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Efficacy of the Fungus, *Metarhizium anisopliae* var. *acridum* against Some Acridid Insects

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ABSTRACT

Acrididae pests are serious agricultural pests that cause considerable damage to food crops and pasture grasses, particularly during outbreaks. New control strategies aim to use of relatively safe materials such as pathogens. In this study, *Metarhizium anisopliae* var. *acridum* (Green Muscle) was applied against some Acrididae pests at different periods in some places in Egypt considered favorable breeding sites to test its efficacy on the target pests under the Egyptian ecological conditions. Results showed that the efficacy of *M. anisopliae* var. *acridum*, in all treatments, indicated that it was a specific bio-pesticide for controlling Acrididae pests but it seems to be slow acting as a bio-agent unlike chemical pesticides. Efficient applications were in case of grasshoppers, desert locust nymphs, desert locust adults or migratory locust adults and then local locust (trees locust), respectively. At field application rates, 50 g/ha dose (diluted in diesel) of *M. anisopliae* var. *acridum* resulted in an optimal mortality of locusts and grasshoppers in the cages during 21 days, followed by 50 g/ha dose diluted in vegetable oil, respectively. Use of diesel for dilatation may be more practical than vegetable oil. Accordingly, Green Muscle® can be useful for developing different elements of an IPM strategy as a preventive measure whereas chemical insecticides will be most effective in curative treatments.

Key words: *Metarhizium*, Acrididae, locust, *Schistocerca gregaria*, Grasshoppers, biological control.

INTRODUCTION

Locusts and grasshoppers (Orthoptera: Acrididae) are serious agricultural pests that cause considerable damage to food crops and pasture grasses in Africa and Western Asia (Showler, 1993), particularly during outbreaks.

At present, the main prevailing method for controlling locust and grasshopper outbreaks involves use of synthetic chemical insecticides. Due to the environmental and pest-resistance problems associated with chemical pesticides, there is an increasing interest for the exploitation of biological control agents, available as commercial products or those still under development.

Consequently, the environmental pollution by chemical pesticides such as; toxicity to non-target organisms (Tingle, 1996) and humans (Pretty, 1996) has led to new strategies and development of environmental friendly alternatives to control locusts and grasshoppers based on microbial control agents (Johnson and Goettel, 1993; Lomer *et al.*, 1997 and Lange, 2005).

Uses of bio-control agents are considered suitable and promising alternatives to chemical pesticides for controlling Acrididae pests (Van Huis, 1992). Recent advances in biological control researches, coupled with improved surveillance and intelligence, could make big differences when the next round in the battle is fought. Such bio-products could make it possible to sharply reduce the amount of chemical pesticides used (FAO, 2007 and Van der Valk, 2007).

The entomopathogenic fungi are considered promising, because some of them could be manipulated as biological pesticides. This allows the possibility of using conventional application technology by aerial spraying, which is already highly developed for locust swarms control. Microbial control agents can be effective for controlling locusts and grasshoppers for many reasons such as; effects on their activity, reproduction, food consumption, development and behavior (Woldewahid *et al.*, 2007).

M. anisopliae var. *acridum* has been registered for use in many parts of Africa (Green Muscle®), and in Australia (Green Guard®) and it is under evaluation in a number of other locust affected countries around the world (Thomas, 2000). The process of mycopathogenesis begins with attachment of the conidia with the cuticle of insect, followed by germination and penetration of the cuticle by the germ tube. Consequently, the insect death results from toxemia or nutrient depletion (Charnley, 1984).

Several authors obtained almost the same results and they reported that *M. anisopliae* var. *acridum* was effective against locusts and grasshoppers in the field, *i.e.* against desert locust, *Schistocerca gregaria* in Mauritania (Langewald *et al.*,1997), against migratory locust, *Locusta migratoria migratorioides* in an aerial treatment in Australia (Hunter *et al.*,1999), against Australian plague

locust, *Chortoicetes terminifera* (Hunter *et al.*, 2001), against variegated grasshopper, *Zonocerus variegates* in Benin (Douro-Kpindou *et al.*, 1995), against wingless grasshopper, *Phaulacridium vittatum* in Australia (Milner *et al.*, 1994), against grasshoppers particularly Rice grasshopper, *Hieroglyphus daganensis*, in northern Benin (Lomer *et al.*, 1997) and against Sahelian grasshoppers (Kooyman *et al.*, 1997).

