as fresh food supply. Each cage was equipped internally with 60-W electric lamb to lighten the cage in daily regime of 8:16 D: L, laboratory temperature was maintained at 30±2°C. The relative humidity ranged 70 - 80% (Hunter-Jones, 1961).

Treatments

1. Soil treatment (indirect treatments)

Plastic cups 300ml volume comprised a mixture of 100g sterilized soil moistened with the tested concentration of the fungus and with suitable volume of water for keeping the humidity of the soil at about 75-85% R.H. to obtain the desired concentrations.

Clusters of locust eggs (30 eggs/each) were embedded, each, in the treated soil, into the experimental cup. The cups were covered with muslin cloth and incubated at 28±2°C until hatching. Daily examination of cups was carried out for monitoring hatching percentages and adjusting humidity as well. After hatching, number of survivor and dead nymphs were recorded daily and calculated, cumulatively after certain day's intervals. The hatched 1st instar nymphs were collected and transferred to a clean wooden cage supplied with fresh clover for feeding. The development of the nymphs was followed-up daily until death. The cadavers were removed from the cages, then surface sterilized in 5% sodium hypochlorite and 75% ethanol solution and rinsed in a plenty of sterile distilled water, then left to dry for 48h (Douroukpinduo *et al.*, 1995). After drying, they were kept in humid conditions in clean desiccators at room temperature to examine whether they died because of fungus infection or not according to Luz and Farques (1998). The mortality percentages of hatched nymphs were calculated 4, 6, 8 and 10 days post hatching. The whole experiment was replicated five times for each concentration of each fungus.

2. Nymph treatment (direct treatment)

Two groups of aforementioned described wooden cages, were prepared for nymphal rearing and treatments. At the 1st group cages, each cage comprised 25 individuals of the 1st instar nymphs, supplied with food (clover), then sprayed separately with each of the tested concentration (4, 2 and 1 g/L) of each fungus suspension (direct treatment). The 2nd group of cages were sprayed with water only and used as check. The whole experiment was replicated five times. Three days post treatment; nymphs were transferred into clean cages supplied with fresh clover for feeding. Daily examination was carried out till the death of the nymphs. The cadavers were removed from the cages and then treated as mentioned above. The mortality percentages of nymphs were calculated 5, 7, 15 and 17 days post treatment.

Statistical analysis

All experiments were subjected to analysis of variance (ANOVA), using SPSS computer program, and the means were compared using Duncan's Multiple Range test. Percentage mortality was corrected using Abbott formula (Abbot, 1925) for determining LT_{50} (median lethal time for 50% mortality) values and by using probit analysis (Finney, 1952).

RESULTS AND DISCUSSION

Generally, no mortality was observed before the 4th day post treatment.

Effect of soil treatment on egg hatching

Data in table (1) indicated slight effects of the two tested entomopathogenic fungi on the locust's

eggs. At the highest concentration (4g/L) of both fungi, percentages of un-hatched eggs did not exceed 13.33 and 9.33%, for Biooranza and Biovar, respectively.

Table (1): Effect of *Metarhizium anisopliae* and *Beauveria bassiana* on *Schistocerca gregaria* (Forskal) eggs (Mean ± SE)

Concentr-ation (g/L)	% un-hatched eggs		T-value
	Bioranza	Biovar	
4	13.33±2.36 a A	9.33±1.91 a A	1.310 ^{NS}
2	8.67±3.09 a A	5.33±1.33 b A	0.990 ^{NS}
1	1.99±0.82 b A	2.67±1.25 bc A	0.448 ^{NS}
Check	00.00±0.00 b A	00.00±0.00c A	0.000 ^{NS}
F-value	9.542**	8.912**	

- Means in a column followed with the same SMALL letter(s) are not significantly different at 5% level of probability
- Means in a row followed with the same CAPITAL letter(s) are not significantly different at 5% level of probability

** = Highly significant NS= Not significant

Effect of soil treatment on first nymphal instar (indirect treatment)

Effects of the fungi on the newly hatched nymphs from eggs embedded in fungi-treated soil were evaluated four days post hatching for both fungi (Table 2 and Fig. 1a & b). Thereafter, the mortality of the nymphs increased gradually as post treatment period increased. Data in table (2) showed significant differences between the efficacy of the two products at both highest and lowest concentrations.

