

CRC/EMPRES Final Report on Research Project: (PR27209)

Final Report

1. General information	
Final Report	
Reported period:	August 2003 to July 2005
Report prepared by:	Gamal M. Abdelatef (PI) Research assistant at Plant Protection Research Institute
Title of the research project	Effect of Green Muscle on Locust and Grasshoppers
Expected duration for the research project:(month/year to month/year)	August 2003 to July 2005
Supervised by	Prof. Dr. Gamal Zaki Taha Senior Researcher, Locust and Grasshopper Research Dep., Plant Protection Research Institute

2. Over view:

2.1 Purpose of the research project

The project focuses on the use of beneficial micro-organisms (pathogens) as biological agent for locust and grasshopper control specially Green Muscle, to achieve:

1. Reduction of insecticides used in locust control.
2. Demonstration of new environmentally safe biocontrol agent.
3. Reduction of control costs.

2.2 Narrative summery:

The preventative strategy of locust control defined as the elimination of hoppers and adult before encroachment into crop areas (**Grant 1989**). Although the arthropod predators and parasitoids of locust had a major role in hastening the end of plagues they were unsuitable for manipulation particularly in migratory pest such as the Desert Locust, since DL were poor

targets for classical biological control. Pathogens showed more promises, because some of these could be manipulated as biological pesticides. This allows the possibility of using conventional application technology, including aerial spraying, which is already highly developed for locust control (**Prior and Greathead 1989**). Interest in using pathogens as biological control agents has grown since the last major locust plague of the 1980s. Millions litres of chemical pesticides were used to combat this plague, which led to an outcry among environmental groups (**Greathead 1992 a**). Green Muscle is safe to use compared with many pesticides. Under field conditions, ecotoxicological studies demonstrated no negative effects, it appears also that the Green- Muscle isolate is free of destruxins (toxic metabolites). Green Muscle may be the best candidate to be used in Desert Locust control (**Langewald *Et. Al.*, 2003**).

In the present project the efficacy of Green-muscle was studied against Desert Locust and grasshoppers under the Egyptian field conditions.

2.3 Introduction

Locust and grasshoppers have long been characterized as omnivorous creatures which devoured all green vegetation. About 4.6 million ha. of land in 10 countries received aerial or ground insecticides treatments in 1986-1987 against Desert Locust, and 10 million ha. were sprayed in 1988, the large scale and repeated application of insecticides raised concern about the possible impact on the environment as well as human health (**Greathead 1992 a**). The scale of the problem is immense and the volume of chemical pesticides used in control has caused great concern to donors and the afflicted countries (**Anon., 1990**). The present control methods of Desert Locust and Grasshoppers in Egypt were aimed to reduce sufficient numbers of grasshoppers individuals by using chemical insecticides, so that the total

amount lost in crops is reduced to acceptable level. Microbial control agents can be effective in controlling grasshoppers for many reasons such as effects on the activity, reproduction, consumption of food, development, and behaviour (**Abdelatif 1998**). Locust and grasshoppers like most of insects are host to numerous micro organisms, the most extensively studied pathogens are the protozoa *Nosema locustae* and deuteromycete fungi such *Metarhizium anisopilae*, *M. anisopilae* var. *acridum* (= *Metarhizium flavoviridae*) and *Beauveria bassiana* (**Greathead 1992 b**). All fungi isolates highly virulent to *S. gregaria* which have been tested are in the genus *Metarhizium* and have originated from Orthoptera. The isolate that was adopted as a standard and used in all assays for comparison is *M. anisopilae* var. *acridum* IMI330189 which originated from the grasshoppers *Ornithacris cavroisi* collected in Niger. No highly virulent isolates to locust have yet been found originating in non Orthopteran hosts (**Prior, 1992**). There are four major constraints on candidate pathogens for locust control 1- It must have acceptable host specificity and be safe to non target organisms. 2- It must be suitable for production in large quantity. 3- It must have the ability to challenge the target organism effectively, under harsh environmental conditions. 4- It must cause acceptable rapid mortality. The use of pathogens may offer an environmentally sound method for the management of grasshoppers and locusts, especially hyphomycete fungi which are the most promising candidates (**Prior & Greathead, 1989**). Green muscle is regarded as being of low risk to humans and livestock while having few effects on other non-target organisms. Green muscle is now registered against locusts and grasshopper in south Africa (**FAO. 2002**).

3. Summary

Green Muscle was used against Desert Locust at Elba mountain area and

Grasshoppers at Sharq El-Owainat, Baharia Oasis and Mansura District, to evaluate its efficacy under the Egyptian environmental conditions.

1-Against Desert Locust nymphs:

At the first trial Green Muscle was used at 2 rates of application Zero, 25 and 50 g. active spores/ha diluted in vegetables oil. Each treatment was applied to 3 plots 2500 m² each, using Hand-held spinning disc sprayer (Micron Ulva +). After treatment, as soon as possible, 300 treated 5th nymphal instar were collected and kept in 3 field cages (2 X 1.4 X 1m). The efficacies of Green Muscle were 9.84, 52.69 and 75.82 % after treatment with 25 g spores/ha at 1st, 2nd and 3rd week after treatment, respectively. While in case of 50 g spores treatment it were 25.56, 68.64 and 89.64 %, at 1st, 2nd and 3rd week after treatment, respectively. There were significant differences ($\alpha = 5\%$) between application rates.

At the second trials 5 treatments were carried out in larger areas against 3rd, 4th and 5th nymphal instars, each treatment was applied into 4 ha using Knapsack mist blower sprayer equipped with micronair AU 8000. The treatments were **1-** control (sprayed with vegetable oil) **2-** diesel alone **3-** twenty five g active spores/ha (vegetable oil used as diluents) **4-** twenty five g active spores/ha (diesel used as diluents) **5-** fifty g active spores/ha (vegetable oil used as diluents). The efficacies of treatments 2, 3, 4 and 5 were 3.53, 4.71, 21.18 and 21.18, respectively, at 1st week. While in the 2nd week it were 1.39, 40.28, 52.78 and 66.67%, respectively. At the 3rd week it were 3.03, 71.21, 81.82 and 87.88 %, respectively.

2- Against Grasshoppers:

Preliminary trials were carried out against grasshoppers *Chrotogonus homalodemus*, *Acrotylus insubricus* and *Aiolopus strepens* at Sharq El-Owainat and Baharia Oasis, using 50 g active spores/ha. The efficacies were

29.41, 41.56 and 60.32 % 1, 2 and 3 weeks after treatment, respectively.

Other trials were done at Baharia Oasis, using 25 and 50 g active spores/ha, on a large scale area using Knapsack mist blower sprayer equipped with micronair AU 8000. Also two times of applications were done (09:00 and 18:00 hrs) to study the effect of application time of Green Muscle. The efficacies ranged between 71.11-82 and 79.55-84% after 21 day post treatment, respectively, also there were no significant differences ($\alpha = 5\%$) between morning and evening application time.

Five treatments were carried out against grasshopper *Euprepocnimes plorans*, each treatment was applied into about 0.25 ha using Knapsack mist blower sprayer equipped with micronair AU 8000. The treatments were **1-** control (sprayed with vegetable oil) **2-** diesel **3-** twenty five g active spores/ha (vegetable oil used as diluents) **4-** twenty five g active spores/ha (diesel used as diluents) **5-** fifty g active spores/ha (vegetable oil used as diluents). The efficacies of treatments 2, 3, 4 and 5 were 2.5, 7.69, 16.24 and 18.8, respectively, at 1st week. While in the 2nd week it was 1.90, 48.57, 60.95 and 64.76 %, respectively. At the 3rd week it was 4.95, 63.37, 82.18 and 90.10 %, respectively.

4. Materials and Methods

1- Monitoring Locust and grasshoppers

Locust and Grasshoppers were surveyed two times during the project duration, to explore the population situation also to choose suitable places for the field trials. The first survey was conducted during the period 10th of October to 17th of October 2003, at area from Baharia oasis north to Sharq El-Owainat south were surveyed for locust and grasshopper (see fig. 1). Second survey during 6th of December to 14th of December 2003, to evaluate the Desert locust situation at eastern and western shores of Lake Nasser as a

result of previous report that indicated heavy densities of immature and mature adults during the last week of October which may laid eggs by this time and hoppers expected to hatch during the first week of December. Garmin GPS 12 XL was used to determine the co-ordinates. Numbers of grasshoppers were determined by counting grasshoppers or locust in virtual 1 m.², these were repeated for 10- 25 times then the mean were calculated. Samples of grasshoppers were taken for classification.

2- Green Muscle samples and spores viability test

Samples of Green muscle were kindly provided by (Biological Control Products) South Africa and stored at 5 ° C. To study the viability of Green muscle spores, series dilutions were made by using shellsol oil under sterile air conditions, then 100 micro litter of each dilution were spread in petri dish containing selective media consist of: 0.36 g. KH₂PO₄, 1.42 g. Na₂HPO₄ 12H₂O, 0.62 g. MgSO₄ 7H₂O, 1 g. KCL, 0.70 g. NH₄NO₃, 10 g. Maltose, 5 g. Yeast extract, 18g. Agar-Agar and 0.5 g. Chloramphenicol per 1000 ml of distilled water, 3 petri dishes of each dilution were examined for spore germination after 24 hours at 27° C. The viability was calculated, and dose was modified to achieve 25 and 50 g. viable spores /ha.

To determine the effect of diesel on the viability of Green Muscle spores, series of dilutions of spores were made by using diesel under sterile conditions then 100 micro litter of each dilution were spread in Petri dish containing the previous selective media, 3 Petri dishes of each dilution were examined for spore germination after 24 hours at 27° C. The viability was calculated.