This study aims to evaluate the efficacy of the fungus, *Metarhizium anisopliae* var. *acridium* (Green Muscle), against locusts and grasshoppers (Orthoptera: Acrididae) under Egyptian ecological conditions, could be useful for developing different of an IPM strategies, both optimizing time and place of treatment and combining or replacing the use of chemical insecticides.

MATERIALS AND METHODS

1. Experimental insects and locations of field trials:

During 2007, locusts and grasshoppers preliminary surveys were carried out several times in some places which are considered favorable breeding sites for the desert locust and grasshoppers. Such sites may expect to have risks after rains fall. Small-scale breeding sites occurred in; Baharia Oasis (Egyptian Western Desert), along both sides of Lake Nasser (South of Aswan City), Sharq El-Owainat region (South of Western Desert), and Abu-Ramad region (Red Sea Coast, near Elba Mountains and Egyptian-Sudanese border). Therefore, three field trials were carried out under cages to evaluate different effects of the fungus, *M. anisopliae* var. *acridum* (Green Muscle®), against locusts and grasshoppers under the Egyptian environmental conditions in three sites: Baharia Oasis in June 2007; Sharq El-Owainat region in November 2007 and Abu-Ramad region in December 2007.

2. Entomopathogenic Fungus:

The LUBILOSA (LUtte BIologique contre les LOcustes et les SAuteriaux) program has developed an oil formulation containing the fungal pathogen, *Metarhizium anisopliae* var. *acridum* (Deuteromycotina: Hyphomycetes), a specific bio-pesticide for control of locusts and grasshoppers (Bateman, 1997; Lomer *et al.*, 1999; Lomer *et al.*, 2001; Langewald *et al.*, 2003 and Van der Valk, 2007).

Green Muscle® is one of the commercial products used as a bio-pesticide (LUBILOSA, 1999) which contains spores of *M. anisopliae* var. *acridum*, strain IMI 330189, which was originally isolated from a dead locust in West Africa. This product contains spores of the fungus available in special formulation suitable for dry and desert conditions. It was kindly provided by the General Department for Locusts and Agro-Aviation Affairs, Egyptian Ministry of Agriculture in co-operation with FAO from the Biological Control Products of South Africa (Pty) Ltd. It stored at 4 °C to time of applications as recommended by the Green Muscle User Manual (Bateman and Alves, 2000).

Green Muscle® is available in special two formulations suitable for dry and desert conditions: 1) as dry conidial technical powder (TC) and formulated in oil on demand; 2) as oil miscible flow-able concentrate of fungus spores (OF), when diluted in appropriate oil/diesel, it becomes suitable for use with ULV spraying on demand.

3. Fungus Formulation:

Dry conidial technical powder (TC) (the aluminum sachet) containing *M. anisopliae* var. *acridum* (Green Muscle®) spores was one kilogram. The content of sachet was added to an appropriate amount of vegetable (sunflower oil) or diesel oils and delivered inside the sachet by a big syringe to avoid spillage of the spore dust and then the contents were squeezed into a clean bucket. The sachet was washed with more oil or diesel to transfer all the residual spores into the bucket. A total of twenty liters of oil or of diesel were used for each one kilogram of (Green Muscle®) spores. This initial stock mixture was prepared at night for using in the field next day.

For field application, 50 g/ha + 1 liter of the stock mixture was diluted in 1 liter of oil or diesel and resulting mixture was applied at two liters per ha (FAO, 2005 and 2007).