Bioranza almost possessed complete reduction (100%) after 10, 12 and 18 days post nymphal hatching, at 4, 2, 1/L concentrations, respectively (Fig. 1a). The corresponding figures for Biovar were 96, 96 and 100% reduction after 10, 18 and 22 days, respectively (Fig. 1b). Data in table (3) showed that, at the highest concentration level (4g/L), LT_{25} and LT_{50} values for Bioranza were 3.520 and 4.836 days and 4.589 and 6.026 days for Biovar, respectively. At 2g/L concentration level, the corresponding figures were 4.675 and 6.319 days for Bioranza and 5.429 and 9.644 days for Biovar, respectively. At the least concentration level (1g/L), they were 5.250 and 11.283 days for Bioranza and 8.422 and 15.525 days for Biovar, respectively.

From the abovementioned results, it could be concluded that the effect of Bioranza (*M. anisopliae*) formulation was much rapid than the Biovar (*B. bassiana*) for controlling the 1st instar nymphs of the desert locust *S. gregaria* resulting from treated eggs.

First nymphal instar treatment (direct treatment)

Newly hatched nymphs, within 24 hours, were sprayed with three fungal concentrations (4, 2 and 1g/L). Data in table (4) clarify that, no mortality was observed 4 days post treatment at all tested concentrations. Recorded percentage mortality after 5 days of treatment were 13.44 and 7.40 for Bioranza and Biovar at the higher concentration (4g/L) %, respectively, increased gradually with increasing post treatment time. Statistical analysis of the obtained results indicated that there were significant differences between the two products at all the tested concentrations and time of check. Also, significant differences were observed among the tested concentrations (Table 4 and Fig. 2a & b).

Data in table (5) showed that the LT_{25} and LT_{50} values were 5.845 and 8.458 days for Bioranza at the higher concentration and 7.959 and 12.919 days for Biovar, respectively. The LT_{25} and LT_{50} values for the least concentration (1g/L) were 9.232 and 15.626 days for Bioranza and 11.452 and 18.261 days for Biovar, respectively.

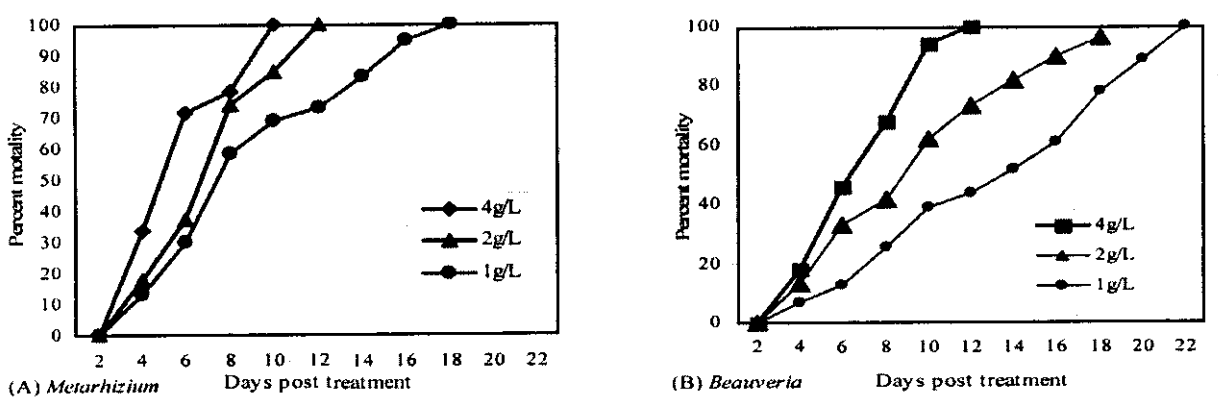

Fig. (1): Effectiveness of the Bioranza and Biovar fungi products on the 1st nymphal instar of *Schistocerca gregaria* (Forskal) treated indirectly.

