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Reduction of Feeding by the Gregarious Nymphs of the Dessert Locust *Schistocerca gregaria* (Forskal), Following Infection by the Fungal Pathogens, *Metarhizium anisopliae* Var. *Acridium* (Metch)

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ABSTRACT

The effect of infection by the fungal entomopathogen, *Metarhizium anisopliae*, on the feeding of the gregarious nymphs of the Desert Locust, *Schistocerca gregaria* was investigated in the laboratory in cage studies. A significant reduction in feeding, as indicated by food consumption, was recorded after inoculation with different spore concentrations ($1.3x10^6$, $1.3x10^7$ and $1.3x10^8$ spores/ml). The reduction occurred before any mortality was recorded due to infection. All infected individuals in all the treatments died by day 7. In the concentration of $1.3x10^8$ treatment, four days after inoculation each locust had eaten less amount of food in relation to locusts from the control group equivalent to the amount consumed by the control group on day 2. This reduction in feeding as accumulated mean measured in milligrams dry weight in day 4 was 38.1, 24.4 and 15.3 for the dose rates $1.3x10^6$, $1.3x10^7$ and $1.3x10^8$ spores/ml, respectively in comparison to the untreated control (38.1 mg).the reduction is substantial contribution to the overall effect of the slow acting pathogen. Furthermore, the rapid reduction in feeding indicated that the effect was not simply due to the invasion of the host tissues by the pathogen or production of secondary metabolites. The mechanism behind such reduction is unclear. In the late stages of infection process, it can be attributed to mechanical damage of host tissue. The possibility is that reduction in feeding is associated with behavioral response.

Key Words: Reduction, *schistocerca gregaria*, fungal, pathogens.

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INTRODUCTION

The entomopathogenic fungus *Metarhizium anisopliae* var. *acridium* (Deuteromycotina: Hyphomycetes) in oil-based formulation is used for control of locusts and grasshoppers. It acts through direct contact and field trials in Sudan have proved to be effective on tree locust *(Kooyman and Abdalla, 1998)*.

Metarhizium spp. have show effective control of a number of target species under a range of natural field conditions. These include: The variegated grasshopper, *Zonocerus variegatus* (L.), in the humid zones of southern Benin *(Douro-kpindou et al., 1995)*, the rice grasshopper, *Hieroglyphus daganensis* Krauss, in area of the Niger flood plain in north Benin *(Thomas et al., 1997)* and the Senegalese grasshopper, *Oedaleus senagalensis* (Krauss), in the Saharan zone of southern Niger.

Hoppers of *Schistocerca gregaria* eat about their weight of fresh vegetation each day, this amount increasing from about 20 mg at the beginning of the first instar to about 1.5 gm in the middle of the fifth instar. Actively migrating immature adults need to eat at least their own weight (2-3 g) of fresh vegetation each day and possibly three times as much. The disadvantage of using fungal pathogens to control Desert Locusts is the time taken to kill the insect in relation to pesticides. The main hazards caused by the Desert Locust is consumption of a large amount of food, it was reported that the application of the pathogen was followed by reduction in feeding rate *(Moore et al., 1992)*.

However, effective control may not be determined by mortality rate alone. As a consequence of infection an insect behavior is changed and hence its impact as a pest is reduced. This may also constitute effective control.

Both chemical pesticides and pathogens can affect insect feeding rate. *Haynes (1988)* found examples of both increase and reduction in food intake following exposure to pathogens. Studies by *Moor, et al. (1992)* and *Seyoum, et al. (1994)* have both reported significant reduction in feeding following infection with *M. flavoviride*.

This paper was to show the reduction in feeding rate by different doses of pathogen in a controlled condition in the laboratory cages in the Sudan, The study was conducted using *Schistocerca gregaria* nymphs infected with *Metarhizium anisopliae* var. *acridium*.

MATERIALS AND METHODS

The study was conducted 9 years at International Center of Insect, Physiology and Ecology (ICIPE), at Port Sudan (Sudan). The colony of *Schistocerca gregaria* used was obtained from stock that has been reared at Port Sudan station.

The strain of *Metarhizium anisopliae* var. *acridium* was supplied by Plant Pathology Laboratory, Plant Protection Directorate- Khartoum North- Sudan.