Spore formulation of the (Green Muscle®) was used at 2 rates of applications: 25 g and 50 g/ha. through five treatments applied as follow: 1) control (untreated); 2) 25g/ha (diluted with vegetable oil); 3) 25g/ha (diluted with diesel); 4) 50 g/ha (diluted with vegetable oil) and 5) 50g/ha (diluted with diesel). Conidia viability was assessed one day before application and percent germination was found over 83%. Each treatment was applied in 3 plots, each plot under cage size $1m^3$ (1 x 1 x 1m). Spacing among plots was randomizing to avoid contamination by the spray drift.

4. Field Trials:

Experimental design was a Randomized Complete Block Design (RCBD) with three replications. At the time of trial applications, wind speeds were measured by wind meter (Anemometer)

and the relative humidity (RH) was measured by Psychrometer. Daily numbers of living and dead insects in cages were counted, in daily interval, for 21 days of application as well assessment of mortality was carried out. The results were recorded after one, two and three weeks.

A- First Trial:

This field trial was conducted in June 2007, against relatively high populations of different grasshoppers, mixed with few local locusts in a habitat in the Western Desert at Baharia Oasis. The coordinates of the field trial site was (28 21 N/28 51 E). Treated area was cultivated by alfalfa (*Medicago sativa*) in sandy loam soil. Population densities were randomly, 15 insects of different grasshoppers/m^2 and 5 insects of local locust/m^2. Numbers of grasshoppers and local locust were collected, counted and placed in each cage (1 m^3). The grasshoppers were represented by different species: *Acridella nasuta, Acrotylus insubricus, Chrotogonus homalodemus*, *Euprepocnemis plorans, Hetracris annlosa* and *Aiolopus strepens*, while the local locust was *Anacredium aegyptium*. Micron Ulva+ hand-held spinning disc sprayer ULV was used to apply this field trail after being calibrated.

All precautionary measures were taken during spraying. The wind speed was 5–6 m/s, the temperature was 31 C°, and RH was 63% at the time of application (16:40).

B- Second Trial:

It was conducted in Sharq El-Owainat region during the second half of November 2007 against immature and mature adult groups of desert locust, *S. gregaria* mixed with few of migratory locust, *Locusta migratoria migratorioides*. The coordinates of the field trial site was (22 19 N/28 45 E). Treated area was cultivated by wheat (*Triticum aestivum*) in a sandy soil. Population densities were 20 insect adults/ m^2, mixed with desert locust and migratory locust were placed in each cage (1 m^3). Knapsack mist blower was used to apply this field trail after being calibrated and all precautionary measures were also taken during application. The wind speed was 6–7 m/s, temperature was 24 °C, and RH was 67% at the time of application.

C- Third Trial:

It was carried out in Abu-Ramad region in December 2007, after a small-scale breeding occurred during the 1st half of November 2007 in Wadi Diib near the Red Sea coast and the Egyptian-Sudanese border against solitaries' hoppers, scattered immature and mature adults. The coordinates of the field trial site was (22 24 N/36 24 E). Treated area was cultivated by acacia trees, with light grasses, in a sandy soil. Population density was 20/m^2 of desert locust, *S. gregaria,* (10 consisted of 2nd to 5th instars of hoppers and 10 immature and mature adults/ m^2). The insects were collected, counted and placed in each cage (1 m^3). Micron Ulva + hand-held spinning disc sprayer ULV was used to apply this field trail and all precautionary measures were taken during the spray. The wind speed was 3–4 m/s, temperature was 26 °C, and RH was 72% at the time of application.

RESULTS AND DISCUSSION

Survey results indicated, low numbers of local locust scattered and mixed with high densities of grasshoppers at Baharia Oasis during June 2007. No locusts were found during the surveys carried out along both sides of Lake Nasser by mid- September 2007. Immature and mature adult groups of desert locust, mixed with few numbers of migratory locust at Sharq El-Owainat region in November 2007 and isolated hoppers of all instars, scattered immature and mature adults were found at Abu-Ramad region in December 2007.