Table (2): Effect of *Metarhizium anisopliae* and *Beauveria bassiana* on the mortality of the newly hatched nymphs of *Schistocerca gregaria* (Forskal) resulted from treated eggs (indirect treatment)

Days post treatment	Concentration (g/L)	Mean percent mortality ± SE		T-value (df=8)
		Bioranza	Biovar	
4	4	33.60±0.98 a A	18.40±2.29 a B	6.095**
	2	17.80±1.50 b A	13.20±1.32 b A	2.061 NS
	1	13.20±1.66 c A	7.15±1.21 c B	3.378**
	Check	00.00±0.00 d	00.00±0.00 d	
	F-value	129.461**	29.675**	
6	4	71.60±3.89 a A	46.00±3.49 a B	4.894**
	2	37.60±2.20 b A	33.00±2.98 b A	1.240 NS
	1	30.20±1.71 c A	12.96±0.82 c B	9.064**
	Check	00.00±0.00 d	00.00±0.00 d	
	F-value	150.654**	77.070**	
8	4	78.20±3.34 a A	67.40±3.91 a A	2.102 NS
	2	74.40±5.64 a A	41.60±2.99 b B	5.140 **
	1	58.40±3.36 b A	25.44±1.51 b B	8.954 **
	Check	00.00±0.00 c	00.00±0.00 c	
	F-value	96.773**	120.806**	
10	4	100.00±0.00 a A	94.00±2.28 a B	2.631 *
	2	85.20±2.94 b A	62.00±1.38 b B	6.951 **
	1	69.20±2.37 c A	39.20±2.42 c B	8.854 **
	Check	00.00±0.00 d	00.00±0.00 d	
	F-value	547.884**	461.928**	

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Table (3): LT-values of hatched *Schistocerca gregaria* (Forskal) nymphs resulted from eggs treated with different tested concentrations of *Metarhizium anisopliae* and *Beauveria bassiana* (indirect treatment)

Concentration	LT values	Bioranza (*Metarhizium anisopliae*)	Biovar (*Beauveria bassiana*)
		Time (in days)	
4 g/L	LT_{25}	3.520	4.589
	LT_{50}	4.836	6.026
	Slope	4.896	5.699
2 g/L	LT_{25}	4.675	5.429
	LT_{50}	6.319	9.644
	Slope	5.154	2.703
1 g/L	LT_{25}	5.250	8.422
	LT_{50}	11.283	15.525
	Slope	2.030	2.540

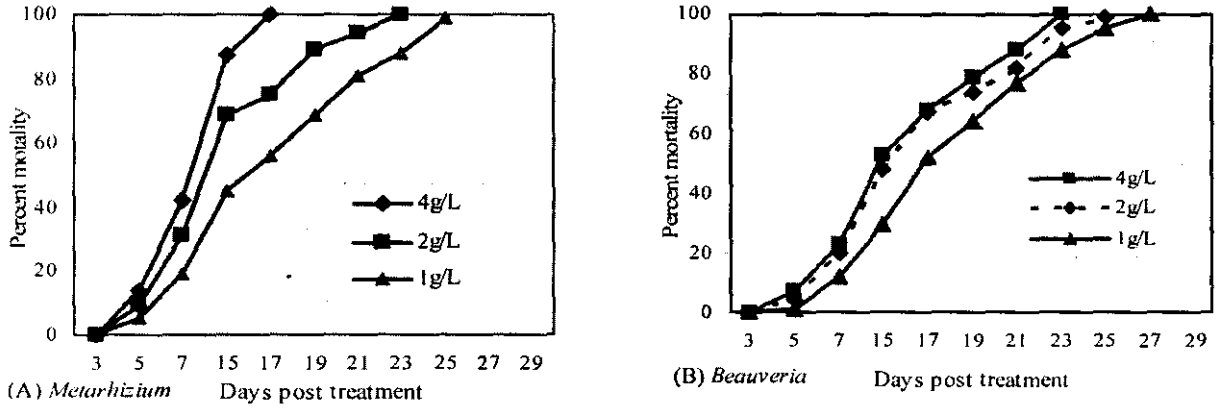

Fig. (2): Effectiveness of Bioranza and Biovar fungi products on the 1st nymphal instar of the desert locust *Schistocerca gregaria* (Forskal) (direct treatment).

Table (4): Effect of the commercial fungi products, bioranza (*M. anisopliae*) and Biovar (*B. bassiana*) against newly hatched nymphs of *S. gregaria* (direct treatment)