3- Effect of Green Muscle on Desert Locust and Grasshoppers in the field

Three preliminary field trials were conducted to study the effect of

Green muscle on Egyptian Grasshoppers. Also 2 trials were conducted against Desert Locust nymphs and several trials against Grasshoppers.



Figure (1) Map of Egypt indicating research sites

A- Preliminary field trials:

The first trial was conducted in area called Mandesha at Bahariyya oasis (N 28° 20.347” E 028° 57.038”): treated area was 1408 m², cultivated with *Alfa alfa*, sandy soil. The population density was determined by counting grasshoppers in virtual 1 m², this was repeated for 5 - 10 times then the mean was calculated. The collected samples of grasshoppers were

discriminated into species and samples of the species were sent to Taxonomy Department at Plant Protection Research Institute for classification. Diesel (as recommended in the Green Muscle user manual, also in personal communication with Mr. Kooyman, Christiaan (IITABE)) used as diluents, the final volume of the spray was 340 ml. C5 Berthoud sprayer (the only available sprayer by that time) with black nozzle was used to spray Green muscle, the flow rate was 150 ml./minute. Check plot was not treated by diesel (personal communication with Mr. Kooyman (IITABE)), however, in another experiment, plots treated with diesel only showed some effect on Desert Locust (nymphs) and Grasshoppers (nymphs and adults) refer to tables 17 and 31). Samples of insects were collected at the 2nd day of the treatment from both treated and untreated areas and kept in plastic cages. The insect populations were counted every week for 3 weeks. The caged insects were taken to Locust and Grasshopper Research Department's lab, kept under 31° C and observed for mortality twice daily for 21 day after treatment.

Second trial was conducted in area called Segam at Bahariyya oasis (N 28° 22.171" E 028° 59.306"): treated area was 4186 m², the final volume of the spray was 1015 ml., cultivated with *Alfa alfa*, sandy soil, the same procedures of the first trial were done.

The third trial was conducted in Sharq El-Owainat (N 22° 18.503" E 028° 45.518"): treated area was 3140 m², the final volume of the spray was 750 ml., cultivated with eggplants and green peppers, sandy soil, there wasn't any other area to be used as control due to the lake of grasshoppers infestation because it was at the end of the season and there were many successful control operations), so the untreated insects were collected before treatment then after the treatment with 24 hr. treated insects were collected.

Treated and untreated insects were also taken to the lap. and observed for mortality for 21 days.

B- Effect of Green Muscle on Desert Locust Nymphs

Two field trials were conducted to study the effect of Green Muscle against Desert Locust nymphs in Elba Mountains (N 22° 12'16.8" E 036° 25'22.4"). Two sprayers were used according to plots area, for the 1st trial a Hand-held spinning disc sprayer was used (small plots area) and for the 2nd trial a Knapsack mist blower sprayer was used (larger area).

The first trial was done at Todhi Valley (cultivated with drying grasses, sandy soil), Green Muscle was used at rates 0, 25, 50 gram of active spores/ha. Sunflower oil was used as diluents. Each rate of application was applied into 2 plots 50 X 50 m. At the day before treatment the plots were allocated, the sprayer was calibrated and the swath width was determined. Hand-held spinning disc sprayer Micron Ulva + was used for the application, swath width was 5 m. The Desert Locust populations at the trial area were consisted of 5th instar nymphs and adults (settled in that valley), after treatments, three field cages (fig. 2 and 3) 2 X 1.4 X 1 m, were placed in every plot over green vegetation, then as soon as possible 100 of 5th instar nymphs were collected from the inside of sprayed area and kept in every cage, the adult insects were excluded. Mortality was observed daily for 21 day after treatment.

The second trial was at Eldaeb Valley (cultivated with drying grasses, sandy soil), Five treatments of Green Muscle were applied, The treatments were: **1-** control (sprayed with vegetable oil) **2-** diesel alone **3-** 25 g active spores/ha (vegetable oil used as diluents) **4-** 25 g active spores/ha (diesel used as diluents) **5-** 50 g active spores/ha (vegetable oil used as diluents).



Figure (2) Field cages 2 X 1.4 X 1 m, during the preparation.



Figure (3) Field cages spread over the trial area at Todhi Valley.

Each treatment was applied into 4ha. At the day before treatment the plots were allocated, the sprayer was calibrated and the swath width was determined. Knapsack mist blower sprayer equipped with micronair AU 8000 was used for the application. The Desert Locust populations at the trials areas were consisted of 3rd, 4th and 5th instar nymphs and very few adults and ranged between 10-15 nymph/m² (were marching). After treatments, samples of treated nymphs were collected randomly from each treated area and kept in 10 plastic cages (fig. 4) 10 nymph per cage, treated nymphs were fed daily on grasses from same treated area which it were collected from. Mortality was observed daily for 21 day after treatment.

In both trial we couldn't conduct nymphs count in the treated areas, because it was impossible to keep treated insects inside there treated areas

C- Effect of Green Muscle on Grasshoppers

These field trials were conducted at Bahariyya Oasis (N 28° 20.347" E 028° 57.038") on different grasshoppers' species and at Mansura District (N 31° 02.055" E 031° 23.435") on *Euprepocnimes plorans*.

Bahariyya Oasis trials: Two sprayers were used in these trials: Hand-held spinning disc sprayer (Micron Ulva +) and knapsack mist blower sprayer equipped with micronair AU 8000. Also two times of application were done (09:00 and 18:00 hrs) to study the effect of application time of Green Muscle.

Micron Ulva trials: The first trial was conducted at 09:00. and the rates of applications were 0 and 50 gm active spores/ha, the treatments were applied into two separate areas each 50 X 50 m, cultivated with *Alfa alfa*, sandy soil, then samples of grasshoppers were collected from the center of the treated area and kept in field cages, three plastic cages were used to every treatment each contain 25 grasshoppers. The same were done fore the

second trial which conducted at 18:00 hrs (sunset about 19:00 hrs).



Figure (4) The plastic cage (bottomless plastic jar covered with white long tube of white textile)

Knapsack mist blower trials: Two trials were done at 09:00 and 18:00 hrs, at application rates 0, 25 and 50 g active spores/ha, into areas ranged from 4500 to 1000 m². Samples of insect were collected from the treated area after 48 hrs and kept in plastic cages (3 cages per treatment and each contain 25 grasshoppers) and observed daily.

The plastic cages were kept in the Locust Base at El-wahat Al-Bahria; each cage was fed daily on grasses and alfa alfa from relevant treated plots. Also the mortality was recorded, the cadavers were removed and discriminated into species and counted to determine the effect of Green Muscle on each species. The cadaver's surfaces were sterilized and kept in

petri dishes for 24 Hr. then sterilized moistened pieces of cotton were placed in every Petri dish.

Also numbers of grasshoppers were counted in 10-15 virtual square meters before and after treatment for 21 days. A technique was used to determine the number of grasshoppers in constant area and to collect samples of grasshoppers, by a net sack like made of textile and form box like, it's dimensions were 2 X 1 X 2 m, this sack was held by two men who run up winded -which it cause the sack to be open- for 20 m (fig.5) then the number of grasshoppers in the sack were counted. This procedure was repeated daily in 3 different parts of each treatment area.

Mansura district trials: Five treatments of Green Muscle were applied against grasshopper, *E. plorans*, the treatments were: 1- control (sprayed with vegetable oil) 2- diesel alone 3- 25 g active spores/ha (vegetable oil used as diluents) 4- 25 g active spores/ha (diesel used as diluents) 5- 50 g active spores/ha (vegetable oil used as diluents). Each treatment was applied into 0.25 ha cultivated with drying flowered clover, clay soil. The grasshoppers' population were 3rd nymphal instar and few 2nd and 4th nymphal instars and ranged between 15-27 nymph/m². At the day before treatment the plots were allocated, the sprayer was calibrated and the swath width was determined. Knapsack mist blower sprayer equipped with micronair AU 8000 was used for the application. Samples of treated nymphs were collected randomly after treatment from each treated area and kept in 12 plastic cages (10 nymph per cage), each plastic cage were feed daily on treated grasses with the same relevant treatment. Mortality was observed daily for 21 day after treatment. Counting nymphs couldn't be conducted in the treated areas, because, when clover dried the farmers take it off and prepare the land to cultivate the new crop, that cause migration of the

nymphs to another area.



Figure (5) Two men holding net sack like and running up wind.

4- Green Muscle efficacy calculations and statistical analysis

The daily numbers of living insects in cages were subjected to Analysis of variance using Procedure GLM. Duncan's Multiple Range Test was used to calculate the significant differences between means at Alpha = 0.05 (SAS 1999).

Data of caged insects were subjected to Schneider-Orelli formula and those data from field were subjected to Henderson and Tilton formula to calculate the efficacy of Green Muscle (Püntener 1981).

5. Results

1. Monitoring Locust and grasshoppers

1st Survey: Tables (1, 2, 3 and 4) show coordinates of the survey sites, vegetation, kind of soil, predominant species of grasshoppers and locust, % of every species population to the total number of all species.

2nd Survey: There was no locust nor grasshoppers founded during the

second survey. Analysis of reports and investigations were made during the survey suggested that. The huge number of locust desert locust reported during the last week of October may be part of swarm carried by wind to the southern part of Egypt, and it was controlled successfully before laying eggs.

2- Effect of diesel on the viability of Green Muscle spores

Table (5) shows number of germinated spores out of 100 spores counted. Analysis of variance of those data showed there was no significant difference on the germination of Green Muscle spores between diesel and shellsol oil after 24 hr. Thus diesel could be used as diluents. In case of Shellsol oil the average of number of germinated spores was 81.2 spores and in case of diesel it was 79.93 spores out of 100 spores.