Data collected were transformed using square root transformation and analyzed by SAS statistical program (SAS, 1990).

A. First Trial:

Fungal growth on cadavers of insects was evident in the treatments with (Green Muscle®) 50 g/ha (occurred amongst days 12-13 onwards), while with 25 g/ha, it occurred amongst days 15-16th onwards in all cages. Moreover, numbers of dead infected grasshoppers increased slowly and the majorities of the treated grasshoppers were much less active than the untreated ones (control treatment).

Table (1) shows the effect of Green Muscle® on mortality percentage of different grasshoppers. Data in this table indicate that the efficacy percentages of the fungus on different grasshoppers' population were 8.4, 41.2 and 60.8 % post treatment with 25 g/ha diluted in vegetable oil and were 14.7, 54.2 and 77.3 % after treatment with 25 g/ha diluted in diesel, in the 1st, 2nd and 3rd weeks post treatment, respectively.

Meanwhile, the efficacy percentages were 19.2, 58.6 and 86.5 % after treatment with 50 g/ha diluted with vegetable oil and were 24.7, 65.2 and 94.4 % after treatment with 50 g/ha diluted in diesel in the 1st, 2nd and 3rd weeks post treatment, respectively. Highest mortality was recorded for *A. insubricus*, *A. strepens* and *C. homalodemus*.

Table (2) shows the effect of the fungus on mortality percentage of local locust (trees locust), *A. aegyptium*. Data indicate that the efficacy

percentages on the local locust, *A. aegyptium* were 4.3, 21.6 and 37.1 % post treatment with 25 g/ha diluted in vegetable oil, compared to 9.6, 27.4 and 42.1% post treatment with 25 g/ha diluted in diesel in the 1st, 2nd and 3rd weeks post treatment, respectively.

Efficacy percentages were 14.2, 36.8 and 58.4 % post treatment with 50 g/ha diluted in vegetable oil, and 19.0, 47.2 and 71.0 % post treatment with 50 g/ha diluted in diesel in the 1st, 2nd and 3rd weeks post treatment, respectively. Highest mortality was recorded among nymphs of the local locust, *A. aegyptium*.

B. Second Trial:

Also, fungal growth was more evident on insect cadavers in treatments with 50 g/ha of Green Muscle®, occurred amongst days 13-14th, while in case of 25 g/ha, it occurred amongst days 16-17th onwards, in all treated cages. The activity of insects in treated plots was less than untreated ones (control treatment).

Table (3) shows the effect of the fungus on mortality of immature and adult stages of desert locust, *S. gregaria* and migratory locust, *L. m. migratorioides*. Efficacy percentages of *Metarhizium* against immature and adult stages of the two locusts were 6.5, 26.1 and 38.6 % post treatment with 25 g/ha diluted in vegetable oil and were 11.1, 32.7 and 46.2 % in case of 25 g/ha diluted in diesel in the 1st, 2nd and 3rd weeks, respectively. Efficacy percentages were 17.4, 43.9 and 62.4 % post treatment with 50 g/ha diluted in vegetable oil and they were 23.2, 56.3 and 77.5 % post treatment with 50 g/ha diluted in diesel in the 1st, 2nd and 3rd weeks after treatment, respectively.

C. Third Trial:

Activity of insects was less appearing in the treated plots inversion to those in the control and the fungus growth was as the same forerunner more evident among insect cadavers in treatments with 50 g/ha occurred amongst 13-14th days, while in case of 25 g/ha, it occurred amongst 17-18th days onwards, in most cages.

Data in table (4) indicate that the efficacy percentages of *Metarhizium* against immature and adult stages of desert locust, *S. gregaria* were 11.4, 37.2 and 64.6% post treatment with 25 g/ha diluted in vegetable oil, while they were 19.7, 46.5 and 75.2 % post treatment with 25 g/ha diluted in diesel in the 1st, 2nd and 3rd weeks post treatment, respectively. While the efficacy percentages were 28.6, 53.1 and 81.5 % post treatment with 50 g/ha diluted in vegetable oil and they were 33.4, 62.3 and 89.1 %

Table (1): Efficacy of *M. anisopliae* var. *acridum* (Green Muscle®) against different grasshoppers at Baharia Oasis, Egypt in June 2007.

