International Journal of Agriculture Innovations and Research
Volume 2, Issue 6, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 01/05/2014 | Accepted on : 16/05/2014 | Published : 21/05/2014

The Effect of *Metarhizium flavoviride* and Phenylacetonitrile on Food Consumption and the Daily Behavior of the Gregarious Nymphs of *Schistocerca gregaria*

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***Abstract* – *Metarhizium flavoviride*, strain Mfl5 spores were formulated in cotton seed oil at a concentration of 0.9 X 107 spores/ml and applied topically under the pronotal shield of the fourth nymphal instars of the desert locust *Schistocerca gregaria*. The amount of food consumed was calculated daily for six days where all treated nymphs were died. The reduction in the amount of food consumed was more than 50% compared to the amount consumed by non-treated nymphs, and that treated with oil only. Also the effect of integration of the adult aggregation pheromone major component, phenylacetonitrile, with the entomopathogenic fungus M. flavoviride on the daily behaviour of the desert locust nymphs was assessed. It was found that phenylacetonitrile alone increased the activity of the stimulated nymphs. While the fungus treated ones were more static, those under PAN stimulation spend most of the time moving and consuming more food. Nymphs treated with M. flavoviride in the presence of PAN stimuli are the most static ones and the least in moving and food consumption.**

***Keywords* – Desert Locust Nymphs, Food Consumption, Metarhizium Flavoviride, Phenylacetonitrile.**

I. INTRODUCTION

One of the most important characteristics contributing to the status of the desert locust *S. gregaria* as a major pest is the amount of food consumed daily. Desert locusts eat about their own weight of green vegetation daily, this amount vary from 20 mg at the beginning of the first nymphal instar to about 1.5 g in the middle of the fifth instar [15]. Actively migrating immature adult needs to eat at least 2-3 g of fresh vegetation each day and possibly three times as much [13]. In some countries they are the determining factor between sufficient food and starvation in plague years. The desert locust is a polyphagous pest consuming cultivated as well as wild plants, among which are the main staple foods for people in the affected areas. A major invasion of swarm may weigh 100.000 tons and eat this much green vegetation each day [13]. Gregarization of the desert locust is brought about by mean of pheromones. Pheromones are such chemicals which are produced by an organism influencing the behaviour of other members of the same species [6]. The use of pheromone type compounds in insect control was examined in U.S.A. [4]. Recently some investigators [11], showed that the production of the adult aggregation pheromone is confined to the older males. Torto *et al*. [11] named the composition of the aggregation pheromone as; phenylacetonitrile which is the dominant component, present in much as 75-85% in the volatile emissions of older males, along with benzaldehide, guaiacol and phenol.

The behaviour of individual hopper in bands takes several kinds of general activities and it has a more or less regular daily pattern. Roosting (i. e., they are off the ground, resting on plants, bushes or stones) occurs at night and in the early morning. Roosting also occurs during the middle part of the day when the temperature exceeds about 36ºC. Ground grouping, in which hoppers are concentrated in dense groups on the ground and are mainly stationary, is seen in the morning when the hoppers come down from bushes, and again in the evening before they roost at the night, it may occasionally occurs at other times of the day. Hoppers usually spend the great part of the day marching [16].

The main feeding period occurs when the hoppers go up into the bushes for their evening roost. They also feed when marching by stopping briefly to eat low vegetation in their line of marching. Sometimes feeding is seen on a considerable scale in the middle part of the morning. Very little occurs during the hotter, middle part of the day [16]. This paper investigates the effect of *M. flavoviride* infection and the stimulation of the gregarious nymphs with the major aggregation pheromone on the daily behaviour and the amount of food consumed.

II. MATERIALS & METHODS

A. Rearing of experimental insects: Colonies of the gregarious locust *S. gregaria* (forskål), were established at ICIPE- Portsudan station (Sudan). They were daily fed fresh *Pennisetum typhoides* (dukhun), *Medicago sativa* (barseem) seedlings, *Heliotropium sp* (gerara) and dry wheat bran. Third and fourth nymphal instars were used in the experiments.