3- Effect of Green Muscle on Desert Locust and Grasshoppers in the field

A- Preliminary field trials

First trial The most dominant species were *Chrotogonus homalodemus*, *Acrotylus insubricus* and *Aiolopus strepens*, the percentage of each species were 53.5, 30.6 and 13.4 % respectively. Table (6) and figure (6) show the mean number of grasshoppers per square meter in both treated and untreated areas at 0, 7, 14, 21 days after treatment. Efficacy percentages of Green Muscle on grasshoppers' population (30, 55.88, 64.71% after 0, 7, 14, 21 days after treatment respectively) can also be shown on Table 6. Table (7) shows % of mortality in treated and untreated caged insects and the corrected mortality at 7, 14, 21 days after treatment. The corrected mortality, as shown on table 7, was 29.41, 41.56 and 60.32 %, after 7, 14, 21 days after treatment respectively.

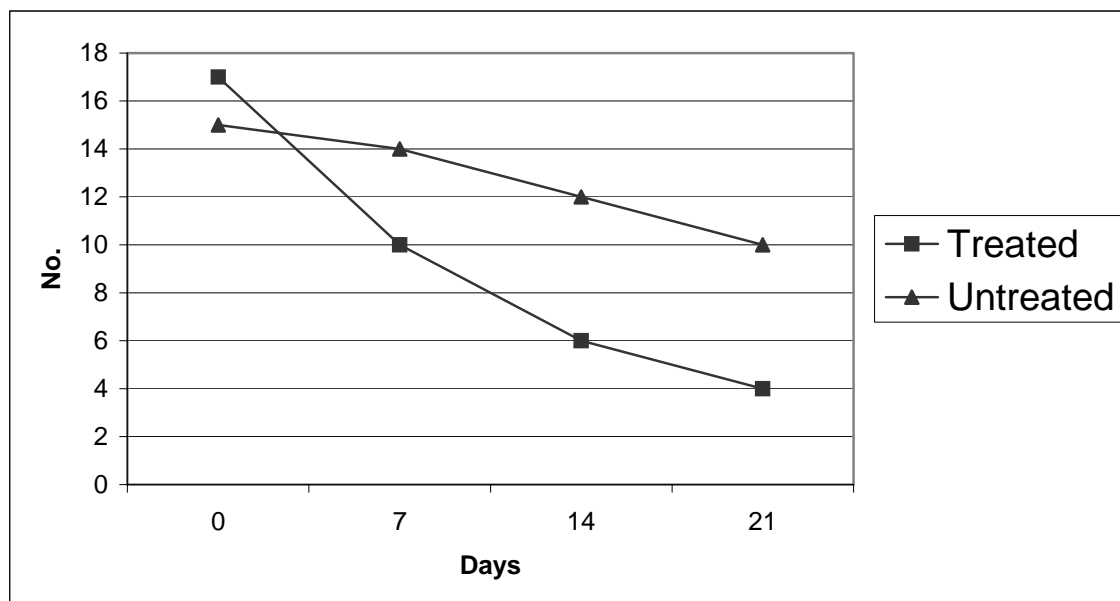


Figure (6) Number of grasshoppers per square meter in both treated and untreated area at 0, 7, 14, 21 days after treatment.

Second trial Same species were presented as the first trial. Table (8) and figure (7) show the mean number of grasshoppers per square meter in both treated and untreated area at 0, 7, 14, 21 days after treatment also efficiency of Green muscle on the grasshopper population. Efficacy of Green Muscle was 67.27 % after 21 days post treatment. Table (9) shows % of mortality in treated and untreated insects and corrected mortality at 7, 14, 21 days after treatment for the caged insects. Mortality in caged treated insects reached 72.5 % after 21 days post treatment, and in caged untreated insects mortality reached 15 %, the corrected mortality was 67.65 %.

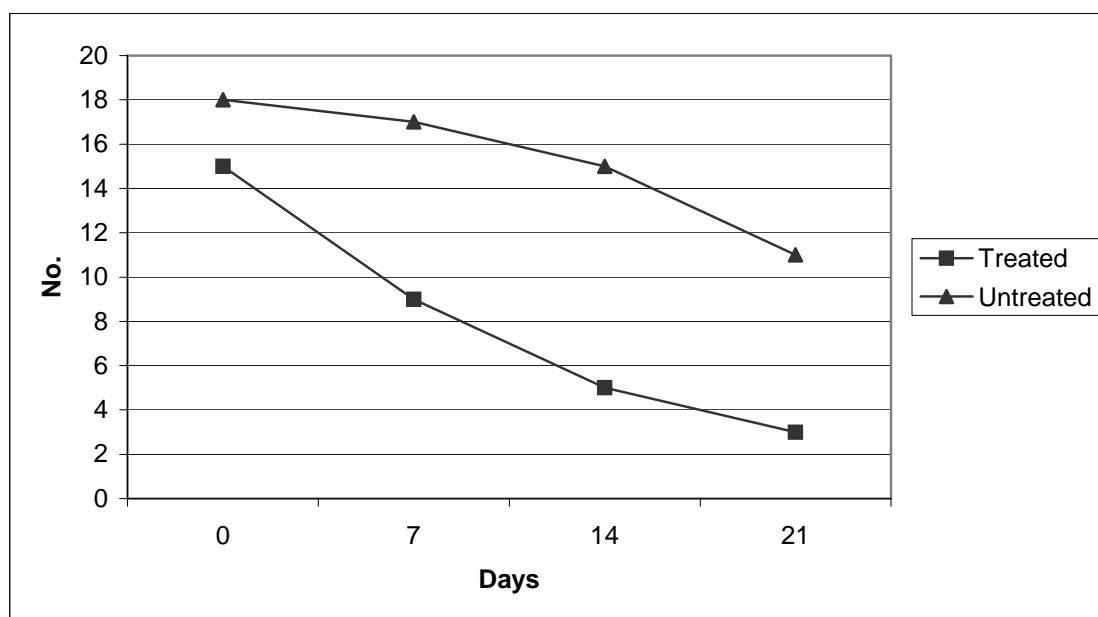


Figure (7) Number of grasshoppers per square meter in both treated and untreated area at 0, 7, 14, 21 days after treatment.

Third trial The most dominant species were *Aiolopus strepens* and *Acrotylus insubricus*, the percentage of each species were 50.8 and 35.9 % respectively. Table (10) and figure (8) show % of mortality in treated and untreated insects and corrected mortality at 7, 14, 21 days after treatment for the caged insects. Mortality in caged treated insects reached 92.22 % after 21 days post treatment while in caged untreated insects mortality reached 49.6 %, the corrected mortality was 84.56 %. The corrected mortalities were 25.92, 40.75 and 84.56 % after 7, 14 and 21 days respectively.

Since the area of the trial was isolated, the chance for migration into or out of the plot was very slim. All the cadavers from treatment turned to pink colour but not all of them showed fungal growth. 90 percent of *A. insubricus*, 85 % of *A. strepens* and only 10 % of *C. homalodems* cadavers

showed fungal growth. There were 2 treated *A. insubricus* cadavers showed growth of *Aspergillus flavus*. While there were no *Metarhizium* or any other fungal growth in untreated cadavers except in 3 *A. strepens* and 1 *A. insubricus* cadavers showed growth of *Aspergillus flavus*. The random samples which taken from treated cadavers showed growth of *Metarhizium* on the selective media.

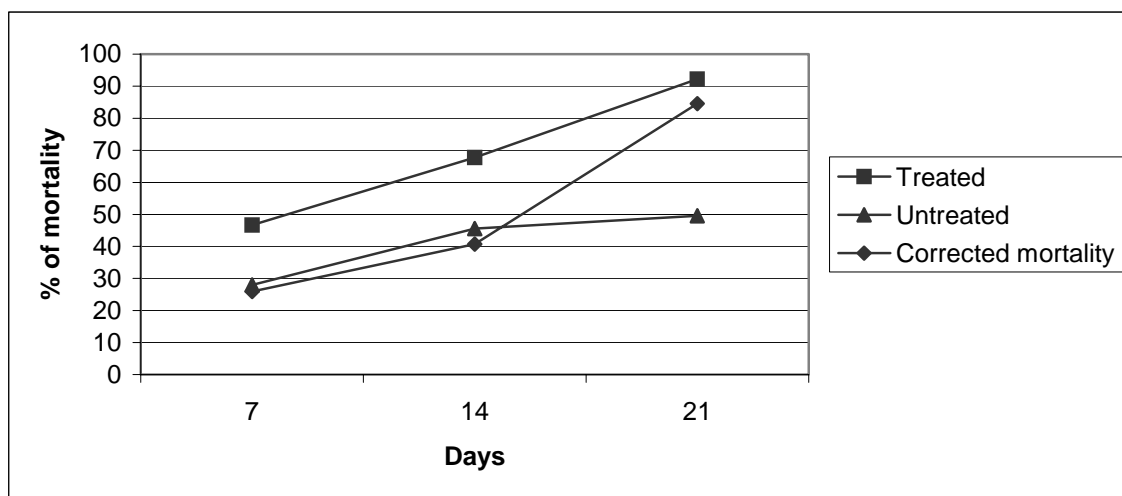


Figure (8) Percentage of mortality in treated and untreated insects and corrected mortality at 7, 14, 21 days after treatment for the caged insects

B- Effect of Green Muscle on Desert Locust Nymphs

First trial Data on table (11) shows the mean number of living nymphs, accumulative mortality, efficacy of Green Muscle on Desert Locust 5th nymphal instar in rates of 0, 25, 50 gram of active spores/ha diluted with vegetable oil. Also figure (9) shows number of living nymphs. It could be noticed that treatment with 50 g. active spores/ha., resulted in 68.64 % and 89.6 % reduction after 14 and 21 days after treatment respectively. While in case of 25 g active spores/ha., the reduction was 52.7 and 75.8 % 14 and 21

days after treatment respectively. Also, it's clear that the time requested to cause 50 % reduction is 11 and 14 day after treatment with 50 and 25 g active spores/ha, respectively.