Days post treatment	Concentration (g/L)	Mean percent mortality ± SE		T-value (df=8)
		Bioranza	Biovar	
5	4	13.44±1.22 a A	7.40±0.70 a B	4.289**
	2	8.54±0.97 b A	4.25±1.09 b B	2.758*
	1	5.09±0.99 c A	1.39±0.41 c B	3.472**
	Check	0.00±0.00 d	0.00±0.00 c	
	F-value	37.658**	31.018**	
7	4	41.45±2.21 a A	23.26±1.93 a B	201**
	2	31.30±2.32 b A	20.06±1.43 a B	4.121**
	1	18.97±1.98 c A	11.89±0.81 b B	3.307*
	Check	0.00±0.00 d	0.00±0.00 c	
	F-value	89.506**	67.054**	
15	4	87.30±2.17 a A	52.86±1.88 a B	12.005**
	2	68.47±2.34 b A	47.36±2.57 a B	6.077**
	1	44.36±3.07 c A	30.29±2.06 b B	3.810**
	Check	0.00±0.00 c	0.00±0.00 c	
	F-value	290.438**	157.491**	
17	4	99.22±0.33 a A	68.56±2.74 a B	11.103**
	2	76.02±1.79 b A	66.76±3.54 a B	2.335*
	1	56.57±1.60 c A	52.32±3.07 b A	1.226^{NS}
	Check	0.00±0.00 d	0.00±0.00 c	
	F-value	121.915**	139.911**	

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- Means in a row followed with the same CAPITAL letter(s) are not significantly different at 1% level of probability
** = Highly significant * = Significant NS= Not significant

Table (5): LT-values of newly hatched *S. gregaria* nymphs treated at different concentrations of bioranza (*M. anisopliae*) and Biovar (*B. bassiana*) (direct treatment)

Conc.	LT values	Bioranza (*Metarhizium anisopliae*)	Biovar (*Beauveria bassiana*)
		Time (in days)	
4 g/L	LT_{25}	5.845	7.959
	LT_{50}	8.458	12.919
	Slope	4.204	3.206
2 g/L	LT_{25}	6.900	8.843
	LT_{50}	10.689	13.925
	Slope	3.549	3.421
1 g/L	LT_{25}	9.232	11.452
	LT_{50}	15.626	18.261
	Slope	2.951	3.328

It could be concluded that the entomopathogenic fungus, *M. anisopliae* was more effective than *B. bassiana* when applied either as soil treatment (indirect) or spraying (direct treatment). In the indirect treatment, after 4 days of treatment, *Metarhizium* was about 1.4 to 1.9 times more effective than *Beauveria*. Also, after 8 days of treatment, *Metarhizium* was still more effective. The achieved mortality was about 1.8 and 2.3 times than *Beauveria* at 2 and 1g/L concentration levels, respectively. The same trend was observed in case of the direct treatment, where *Metarhizium* was still more effective than *Beauveria*. The achieved mortality ranged between 1.8 and 3.7 times after 5 days of treatment, reduced to reach 1.4 and 1.7 after 15 days of treatment that means that *Beauveria* needed much time to induce its effects.

The obtained results indicated that *Metarhizium* spp. was more effective than *Beauveria* spp. when applied separately against eggs and the first nymphal instar of *S. gregaria*. The low effectiveness of *Beauveria* against the desert locust, were partially in accordance with those of Krall and Nasseh (1990) and Nasseh *et al.* (1991) who reported that *Nosema locustae* and *B. bassiana* were not effective biological control agents against *S. gregaria* under their experimental conditions; as well with those obtained by Balogun and Fagade (2004) who mentioned that the incidence rates of isolated fungi from the grasshoppers, *Zonocerus variegatus* cadavers was 20% for *Metarhizium* and 18% for

B. bassiana, but they were contradicting with our results when they mentioned that *Beauveria* spp. was more effective than *Metarhizium* sp. in locust control (may be to virulence of isolate).

Also, obtained results clarified that, *Metarhizium* spp. was more effective than *Beauveria* spp. against *S. gregaria* which agree with those reported by Bateman and Aves (2000) when tested two rotary atomizers commonly used for small scale ULV spraying in Africa for rate and volume of application of formulated *Metarhizium* against *S. gregaria.* Also, the results agree with those reported by Milner (2000), who mentioned that commercial formulations based on *Metarhizium* spp. are effective enough to control locusts and grasshoppers in Australia, Fargues *et al.* (2001), evaluated the effect of pathogenic activity of the liquid culture media of *Metarhizium* spp. towards locusts and grasshoppers, Arthurs and Thomas (2001), Arthurs *et al.,* (2001), Simon and Matthew (2001), Kooyman (2003) examined *Metarhizium* against *S. gregaria* in semi-field experiments. Meanwhile, Klass *et al.* (2007) developed a temperature-dependent model to predict the field performance of *Metarhizium* the key fungal pathogen used as locust biopesticides.

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