B. The cages: Cages with wooden frames were used for rearing insects and also for conducting the experiments. They measured 35x35x37.5 Cm, fitted with a wire mesh from all sides except the anterior one which is covered with a sliding door having a circular opening fitted with a cloth sleeve. The cages were cleaned daily.

C. The fungus: The fungus used in this study is *M. flavoviride* (Mfl5), which was originally isolated from the African migratory locust *Locusta migratoria migratoriodes* collected from south-west of Madagascar. Cultures to be used in these experiments were grown in petri-dishes containing Sabouraud'a dextrose agar (SDA) medium at 28º C., for 15 days before preparation of the initial suspension.

D. Preparation of the inoculums: The spores of the fungus were washed from the culture using 0.05% Tween 80. The required concentration (10^7 spores/ ml) was calibrated with the haemacytometer (Webber & Sons, 784) and then formulated in cotton seed oil. 2 µl of the prepared inoculums were applied under the pronotal shield of the test nymphs (4^{th} instar). Cotton-seed oil was applied the same way as for the control insects.

E. Effect of infection on food consumption: Ninety nymphs (4^{th} instar) of the desert locust were divided into three groups. Group 1, was kept untreated as control; group 2 was treated with cotton-seed oil only, group 3 was inoculated with Mfl5 spores suspension at a concentration of 0.9 x 10^7 spores/ml. each group was subdivided into three cages (10 nymphs/ replication or cage). The cages were arranged randomly on the laboratory bench. Every day a weighed amount of fresh seedlings were given to each group. Another amount of weighed seedlings sample was dried on an oven for 24 hours at 90º C. to determine the dry matter contents of the seedlings supplied to the insects. The remained un-eaten food was daily removed from the cages, similarly dried at 90º C for 24 hours and weighed when cool. Then the dry weight of the food consumed could be calculated. The insects were examined daily and dead ones were removed. This was essentially the method of Moore *et al*. [9].

F. Preparation and application of PAN: the required concentration was prepared by dilution of PAN (original concentration was 1%) in acetone + polyethyleneglycol (PEG) + water. To prepare 0.01% PAN, I ml of PAN was added to 59 ml acetone + 20 ml PEG + 20 ml distilled water. 2 ml of the required concentration was taken by a syringe and injected in a small piece of cotton hanging from the cage with a silk wire. It was applied 4 days before the application of the fungus and continued daily till the end of the experiment.

G. Effect of application of PAN and the fungus on the daily behavior: eighteen cages were prepared, each containing 20 nymphs. Every three cages comprised one treatment. The treatments were as follows:

Untreated nymphs as control
Cotton-seed oil as a second control
Pheromone component additive (i.e., acetone + PEG distilled + water)
Pheromone component (PAN)
M. flvoviride (10^4 spores/ ml) + PAN (0.01%)
M. flavoviride alone (0.9 x 10^7 spores/ ml)

Observations were made 4 times each day, where the numbers of nymphs in each activity were counted (i.e., those moving, roosting or feeding). The daily mean of each activity was calculated from these observations and analysis of variance was used to show if there was any significant difference between the treatments, while the least significant difference (LSD) was used to compare between means**.**

IIII. RESULTS

The mean daily food consumption (in milligrams of dry matter) for the three treatments (i.e., fungus-treated, oil-treated and untreated control) is given in Table 1. After six days from inoculation with the fungus (0.9 x 10^7 S/ml) all treated nymphs died, whereas those left untreated were all survived. In the last day of treatment (day six) the amount of food consumed per an untreated or oil-treated nymphs was more than 5-6 times as much as that consumed per an inoculated nymph. Significant differences ($P \leq 0.01$) in the amount of food consumed were observed between the different treatments, time after the treatment as well as their interaction (ANOVA table). The mean daily amount of food consumed per an untreated control nymph (i. e., 80.77 mg) was not significantly different from that consumed by an oil treated one (76.5 mg) but they were both significantly different ($P \leq 0.05$) from that consumed by the fungus treated nymph.