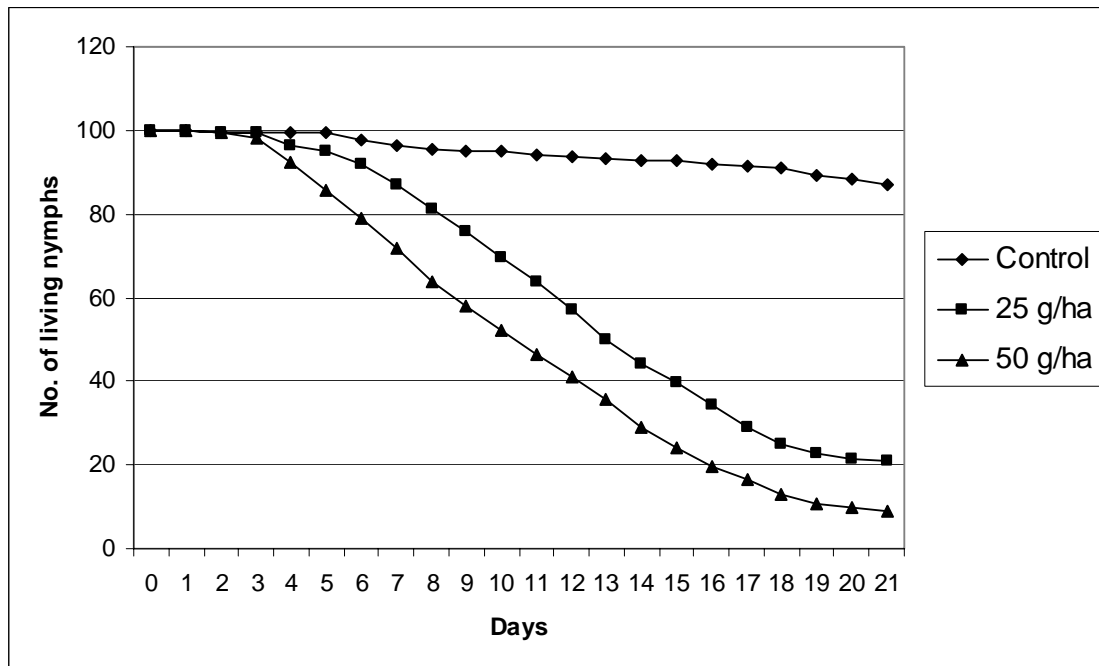


Figure (9) Number of living 5th nymphal instar of Desert Locust after treatment with Green muscle at rate 25 and 50 g spores/ha, also untreated nymphs.

About 38.7 and 45.6 % of treated cadavers with 25 g and 50 g active spore /ha., showed sporeulation respectively, figure (10) shows recently died nymph of Desert Locust 5th nymphal instar (show pink colour which mean that it was died as effect of infection (**Milner 2002**)) also died nymphs after the sporeulation. While there was no evidence of sporeulation from the untreated cadavers, there were about 1.7 % of the treated cadavers with 50 g spores/ha, showed contamination with *Penecillium* spp.

Table (12) shows the analysis of variance for number of living nymphs after treatment and table (13) shows significant differences between the 3

treatments according to Duncan test. Reasonable results could also be obtained from the treatment with 25 g/ha.



Figure (10) Dead nymphs of Desert Locust 5th nymphal instar, recently died nymphs show pink colour (left) and died nymphs after the sporeulation

Second trial Data on table (14) show effect of Green Muscle on 3rd, 4th and 5th nymphal instar of Desert Locust when applied at rates 50 and 25 g spores/hectare diluted with vegetable oil, rate 25 g spores/ha diluted with diesel and treatment of diesel alone. Diesel treatment caused mortality reached to 3.03 % by day 17 after treatment. The efficacies of fungi treatments (50 and 25 g spores/ha diluted with vegetable oil and 25 g spores/ha diluted with diesel) by day 7 after treatment were 21.18, 4.71 and 21.18 %, respectively. By day 14 efficacies were 66.67, 40.28 and 52.78, respectively and by day 17 efficacies were 87.88, 71.12 and 81.82 %, respectively. Table (15) and figure (11) show, the daily mean number of living nymphs after the previous treatments as well as control, while table (16) shows, the analysis of variance for row data of table (15), it's clear that there were high significant differences between the five treatments. Table (17) shows results of Duncan's multiple range test to

compare treatments means, it's clear that all treatments (control- vegetable oil, control-diesel, 25 and 50 g spores/ha diluted with vegetable oil and 25 g spores/ha diluted with diesel) were significantly differ from each other. The lowest mean of insect number was in the treatment of 50 g spores/ha diluted with vegetable oil followed by treatment of 25 g spores/ha diluted with diesel, then treatment of 25 g spores/ha diluted with vegetable oil.

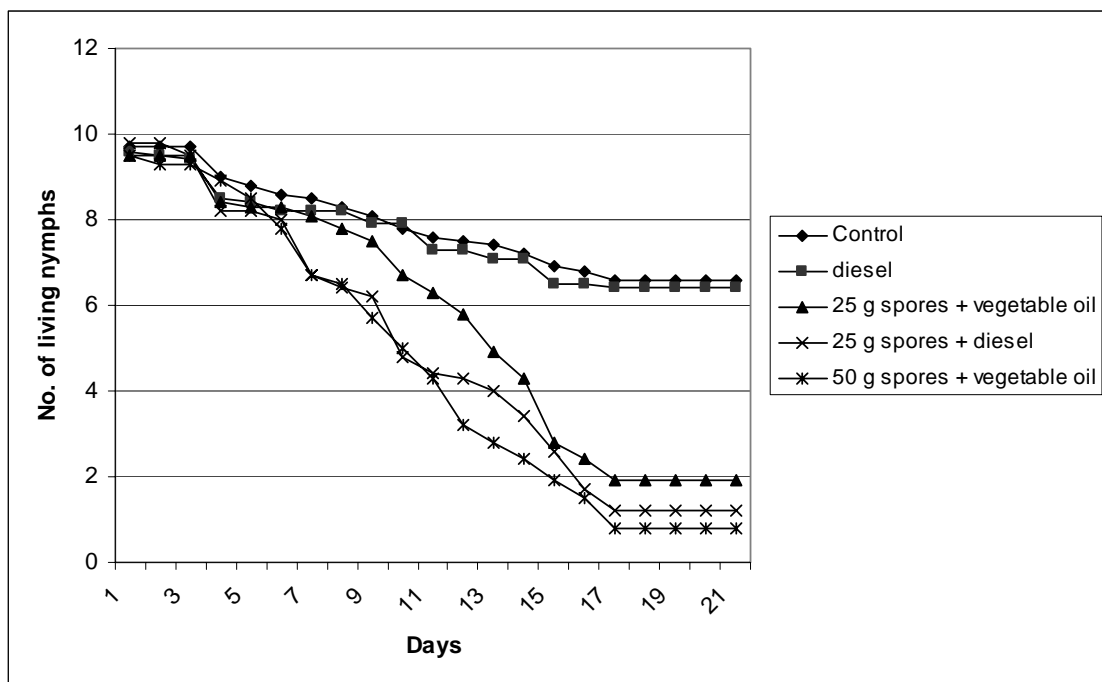


Figure (11) Mean number of living nymphs after treatment with Green Muscle on 4th and 5th nymphal instar of Desert Locust when applied at rate 0, 50 and 25 g spores/ha , rate 25 g spores/ha diluted with diesel and diesel alone.

C- Effect of Green Muscle on Grasshoppers

Bahariyya oasis trials Data in table (18) show the Effect of Green Muscle on different grasshoppers when applied with Hand-held spinning disc sprayer (Micron Ulva +) at 09:00 and 18:00 hrs at the rate 50 g active spores/ha. It's clear that Green Muscle caused reduction reached to 71 % after 12 days post

treatment in both morning and evening treatments. The reduction increased in case of evening treatment and reached to 81.11 % in day 17 while it was 71.11 % in the morning treatment.

Table (19) and figure (12) demonstrates the daily mean number of living grasshoppers during the experimental period after treatment by Micron Ulva + at the rate 50 g active spores/ha at 09:00 and 18:00 hrs. Table (20) shows the analysis of variance for the data shown on table 19, it's clear that there were high significant differences between treatments (control and treated insects), however there were no significant differences between times of application.