Treatment	Mortality Percentage %		
	After 1st week	After 2nd week	After 3rd week
Control	0	0	0
25g V. Oil diluted	8.4	41.2	60.8
25g Diesel diluted	14.7	54.2	77.3
50g V. Oil diluted	19.2	58.6	86.5
50g Diesel diluted	24.7	65.2	94.4

Table (2): Efficacy of *M. anisopliae* var. *acridum* (Green Muscle®) against trees locust, *Anacredium aegyptium* at Baharia Oasis, Egypt in June 2007.

Treatment	Mortality Percentage %		
	After 1st week	After 2nd week	After 3rd week
Control	0	0	0
25g V. Oil diluted	4.3	21.6	37.1
25g Diesel diluted	9.6	27.4	42.1
50g V. Oil diluted	14.2	36.8	58.4
50g Diesel diluted	19.0	47.2	71.0

Table (3): Efficacy of *M. anisopliae* var. *acridum* (Green Muscle®) against desert locust, *Schistocerca gregaria* mixed with migratory locust, *Locusta migratoria migratorioides* in Sharq El-Owainat region, November 2007.

Treatment	Mortality Percentage %		
	After 1st week	After 2nd week	After 3rd week
Control	0	0	0
25g V. Oil diluted	6.5	26.1	38.6
25g Diesel diluted	11.1	32.7	46.2
50g V. Oil diluted	17.4	43.9	62.4
50g Diesel diluted	23.2	56.3	77.5

Table (4): Efficacy of *M. anisopliae* var. *acridum* (Green Muscle®) against desert locust, *Schistocerca gregaria* nymphs at Abu-Ramad region, December 2007.

Treatment	Mortality Percentage %		
	After 1st week	After 2nd week	After 3rd week
Control	0	0	0
25g V. Oil diluted	11.4	37.2	64.6
25g Diesel diluted	19.7	46.5	75.2
50g V. Oil diluted	28.6	53.1	81.5
50g Diesel diluted	33.4	62.3	89.1

when diluted in diesel in the 1st, 2nd and 3rd weeks post treatment, respectively.

Data in table (5) indicate that the efficacy percentages of *Metarhizium* against desert locust, *S. gregaria* nymphs (between 3rd - 5th nymphal instars) were 6.1, 24.7 and 41.2 % post treatment with 25 g/ha diluted in vegetable oil, compared to 10.3, 35.3 and 50.4 % when diluted in diesel in the 1st, 2nd and 3rd weeks post treatment, respectively. While efficacy percentages were 16.4, 42.2 and 69.3 % post treatment with 50 g/ha diluted in vegetable oil, and were 20.6, 54.5 and 74.6 % when diluted in diesel in the 1st, 2nd and 3rd weeks post treatment, respectively.

As most cadavers of treated insects, typical symptoms of fungal infection included dry cadaver, body coloring and the fungal growth which was more evident in treatments with (Green Muscle®) 50 g/ha. than 25g/ha. in all trails occurred (Fig. 1). Insects' mortality in the control was natural and as was as a result of natural ecological conditions.

Analysis of variances of the obtained data showed significant differences among the five treatments. Lowest mean of insects number affected was in all treatments with 25 g/ha diluted i vegetable oil. While, the highest one was in all treatments of 50 g/ha diluted in diesel. The data convergent approximately in most of the treatments with 25 g/ha diluted in diesel and the treatments of 50 g/ha diluted in vegetable oil.

Diesel treatment with 50 g/ha caused the highest mortality after 21 days under the Egyptian ecological conditions reaching 94.4, 89.1, 77.5, 74.6 and 71.0%, in case of grasshoppers, desert locust nymphs, desert locust mixed with migratory locust, desert locust adults and local locust, respectively. Pursue this, vegetable oil treatment with 50 g/ha caused the second higher mortality after 21 days reaching 86.5, 81.5, 69,3, 62.4 and 58.4%, in case of grasshoppers, desert locust nymphs, desert locust adults, desert locust mixed with migratory locust and local locust, respectively.