Table 1: Mean daily food consumption (mg dry weight) of the 4^{th} nymphal instar of the desert locust after inoculation with *M. flavoviride* (Mfl5 at 0.9 x 10^7 S/ml)

Days after treatment	Treatments		
	Control (untreated)	Oil-treated	Fungus inoculated (0.9 x 10^7 S/ml)
1	102.6	127.2	96.9
2	043.3	038.9	16.5
3	088.5	053.6	22.7
4	080.9	061.2	33.8
5	085.8	087.7	34.0
6	083.3	090.4	15.2
Mean	80.77	76.53	36.03

Fig.1 shows the cumulative amount of food consumed per a nymph inoculated with the fungus (Mfl5) compared to that consumed by a nymph in the control groups. The cumulative amount of food consumed by the fungus-inoculated nymph in six days was equal to 45.2 and 47.7% of the amount consumed by an untreated and an oil treated nymph, respectively.

The effects of low dose of fungus combined with PAN, aggregation pheromone major component, on the desert locust nymphs daily behaviour were shown in Table 2. Significant differences ($P \leq 0.05$) were illustrated in the number of nymphs moving in the different treatments. Those received, respectively, oil treatment or pheromone additives were not statistically different in their movement activity from those in the untreated control, but they were significantly different from those in the other treatments.

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Volume 2, Issue 6, ISSN (Online) 2319-1473

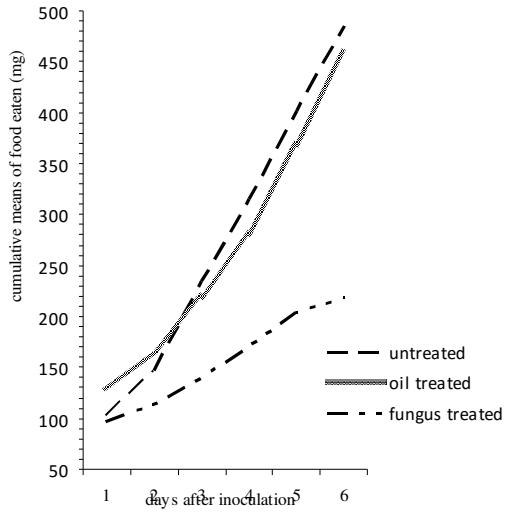

Fig.1. Cumulative means of food eaten (mg) per nymph (4 th instar) exposed to 0.9 x 10^7 S/ml *M. flavoviride* (Mfl5)

Nymphs exposed to PAN treatment only were significantly more active than those in all other treatments. The effect of fungus inoculation either alone at a high dose (0.9 x 107 S/ml), or combined at a low dose (104 S/ml) with PAN stimulation caused a significant reduction in the movement activity of the nymphs. It was also observed that a moving nymph may stimulate the movement of static ones when it touches them.

The results in Table 2 also indicate the number of roosting desert locust nymphs as affected by a low dose of fungus (104 S/ml) plus PAN compared to untreated control, PAN alone or high dose fungus ()0.9 x 107 S/ml) alone. The statistical analysis illustrated significant differences ($P \leq 0.05$) between treatments. Nymphs under PAN stimulation had the tendency to become restless, so they were significantly less roosters compared to the others. On the other hand, nymphs under the effect of both low fungus dose (104 S/ml) and pheromone component (PAN) showed significantly greater number of roosters than nymphs in the other categories.

Table 2: Effect of integration of low doses of *M. flavoviride* (Mfl5) with phenylacetonitrile (PAN) on daily behaviour of desert locust nymphs

Treatments	Mean Number of nymphs ± S.E		
	Moving	roosting	Feeding
Control (untreated)	4.14 ± 0.13	15.247 ± 0.06	0.646 ± 0.17
PAN additives (DW + PEG + acetone)	4. 807 ± 0.19	14.870 ± 0.20	0.375 ± 0.04
Fungus additives (oil)	3.708 ± 0.44	15.407 ± 0.60	0.979 ± 0.32
PAN (0.01%)	9.123 ± 0.83	10.167 ± 0.87	0.708 ± 0.04
PAN 0.01%+ fungus (10^4 S/ml)	1.580 ± 0.02	18.170 ± 0.02	0.250 ± 0.00
Fungus 10^7 S/ml	1.600 ± 0.20	15.560 ± 0.19	0.312 ± 0.00
LSD ($P \leq 0.5$)	1.243	1.375	0.413

PAN, phenylacetonitrile; PEG, polyethyleneglycol; DW, distilled water

Those treated with the high fungus dose in oil formulation without PAN were found to be not significantly different in their roosting activity from the control. It is worth mentioning, however, that some dead nymphs were observed among those inoculated with the high dose fungus alone which might indicate an under estimation of roosters in this category.