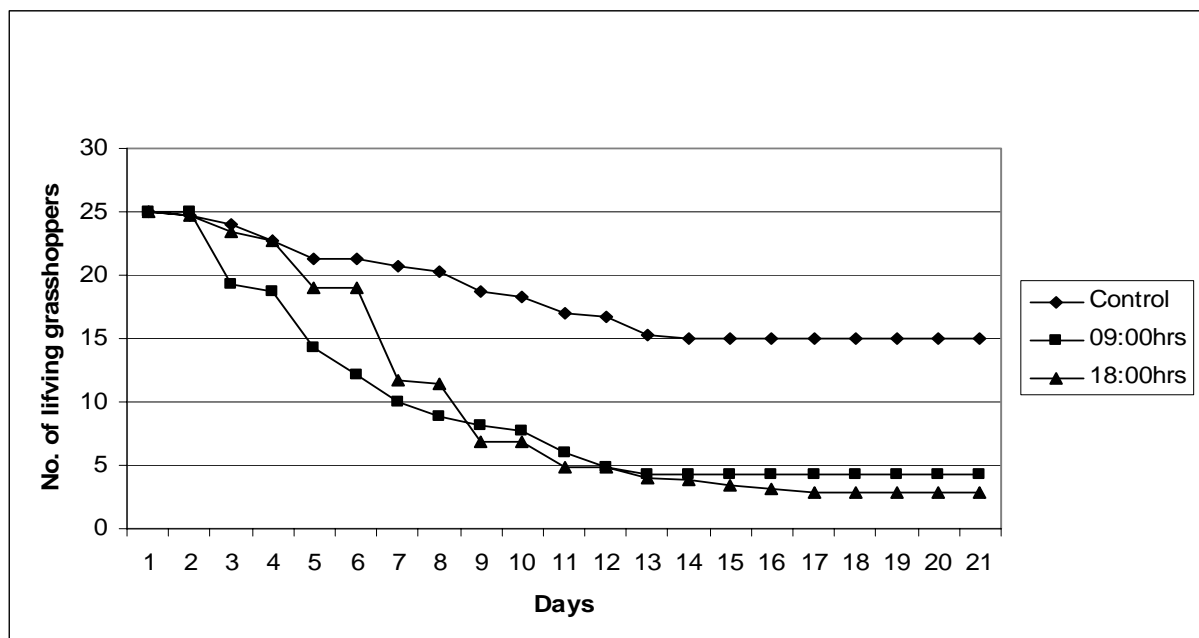


Figure (12) The daily mean number of living grasshoppers during the experimental period after treatment by Micron ULVA + at rate 50 g active spores/ha at 09:00 and 18:00 hrs.

Table (21) shows the effect of Green Muscle on different grasshoppers species when applied with knapsack mist blower at 09:00 and 18:00 hrs at rates 25 and 50 g active spores/ha in case of caged insects the reduction reached to 79.70, 82, 79.55 and 84 % in case of treatment rates 25 and 50 g active spores/ha at 09:00 and 18:00 hrs respectively after 21 day post treatment, while table (22) and figure (13) show the daily mean numbers of living grasshoppers in cages in case of knapsack mist blower sprayer in the same treatments as well as control, it could be noticed from table (23) that there were high significant differences between treatments (zero, 25 and 50 g spores/ha), also table (24) shows that there were high significant differences between each dose and others (zero, 25 and 50 g spores/ha).

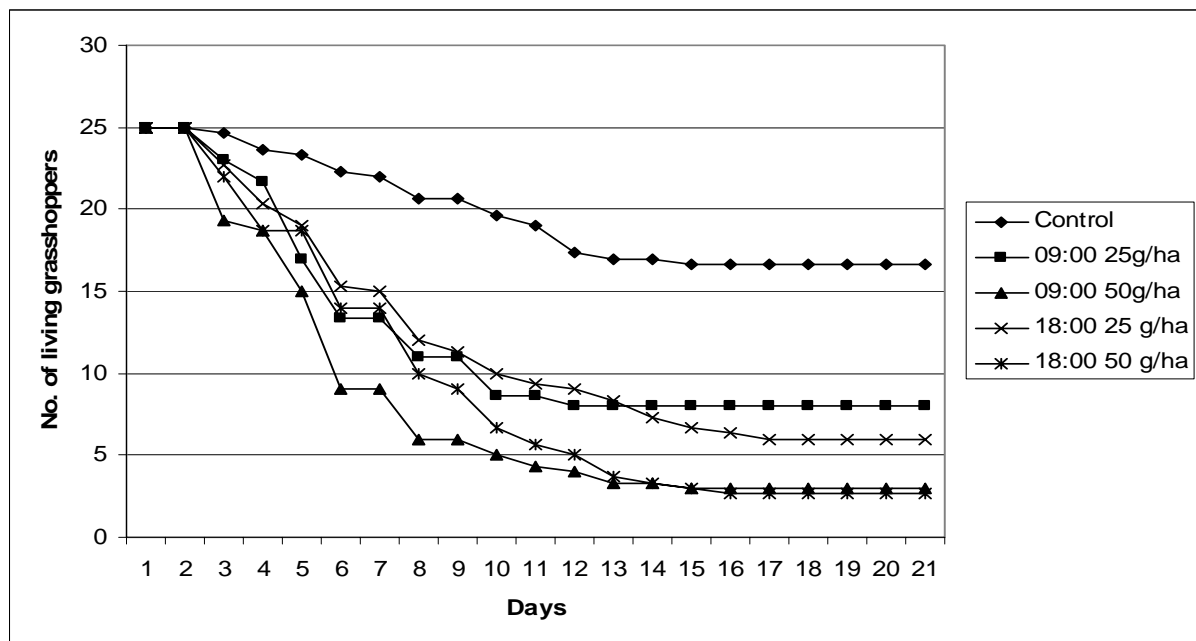


Figure (13) The daily mean numbers of living grasshoppers in cages in case of knapsack mist blower sprayer when Green Muscle applied at 09:00 and 18:00 hrs at rates 25 and 50 g active spores/ha as well as control.

It's clear that there were no significant differences between times of

application.

Table (25) shows the effect of Green Muscle on the field population when numbers of grasshoppers counted per virtual square meter which reached to 90.91, 92.16, 80.74 and 90.74 %, respectively after 21 day, table (26) shows the same effect when counted numbers of grasshopper were measured by the - sack like- net method, which reached to 66.00, 87.01, 67.63 and 76.53 % after 21 day post treatment. Table (27) shows the percentage of mortality of every species to total number of the same species.

Mansura District trials: Data in table (28) show the effect of Green Muscle on 3rd nymphal instar of *E. plorans* when applied at the rates 50 and 25 g spores/ha diluted with vegetable oil, the rate 25 g spores/ha diluted with diesel and treatment of diesel alone at Mansoura district. Diesel treatment caused mortality reached to 4.95 % by day 17 after treatment. The efficacy of fungi treatments (50 and 25 g spores/ha diluted with vegetable oil and 25 g spores/hectare diluted with diesel) by day 7 after treatment was 18.80, 7.69 and 16.24, respectively. By day 14 the mortality reached 64.76, 48.57 and 60.95%, respectively and by day 17 the efficacy reached 90.10, 63.37 and 82.18 % respectively. Table (29) and figure (14) show, the daily mean number of living grasshoppers after the same treatments as well as control, while table (30) shows, the analysis of variance table for row data of table (29), it's clear that there were high significant differences between the five treatments (control-vegetable oil, control-diesel, 50 and 25 g spores/ha diluted with vegetable oil, and 25 g spores/ha diluted with diesel). Table (31) shows results of Duncan's multiple range tests to compare treatments means, it's clear that all treatments were differ significantly from each other.

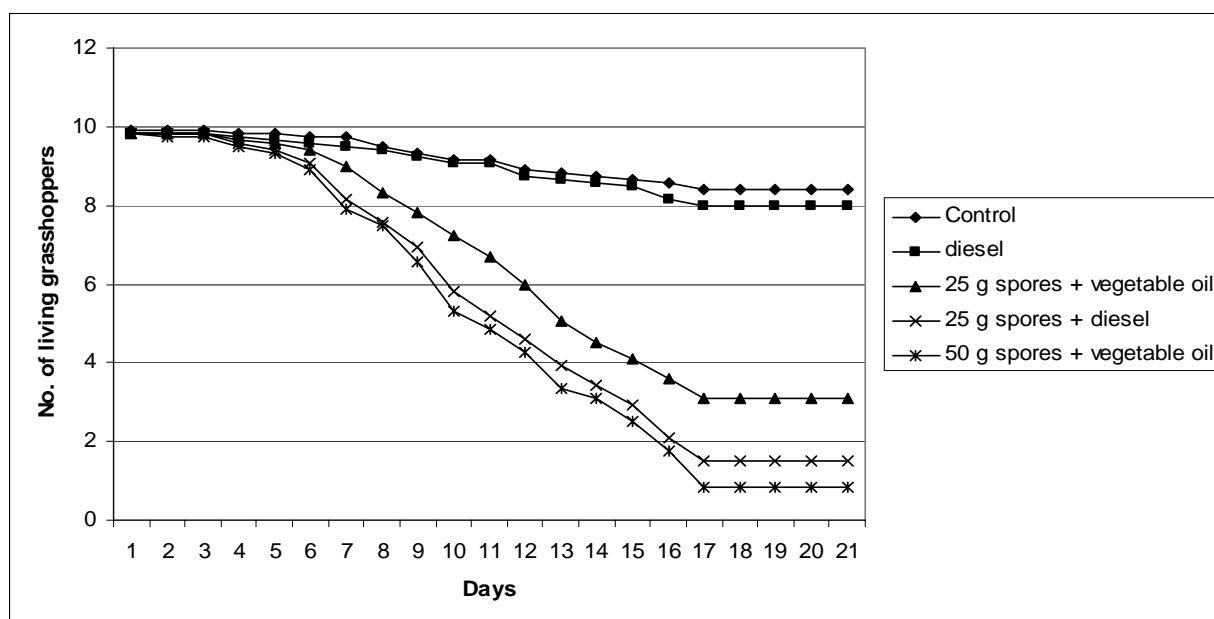


Figure (14) The daily mean number of living grasshoppers of *E. plorans* when Green Muscle applied at rate 50 and 25 g spores/ha, rate 25 g spores/ha diluted with diesel and diesel alone, as well as control.

6. Discussions

Green Muscle that used in this study is a mycon-insecticide product developed by LUBILOSA and based on isolate IMI330189 (Bateman et al., 1998).

In the present study, Green Muscle was tested against Desert Locust and Egyptian Grasshoppers in the field. The mortality was obtained from caged insects as well as, in some cases for grasshoppers, counting population in the field (refer to the results section on tables 6, 7, 8, 9, 21, 25 and 26). Data obtained from caged insects and field counts for grasshoppers were identical. In the preliminary field trials, the percentages of mortality after 21 days, in treated and untreated caged insects in the 3rd trial (at Sharq El-Owainat, see table 10 and Figure 8), reached 84.56 and 49.6 % respectively. The high mortality in the untreated cages may be attributed to insect stress during handling and transportation for a long distance from the field at Sharq El-Owainat to the laboratory of the Plant Protection Research Institute in Cairo. More

over the transported insects may be infected by local pathogens in the laboratory. All cadavers of untreated insects did not show any evidence of pathogenic fungal growth, while all cadavers of treated insects show pink colouration indicating a fungal infection.