The results showed also that, 50 g/ha dose (diluted in diesel) of *M. anisopliae* var. *acridum* (Green Muscle®) resulted in an optimal mortality of locust and grasshoppers in the cages during 21 days, followed by 50 g/ha dose diluted in vegetable oil, respectively. From the economical point of view, use of diesel for dilatation may be more practical than vegetable oil

Efficient applications were in case of grasshoppers treatments and desert locust nymphs, followed by desert locust adults or migratory locust

Table (5): Efficacy of *M. anisopliae* var. *acridum* (Green Muscle®) against desert locust, *Schistocerca gregaria* adults at Abu-Ramad region, December 2007.

Treatment	Mortality Percentage %		
	After 1st week	After 2nd week	After 3rd week
Control	0	0	0
25g V. Oil diluted	6.1	24.7	41.2
25g Diesel diluted	10.3	35.3	50.4
50g V. Oil diluted	16.4	42.2	69.3
50g Diesel diluted	20.6	54.5	74.6

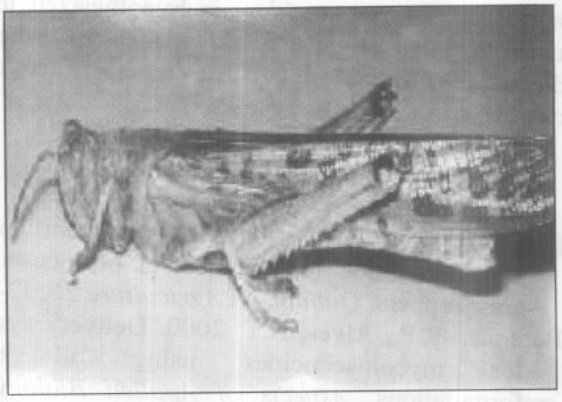

Fig. (1): Typical symptoms of fungal growth on cadaver of *S. gregaria*

adults and then local locust (trees locust), respectively. This could be due to the efficacy of *M. anisopliae* var. *acridum* which may be greater against nymphal instars (smaller size) than the adult insects (biger size) and also may be related to increased susceptibility to the pathogen. Likewise, the effect of the fungus was also greater against young nymphal instars than the older ones.

The efficacy of *M. anisopliae* var. *acridum*, in all treatments, indicated that it is a specific bio-pesticide for controlling Acrididae pests but it seems to be slow acting as a bio-agent unlike chemical pesticides.

Mortalities among the locusts with *M. anisopliae* var. *acridum* were more than the grasshoppers over the long term by three modes, whereas the pests might be infected by: 1) Direct impact, by droplets; 2) Secondary pickup, from vegetation and soil; 3) Horizontal transmission, by recycling the spores produced on infected cadavers.

At field application rates, it is considered safe to non–target Hymenoptera, Coleoptera and Homoptera species (Prior and Streett, 1997), and to mammals (El–Kadi *et al.*, 1993 and Zimmermann, 1993). The fungus can be mass produced relatively easily on artificial solid substrates and when

formulated in oil, can be applied under a wide range of environmental conditions using commonly available pesticide application equipment (Bateman, 1997; Bateman *et al.*, 1998 and Langewald *et al.*, 1997, 1999). There was no side effect of the diesel use as dilutes on viability of fungus spores, it is useful to increase the efficacy impact on the insect.

Accordingly, Green Muscle® can be useful for developing different elements of an IPM strategy, both optimizing time and place of treatment, and combining or replacing the use of chemical insecticides with the use of the fungal control agent *M. anisopliae* var. *acridum* is hypothesized to be most effective as a preventive measure whereas chemical insecticides will be most effective in curative treatments (Eva Nølke, 2006).

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