Generally in all treatments hoppers were more active feeders in the morning when changing the dry food with the fresh one and nymphs almost stopped feeding towards mid-day (2-3 p.m). From the results shown in Table 1, significant differences ($P \leq 0.05$) were observed between different treatments in the number of nymphs exhibiting feeding activity. The PAN stimulated nymphs engaged in feeding were found to be significantly greater than those subjected to the combined effect of PAN and low dose of the fungus inoculum. The untreated controls were also profoundly more actively feeding than those in the two inoculated groups, respectively. The difference between the non-inoculated and PAN stimulated groups were not significant.

V. Discussion

Control strategies of desert locusts and grasshoppers are classically based on the use of chemical pesticides. Although effective in reducing the pest populations and their incidences, pesticides affect the natural enemies [10]. Pesticides are also involved in environmental and health issues, risk of intoxication to farmers and consumers [12]. The concern about the environmental and toxicological issues of the chemical control of locusts and grasshoppers has stimulated interest in development of biological control based on entomopathogens [3].

The behaviour of insects infected with entomopathogens is likely to be different from healthy ones [7]. The changed behaviour may work in favour of control efforts like reduced consumption. In this study *M. flavoviride* Gams & Roszypal, strain Mfl5 was found to decrease the amount of food consumption significantly. This finding was in accordance with that of [9] who studied the food consumption of adult desert locust inoculated with M. flavoviride formulated in oil. The substantial reduction (>50%) of the amount of food consumed by an infected *S. gregaria* nymphs appear to affect the main factor contributing to the status of the desert locust as a major pest. The infection reduces other activities of the pest before death [14] specially the flight ability in the adult desert locust, partially due to the reduced blood trehalose levels [5]. Desert locusts tend to roost with lower number of insects feeding or moving. This indicates the infection

would also lead to reduction of lumen cavity as a result of the extensive growth of the fungus structures inside the insect body. In addition, the competition between the insect and the fungus would further reduces the trehalose levels in the blood. Locust and grasshoppers infected with pathogens raise their body temperature higher than normal by basking longer in the sun or near a light bulb [2]. Such individuals spend therefore more time basking than their healthy counterparts**,** often at expense of feeding [7]. This reduction in food consumption will compensate for the longer term action compared to the chemical pesticides. This reduction in food consumption was also observed on pee leaf miner infected with *Metarhizium anisopliae* as indicated by the number of punctures compared to control [8].

The effect of integration of PAN with the fungus on the daily behaviour of the desert locust, such as roosting, moving and feeding activities monitored 4 times a day illustrated that the treated insects had an increased tendency to roost tthat the initial hyperactivity of insects stimulated by PAN, might have predisposed the insects to the fungus infection to the extent that it brought about a rapid static condition on insects treated with the fungus. This hyperactivity of the gregarious nymphs subjected to PAN was reported by Bashir *et al*., [1], who observed hyperactivity of crowd reared nymphs. They showed that gregarious hoppers exposed to PAN have a tendency to be solitary.

This study shows the possibility of integrated control of the desert locusts. Evaluation of these treatments under field conditions, are needed.

VI. Conclusions

This study shows the possibility to reduce the application of pesticides by integrated strategy combining low doses of pesticides in addition to PAN and low doses of *M. flavoviride*. As this treatment reduces the activity of the treated nymphs so the areas treated with chemical pesticides will be reduced.

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She started her work as Researcher Assistance in the environment and natural resources research institute, national centre for research in 1993. Now she is Assistant Researcher Professor in the same institute. Her research work is directed towards alternatives to chemical pesticides, specially microbial control of insect pests. During 2012 she work as Post Doctoral Fellow in the department of entomology in the Indian agricultural research institute, New Delhi, India, She work on characterization of entomopathogenic strains of *Bacillus thuringiensis* for six months. This fellowship was provided by the CV Raman fellowship for African Researcher.