It was evident from this study that there was no effect of the diesel as diluents on the viability of the Green Muscle spores. However, during the trial it was noticed that diesel causes some mortality to Desert Locust and Grasshoppers. In the economical point of view, the use of diesel as diluents may be more practical than vegetable oil.

Although the present study shows that Green Muscle is a successful candidate to control Desert Locust at the 3rd, 4th and 5th nymphal instars and grasshoppers at nymphal and adult stages, this effect could be greater in younger than the older nymphal instars, this is most probably due to the increased susceptibility of the younger instars to pathogens.

Previous virulence screening tests and trials using *M. anisopliae* var. *acridum* IMI 330189 revealed that 94 % of 17 different orthopteran species were susceptible to the spores, and 95 % - all within acrididae- highly susceptible to the spores (**Prior 1997**). Similar results were obtained from this study and goes in line with other several authors who reported that *M. anisopliae* var. *acridum* (Formerly *M. flavoviride*) was effective against locust and grasshoppers in the field, i.e. against Desert Locust in Mauritania (**Langewald et. al.,1997**), against migratory locusta migratoria 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 (**Lomer et. al., 1993 and Douro-Kpindou et. al., 1995**), against Wingless grasshopper *Phaulacridium vittatum* in Australia (**Baker et. al., 1994 and Milner et. al., 1994**), against grasshoppers

particularly Rice grasshopper *Hieroglyphus daganensis*, in northern Benin (Lomer *et al.*, 1997a) and against Sahelian grasshoppers (Kooyman *et al.*, 1997).

In conclusion, the researcher would like to state that the Data obtained from the caged insects in this research study are in confirmative with previous study (Kooyman and Godonou 1997 and Lomer *et al.*, 1997b) that indicated Green Muscle as a slow acting bio-agent unlike Chemical pesticides.

7. Recommendations

Referring to the results and the conclusion of this study, the following was recommended:

- 1. Green Muscle is recommended to be applied at 50 g spores/ha dose at the application rate of 2.5 L/ha in diesel oil, for the control of Desert Locust hoppers (3rd to 5th instars) and for the control of grasshoppers nymphs and adults.**
- 2. Green Muscle dose could be reduced to 25g spores/ha in moderate density of Desert Locust and grasshoppers in early stages i.e. 1-3 stage instars.**
- 3. The Green Muscle, as a bio-agent, is recommended to be registered in Egypt at the rate 2.5 L/ha of the doses 25g and 50g spores/ha in early infestation of grasshoppers and early outbreak Desert Locust particularly close to organic farms and sensitive areas.**

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Table (1) Coordinates of the survey sites in El-baharia oasis, vegetation, kind of soil, predominant species of grasshoppers and locust, % of every species population to the total number of all species and Mean number of grasshoppers/m².

Site	Coordinates	Vegetation	Kind of Soil	Species	%	Mean No. /M ²
Elkasr	N 28° 20.347" E 028° 57.038"	<i>Alfa alfa</i>	Sandy soil	<i>Chrotogonus homalodemus</i> , <i>Acrotylus insubricus</i>	65.7 30.4	9.2
Elkasr	N 28° 20.513" E 028° 47.466"	<i>Alfa alfa</i>	Sandy soil	<i>Chrotogonus homalodemus</i> <i>Acrotylus insubricus</i> <i>Calliptamuss italicus</i> <i>Heteracris annulosa</i>	50.2 21.3 18.6 8.6	8.3
Bawiti	N 28° 22.187" E 028° 52.528"	<i>Alfa alfa</i>	Sandy soil	<i>Chrotogonus homalodemus</i>	95.6	5.2
Bawiti	N 28° 22.103" E 028° 52.396"	Grass	Sandy soil	<i>Chrotogonus homalodemus</i> <i>Acrotylus insubricus</i> <i>Aiolopus strepens</i> <i>Calliptamuss italicus</i> <i>Heteracris annulosa</i>	24.7 23.2 18.4 17.1 11.2	29.14
Bawiti	N 28° 22.372" E 028° 52.516"	Grass, <i>Alfa alfa</i> and sunflower plants	Sandy soil	<i>Chrotogonus homalodemus</i> <i>Aiolopus strepens</i>	84.5 11.4	30.16
Haiz	N 28° 01.517" E 028° 38.228"	Sugar cane and maize	Clay	<i>Chrotogonus homalodemus</i>	100	1.5
Haiz	N 28° 02.238" E 028° 37.163"	Grass	Clay	<i>Truxalis nasuta</i>	100	0.57

Table (2) Coordinates of the survey sites in El-farafra oasis, vegetation, kind of soil, predominant species of grasshoppers and locust, % of every species population to the total number of all species and Mean number of grasshoppers/m².

Site	Coordinates	Vegetation	Kind of Soil	Species	%	Mean No. /M ²
1	N 27° 03.552" E 028° 54.068"	Maize	Clay	<i>Acrotylus insubricus</i>	100	0.2
2	N 27° 03.410" E 027° 51.589"	Maize	Sand	_____	___	___
3	N 26° 48.535" E 027° 54.105"	<i>Alfa alfa</i> , maize and paper	Clay	_____	___	___

Table (3) Coordinates of the survey sites in Gharb Elmohop, vegetation, kind of soil, predominant species of grasshoppers and locust, % of every species population to the total number of all species and Mean number of grasshoppers/m².

Site	Coordinates	Vegetation	Kind of Soil	Species	%	Mean No. /M ²
1	N 25° 53.465" E 028° 31.408"	<i>Alfa alfa</i>	Clay	_____	___	___
2	N 25° 54.014" E 028° 31.582"	<i>Alfa alfa</i>	Clay	_____	___	___
3	N 25° 54.024" E 028° 32.296"	Maize	Clay	_____	___	___
4	N 25° 54.014" E 028° 31.582"	Rice	Clay	<i>Aiolopus strepens</i>	100	0.3

Table (4) Coordinates of the survey sites in Sharq Eloinat, vegetation, kind of soil, predominant species of grasshoppers and locust, % of every species population to the total number of all species and Mean number of grasshoppers/m².

Site	Coordinates	Vegetation	Kind of Soil	Species	%	Mean No. /M ²
Cleopatra ranch	N 22° 28.259" E 028° 40.563"	Maize	Sand	_____	_____	_____
Elwtanya ranch	N 22° 31.525" E 028° 41.398"	Maize	Sand	_____	_____	_____

Table (5) Number of germinated spores out of 100 spores counted in petri dishes when using Shellsol oil or diesel as a diluents.

Replicat	shellsol oil	diesel
1	92	87
2	71	76
3	89	71
4	82	79
5	76	86
6	84	91
7	73	93
8	86	72
9	91	78
10	83	76
11	77	84
12	86	84
13	80	73
14	64	71
15	84	78
Average	81.2	79.93

Table (6) Number of insects in treatment and check plots/square meter and % of efficacy of Green Muscle in the first trial (data from field).

Days	No of insect in treatment/m ²	No of insect in check/m ²	% of efficacy *
0	17	15	
7	10	14	30.00
14	6	12	55.88
21	4	10	64.71

* Calculated according to Henderson and Tilton formula.

Table (7) Mortality % in treated and untreated caged insects from first trial and % corrected mortality.

Days	% mortality in treated insects	% mortality in untreated insects	% corrected mortality *
7	40	15	29.41
14	55	23	41.56
21	75	37	60.32

* Calculated according to Schneider-Orelli formula

Table (8) Number of insects in treatment and check plots/square meter and % of efficacy of Green Muscle in the second trial (data from field):

Days	No of insects in treatment/m ²	No of insects in check/m ²	% of efficacy *
0	15	18	
7	9	17	37.04
14	5	15	60.00
21	3	11	67.27

* Calculated according to Henderson and Tilton formula.

Table (9) Mortality% in treated and untreated caged insects from second trial and % corrected mortality.

Days	% mortality in treated insects	% mortality in untreated insects	% corrected mortality *
7	37.5	9	31.32
14	53.33	12	46.97
21	72.5	15	67.65

* Calculated according to Schneider-Orelli formula

Table (10) Mortality % in treated and untreated caged insects from third trial and % corrected mortality.

Days	% mortality in treated insects	% mortality in untreated insects	% corrected mortality *
7	46.66	28	25.92
14	67.77	45.6	40.75
21	92.22	49.6	84.56

* Calculated according to Schneider-Orelli formula

Table (11) Effect of Green Muscle on 5th nymphal instar of Desert Locust.

Days	Control		T25			T50		
	No. of living nymphs ^a	Accumulative mortality ^a	No. of living nymphs ^a	Accumulative mortality ^a	% of efficacy ^b	No. of living nymphs ^a	Accumulative mortality ^a	% of efficacy ^b
0	100.00	0	100.00	0	0	100.00	0	0
1	100.00	0	100.00	0	0	100.00	0	0
2	99.67	0.33	99.50	0.50	0.17	99.67	0.33	0
3	99.50	0.50	99.50	0.50	0	98.33	1.67	1.17
4	99.50	0.50	96.50	3.50	3.02	92.5	7.50	7.04
5	99.50	0.50	94.83	5.17	4.69	85.83	14.17	13.74
6	97.67	2.33	92.00	8.00	5.80	79.00	21.00	19.11
7	96.50	3.50	87.00	13.00	9.84	71.83	28.17	25.56
8	95.67	4.33	81.00	19.00	15.33	63.83	36.17	33.28
9	95.17	4.83	75.83	24.17	20.32	58.00	42.00	39.05
10	94.83	5.17	69.67	30.33	26.54	52.17	47.83	44.99
11	94.17	5.83	63.67	36.33	32.39	46.33	53.67	50.80
12	93.67	6.33	57.00	43.00	39.15	40.83	59.17	56.41
13	93.33	6.67	50.17	49.83	46.25	35.83	64.17	61.61
14	93.00	7.00	44.00	56.00	52.69	29.17	70.83	68.64
15	92.67	7.33	39.67	60.33	57.19	24.00	76.00	74.10
16	91.83	8.17	34.33	65.67	62.61	19.83	80.17	78.40
17	91.33	8.67	29.17	70.83	68.07	16.33	83.67	82.12
18	91.17	8.83	24.83	75.17	72.76	12.83	87.17	85.92
19	89.33	10.67	22.83	77.17	74.44	10.50	89.50	88.25
20	88.17	11.83	21.33	78.67	75.80	10.00	90.00	88.66
21	86.83	13.17	21.00	79.00	75.82	9.00	91.00	89.64

^a Mean values calculated from 6 replicates.

^b Calculated by using Schneider-Orelli formula

Table (12) Analysis of variance table for number of living nymphs after treatment with Green Muscle at rates 0, 25 and 50 g active spore/ha.

Source of variance	DF	SS	MS	F	Probability
Days	21	192530.48	9168.11	1558.11	**
Replicates	5	224.16	44.83	7.62	**
Treatment	2	125819.65	62909.82	10691.48	**
Days X treatment	42	68225.56	1624.41	276.07	**
Error	325	1912.33	5.88		
Total	395	388712.20			

Table (13) Duncan test for significance between treatments.

Number of Means 2 3
 Critical Range .5874 .6184

Duncan Grouping	Mean of living nymphs	Number of observations	Treatment
A	94.7045	132	Control oil
B	63.8106	132	25 g /ha
C	52.5379	132	50 g/ha

Means with same letter are not significantly different

Table (14) Effect of Green Muscle on 3rd, 4th and 5th nymphal instar of Desert Locust when applied at rate 50 and 25 g spores/hectare, rate 25 g spores/hectare diluted with diesel and diesel alone.

Days after treatment	diesel	25 g spores + vegetable oil	25 g spores + diesel	50 g spores + vegetable oil
	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a
1	1.03	2.06	-1.03	2.06
2	2.06	2.06	-1.03	4.12
3	3.09	2.06	2.06	4.12
4	5.56	6.67	8.89	1.11
5	4.55	5.68	6.82	3.41
6	4.65	3.49	6.98	9.30
7	3.53	4.71	21.18	21.18
8	1.20	6.02	22.89	21.69
9	2.47	7.41	23.46	29.63
10	-1.28	14.10	38.46	35.90
11	3.95	17.11	42.11	43.42
12	2.67	22.67	42.67	57.33
13	4.05	33.78	45.95	62.16
14	1.39	40.28	52.78	66.67
15	5.80	59.42	62.32	72.46
16	4.41	64.71	75.00	77.94
17	3.03	71.21	81.82	87.88
18	3.03	71.21	81.82	87.88
19	3.03	71.21	81.82	87.88
20	3.03	71.21	81.82	87.88
21	3.03	71.21	81.82	87.88

^a Calculated by using Schneider-Orelli formula.

Table (15) Mean number of living nymphs after treatment with Green Muscle on 3rd, 4th and 5th nymphal instar of Desert Locust when applied at rate 0, 50 and 25 g spores/hectare , rate 25 g spores/hectare diluted with diesel and diesel alone.

Days after treatment	Control -Oil	Control - Diesel	25 g spores + vegetable oil	25 g spores + diesel	50 g spores + vegetable oil
1	9.7	9.6	9.5	9.8	9.5
2	9.7	9.5	9.5	9.8	9.3
3	9.7	9.4	9.5	9.5	9.3
4	9.0	8.5	8.4	8.2	8.9
5	8.8	8.4	8.3	8.2	8.5
6	8.6	8.2	8.3	8.0	7.8
7	8.5	8.2	8.1	6.7	6.7
8	8.3	8.2	7.8	6.4	6.5
9	8.1	7.9	7.5	6.2	5.7
10	7.8	7.9	6.7	4.8	5.0
11	7.6	7.3	6.3	4.4	4.3
12	7.5	7.3	5.8	4.3	3.2
13	7.4	7.1	4.9	4.0	2.8
14	7.2	7.1	4.3	3.4	2.4
15	6.9	6.5	2.8	2.6	1.9
16	6.8	6.5	2.4	1.7	1.5
17	6.6	6.4	1.9	1.2	0.8
18	6.6	6.4	1.9	1.2	0.8
19	6.6	6.4	1.9	1.2	0.8
20	6.6	6.4	1.9	1.2	0.8
21	6.6	6.4	1.9	1.2	0.8

Table (16) Analysis of variance for number of living nymphs listed in table (15) after treatment with Green Muscle.

Source of variance	DF	SS	MS	F	Probability
Treatments	4	937.31764706	234.32941176	215.23	**
Days	16	3399.31058824	212.45691176	195.14	**
Treatments X Days	64	693.44235294	10.83503676	9.95	**
Error	765	832.900	1.088		
Total	849	5862.971			

Table (17) Duncan's Multiple Range Test for variable treatments

Number of Means 2 3 4 5
 Critical Range .2222 .2339 .2418 .2476

Duncan Grouping	Mean of living nymphs	Number of observations	Treatment
A	8.1294	170	Control -oil
B	7.8824	170	Control- diesel
C	6.5882	170	25 g spores
D	5.8353	170	25 g spores + diesel
E	5.5353	170	50 g spores

Means with the same letter are not significantly different.

Table (18) Effect of Green Muscle on different grasshoppers when applied with Hand-held spinning disc sprayer (Micron Ulva +) at 09:00 and 18:00 at rate 50 g active spores/ha (Caged insects)

Days after treatment	09:00	18:00
	% Of efficacy ^a	% Of efficacy ^a
1	0	0
2	-1.35	0
3	19.44	2.08
4	17.65	0.00
5	32.81	10.94
6	42.97	10.94
7	51.61	43.55
8	56.56	43.44
9	56.25	63.39
10	58.18	62.73
11	64.71	71.57
12	71.00	71.00
13	71.74	73.91
14	71.11	74.44
15	71.11	76.67
16	71.11	78.89
17	71.11	81.11
18	71.11	81.11
19	71.11	81.11
20	71.11	81.11
21	71.11	81.11

^a Calculated by using Schneider-Orelli formula.

Table (19) Mean number of living grasshoppers after treatment with (Micron Ulva +) at 09:00 and 18:00 at rate 50 g spores/ha (caged insects).

Days after treatment	Control	09:00	18:00
1	25.00	25.00	25.00
2	24.67	25.00	24.67
3	24.00	19.33	23.50
4	22.67	18.67	22.67
5	21.33	14.33	19.00
6	21.33	12.17	19.00
7	20.67	10.00	11.67
8	20.33	8.83	11.50
9	18.67	8.17	6.83
10	18.33	7.67	6.83
11	17.00	6.00	4.83
12	16.67	4.83	4.83
13	15.33	4.33	4.00
14	15.00	4.33	3.83
15	15.00	4.33	3.50
16	15.00	4.33	3.17
17	15.00	4.33	2.83
18	15.00	4.33	2.83
19	15.00	4.33	2.83
20	15.00	4.33	2.83
21	15.00	4.33	2.83

Table (20) Analysis of variance for number of living grasshoppers listed in table (19) after treatment with Green Muscle at 09:00 and 18:00 at rate 50 g active spore/ha with (Micron Ulva +)

Source of variance	DF	SS	MS	F	Probability
Treatments	1	9464.00000000	9464.000000	2793.31	**
Time	1	7.14285714	7.14285714	2.11	NS
Days	20	15170.0238095	758.5011904	223.87	**
Treatments X Time	1	7.14285714	7.14285714	2.11	NS
Treatments X Days	20	2129.83333333	106.4916666	31.43	**
Time X Days	20	186.85714286	9.34285714	2.76	**
Treatments X Time X Days	20	186.85714286	9.34285714	2.76	**
Error	420	1423.00000000	3.38809524		
Total	503	28574.8571428			

Table (21) Effect of Green Muscle on different grasshoppers when applied with knapsack mist blower at 09:00 and 18:00 at rates 25 and 50 g active spores/ha (The caged insects).

Days after treatment	09:00		18:00	
	25 g /ha	50 g/ha	25 g /ha	50 g/ha
	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a
1	0	0	0	0
2	0	0	0	0
3	8.96	21.62	9.40	10.81
4	12.28	21.13	16.77	21.13
5	36.46	35.71	22.08	20.00
6	54.49	59.70	37.25	37.31
7	53.80	59.09	38.02	36.36
8	65.17	70.97	50.51	51.61
9	65.17	70.97	54.18	56.45
10	78.49	74.58	59.55	66.10
11	77.74	77.19	62.12	70.18
12	80.49	76.92	60.66	71.15
13	80.10	80.39	64.35	78.43
14	80.10	80.39	71.03	80.39
15	79.70	82.00	75.00	82.00
16	79.70	82.00	77.27	84.00
17	79.70	82.00	79.55	84.00
18	79.70	82.00	79.55	84.00
19	79.70	82.00	79.55	84.00
20	79.70	82.00	79.55	84.00
21	79.70	82.00	79.55	84.00

^a Calculated by using Schneider-Orelli formula.

Table (22) Mean number of living grasshoppers after treatment with knapsack mist blower at 09:00 and 18:00 at rate 50 and 25 g spores/ha (caged insects).

Days after treatment	Control	09:00		18:00	
		25 g /ha	50 g/ha	25 g /ha	50 g/ha
1	25.00	25.00	25.00	25.00	25.00
2	25.00	25.00	25.00	25.00	25.00
3	24.67	23.00	19.33	22.67	22.00
4	23.67	21.67	18.67	20.33	18.67
5	23.33	17.00	15.00	19.00	18.67
6	22.33	13.33	9.00	15.33	14.00
7	22.00	13.33	9.00	15.00	14.00
8	20.67	11.00	6.00	12.00	10.00
9	20.67	11.00	6.00	11.33	9.00
10	19.67	8.67	5.00	10.00	6.67
11	19.00	8.67	4.33	9.33	5.67
12	17.33	8.00	4.00	9.00	5.00
13	17.00	8.00	3.33	8.33	3.67
14	17.00	8.00	3.33	7.33	3.33
15	16.67	8.00	3.00	6.67	3.00
16	16.67	8.00	3.00	6.33	2.67
17	16.67	8.00	3.00	6.00	2.67
18	16.67	8.00	3.00	6.00	2.67
19	16.67	8.00	3.00	6.00	2.67
20	16.67	8.00	3.00	6.00	2.67
21	16.67	8.00	3.00	6.00	2.67

Table (23) Analysis of variance for number of living grasshoppers listed in table (22) after treatment with Green Muscle at 09:00 and 18:00 at rate 50 g active spore/ha with (knapsack mist blower)

Source of variance	DF	SS	MS	F	Probability
Treatments	2	7762.1111	3881.05555556	499.33	**
Time	1	10.16931217	10.16931217	1.31	NS
Days	20	11862.3968	593.11984127	76.31	**
Treatments X Time	2	38.67195767	19.33597884	2.49	NS
Treatments X Days	40	1428.44444	35.71111111	4.59	**
Time X Days	20	99.719577	4.98597884	0.64	NS
Treatments X Time X Days	40	67.43915	1.68597884	0.22	NS
Error	252	1958.6667	7.77248677		
Total	377	23227.6190			

Table (24) Duncan's Multiple Range Test for variable treatments

Alpha= 0.05 df= 252 MSE= 7.772487

Critical Range .6918 .7282

Duncan Grouping	Mean of living grasshoppers	Number of observations	Treatment
A	19.7143	126	Control -oil
B	12.1508	126	25g/ha
C	8.8968	126	50g/ha

Means with the same letter are not significantly different.

Table (25) Effect of Green Muscle on different grasshoppers when applied with knapsack mist blower at 09:00 and 18:00 at rates 25 and 50 g active spores/ha (field counting per virtual meter).

Days after treatment	09:00		18:00	
	25 g /ha	50 g/ha	25 g /ha	50 g/ha
	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a
1	2.94	3.41	-3.35	0.86
2	23.05	25.03	6.49	0.86
3	19.12	15.31	4.07	-16.03
4	33.42	21.45	1.39	-34.76
5	46.94	40.48	40.14	46.53
6	54.47	56.70	42.40	37.04
7	49.02	57.49	53.97	52.47
8	44.71	66.38	59.54	57.96
9	77.27	81.04	64.32	62.68
10	79.17	85.59	71.01	68.79
11	81.13	90.26	79.07	76.17
12	82.48	90.26	78.07	76.26
13	84.53	89.84	72.36	81.56
14	88.58	89.68	86.19	83.29
15	88.05	89.21	61.86	80.37
16	89.99	91.65	80.67	90.65
17	90.59	91.88	80.73	90.73
18	91.08	92.30	80.74	90.74
19	90.59	91.88	80.74	90.74
20	90.76	92.03	80.74	90.74
21	90.92	92.17	80.74	90.74

^a Calculated by using Henderson and Tilton formula.

Table (26) Effect of Green Muscle on different grasshoppers when applied with knapsack mist blower at 09:00 and 18:00 at rates 25 and 50 g active spores/ha (field counting by using sack like net).

Days after treatment	09:00		18:00	
	25 g /ha	50 g/ha	25 g /ha	50 g/ha
	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a
1	12.71	48.07	0.76	3.94
2	19.41	48.67	-1.08	6.98
3	27.57	48.35	-0.01	3.85
4	37.51	80.47	13.57	19.89
5	29.61	68.86	25.98	34.56
6	14.99	47.39	20.16	43.71
7	57.05	73.15	39.21	65.96
8	48.21	67.63	14.98	19.51
9	30.45	71.01	34.32	49.52
10	42.71	72.92	41.48	60.65
11	52.82	74.49	36.16	50.86
12	48.04	73.07	28.48	36.74
13	43.33	71.66	62.98	69.32
14	55.37	75.69	36.17	46.00
15	46.84	83.83	32.10	54.04
16	45.59	86.82	57.09	57.34
17	63.46	85.25	63.41	65.52
18	64.64	87.25	63.37	66.79
19	61.75	88.04	64.84	69.49
20	61.75	85.77	67.06	70.54
21	66.00	87.01	67.63	76.53

^a Calculated by using Henderson and Tilton formula.

Table (27) Effect of Green Muscle on the different species of Grasshoppers.

Species	Micron Ulva ^a		Knapsack mist blower ^a			
	09:00	18:00	09:00		18:00	
			25	50	25	50
<i>Chrotogonus homalodemus</i>	20.00	82.35	72.73	70.59	20.00	54.55
<i>Tropidopola cylindrica</i>	80.00	100.00	84.21	72.22	62.50	85.71
<i>Acrida bicolor</i>	-----	-----	62.50	66.67	100.00	100.00
<i>Oxycoryphus compressicornis</i>	100.00	80.00	100.00	100.00	71.43	82.61
<i>Acrotylus insubricus</i>	91.30	91.67	71.43	85.00	84.62	100.00
<i>Heteracris annulosa</i>	----	----	100.00	100.00	----	----
<i>Calaptimus barbarus</i>	----	----	87.50	70.59	100.00	100.00

^a percentage of mortality to the total number of the same species

Table (28) Effect of Green Muscle on 3rd nymphal instar of *E. plorans* when applied at rate 50 and 25 g spores/ hectare , rate 25 g spores/hectare diluted with diesel and diesel alone.

Days after treatment	diesel	25 g spores + vegetable oil	25 g spores + diesel	50 g spores + vegetable oil
	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a	% Of efficacy ^a
1	0.84	0.84	0.84	0.84
2	0.84	0.84	0.84	1.68
3	0.84	0.84	0.84	1.68
4	0.85	1.69	2.54	3.39
5	1.69	2.54	4.24	5.08
6	1.71	3.42	6.84	8.55
7	2.56	7.69	16.24	18.80
8	0.88	12.28	20.18	21.05
9	0.89	16.07	25.89	29.46
10	0.91	20.91	36.36	41.82
11	0.91	27.27	43.64	47.27
12	1.87	32.71	48.60	52.34
13	1.89	42.45	55.66	62.26
14	1.90	48.57	60.95	64.76
15	1.92	52.88	66.35	71.15
16	4.85	58.25	75.73	79.61
17	4.95	63.37	82.18	90.10
18	4.95	63.37	82.18	90.10
19	4.95	63.37	82.18	90.10
20	4.95	63.37	82.18	90.10
21	4.95	63.37	82.18	90.10

^a Calculated by using Schneider-Orelli formula.

Table (29) Mean number of living grasshoppers after treatment with Green Muscle on 3rd nymphal instar of *E. plorans* when applied at rate 50 and 25 g spores/hectare, rate 25 g spores/ hectare diluted with diesel and diesel alone.

Days after treatment	Control	diesel	25 g spores + vegetable oil	25 g spores + diesel	50 g spores + vegetable oil
1	9.92	9.83	9.83	9.83	9.83
2	9.92	9.83	9.83	9.83	9.75
3	9.92	9.83	9.83	9.83	9.75
4	9.83	9.75	9.67	9.58	9.50
5	9.83	9.67	9.58	9.42	9.33
6	9.75	9.58	9.42	9.08	8.92
7	9.75	9.50	9.00	8.17	7.92
8	9.50	9.42	8.33	7.58	7.50
9	9.33	9.25	7.83	6.92	6.58
10	9.17	9.08	7.25	5.83	5.33
11	9.17	9.08	6.67	5.17	4.83
12	8.92	8.75	6.00	4.58	4.25
13	8.83	8.67	5.08	3.92	3.33
14	8.75	8.58	4.50	3.42	3.08
15	8.67	8.50	4.08	2.92	2.50
16	8.58	8.17	3.58	2.08	1.75
17	8.42	8.00	3.08	1.50	0.83
18	8.42	8.00	3.08	1.50	0.83
19	8.42	8.00	3.08	1.50	0.83
20	8.42	8.00	3.08	1.50	0.83
21	8.42	8.00	3.08	1.50	0.83

Table (30) analysis of variance for number of living grasshoppers listed in table (29) after treatment with Green Muscle.

Source of variance	DF	SS	MS	F	Probability
Treatments	4	1783.857	445.9642	1035.97	**
Days	16	3598.251	224.8907	522.42	**
Treatments X Days	64	1266.71	19.79234	45.98	**
Error	935	402.5	0.430481		
Total	1019	7051.318			

Table (31) Duncan's Multiple Range Test for variable treatments

Number of Means 2 3 4 5
 Critical Range .1275 .1342 .1387 .1421

Means with the same letter are not significantly different.

Duncan Grouping	Mean of living grasshoppers	Number of observations	Treatment
A	9.30882	204	Control -oil
B	9.14706	204	Control- diesel
C	7.26961	204	25 g spores
D	6.45098	204	25 g spores + diesel
E	6.17647	204	50 g spores