The fungus was cultured on potato dextrose agar (PDA) and formulated in cotton seed oil. The culture plates were incubated at room temperature (25° -27°c) and species were harvested after 14-21 days and harvested using tween- 80. Using a Counting Chamber (Thomo, Webber, Scientific International Ltd. England). Different concentrations were prepared. Eighty nymphs (4th instar) were divided into four groups each consisting of twenty insects and maintained in a wooden cages (25 x 25x 25 cm) at room temperature (25°27°c) and relative humidity of 74-76%. No discrimination was made between age- class or sex. Each day a weighed amount of fresh millet leaves was given to each nymph. An average of five samples of the millet provided to the nymphs was dried for 24 hr at 80°C, to determine the percentage dry weight of millet, so that the dry weight of food supplied could be calculated, every 24 hr. The uneaten millet was removed from each box, dried at 80°C for 24 hr and weighted. Nymphs were allowed to acclimatize to bioassay boxes for 24 hr prior to inoculation with pathogen. Conidial suspension was made into cotton seed oil. Serial dilutions with final spore concentration of 1.3 x 10^8, 1.3 x 10^7 1.3 x 10^6 spores /ml were made. Each nymph in a group of twenty insects, received 2µ of the conidial suspension applied with a micropipette beneath the dorsal pronotal shield. The control insects were treated with oil only, the treated nymphs were maintained as before. The insects were examined daily and mortality recorded. The dead nymphs were maintained in cages with high RH to provide a saturated atmosphere and encourage sporulation to record mortality due to fungal infection *(Moor et al., 1992)*.

RESULTS

The treated nymphs began to die at day 2 after treatment (Table 1). At the highest dose rate of 1.3x10^8 spores/ml, all treated nymphs died on day 5 with signs of mycosis. With the concentration of 1.3x10^7 spores/ml, death occurred by day 6 and with lowest dose rate 1.3x10^6 spores/ml, death occurred by day 7 with signs of mycosis. Control mortality was low and with no signs of mycosis. In general the locust in the rearing cages of the stock showed no signs of infection prior to or during the experiments. The daily food consumption in milligrams of dry weight for the four treatments is given in (Table 2). There was no significant difference in food consumption in the highest dose 1.3x0^8 spores/ml and the control from day 1 to 3, but it was significantly different in day 4 and 5 of the experiments prior to inoculation. With the second highest dose rate (1.3x0^7spores/ml). Food consumption did not differ between treated and control insect for the first three days of the experiment, but did differ from day 4 to day 6. At the lowest dose (1.3x0^6 spores/ml), food consumption was reduced from day 4 and was significantly different from the control up to day 6. When food consumption presented as accumulated food eaten after inoculation, a clear trend was seen (Table 3). In the 1.3 x0^8 spores/ml, treatment by day 4 after inoculation each locust had eaten less amount of food in relation to locust from control group by day 2 . The total difference in food consumption was even greater as the control locust continued to eat. In the 1.3 x0^7 spores/ml concentration. Treatment, by the day 5 the treated locust had eaten almost the same amount of food eaten at day 3 by compared to a locust from the control locust. This was 33.2% less food as that consumed by the control locusts. The same trend could be seen with the lowest dose 1.3 x0^6 spores/ml treatments were food consumed was 38.1% less than that of the control locusts.

Table 1: Accumulated total number of nymphs dead from treatment by different doses of *Metarhizium anisopliae*.

Days after inoculation	Treatment			
	Control	1.3x0^6 spores/ml	1.3x0^7 spores/ml	1.3x0^8 spores/ ml
1	0	0	0	0
2	0	0	0	1
3	0	0	0	2
4	5	4	6	13
5	6	9	14	20
6	6	18	20	20
7	6	20	20	20

Note: Control insect were treated with oil only.
Temperature (24-27° c).

Table 2: Daily food consumption per nymph inoculated with different concentrations of *Metarhizium anisopliae*.

Days after inoculation	Treatment			
	Control	1.3x10^6 spores/ml	10^7 1.3 spores/ml	1.3x10^8 spores/ ml
1	3.58± 7.9	6.09±20.4	3.7±5 8.0	0.6± 0.6
2	3.42 ± 12.1	4.19 ± 8.4	4.08±12.4	3.98±13.9
3	2.42± 4 .24	4.56±9.3	2.97±3.9	0.8 ± 0.8
4	11.9 ±37.4	0	0	0
5	5.56 ±13.8	0		0
6		0	0	0

Note: Food consumption measured in milligrams dry weight ±SE.

Table 3: Cumulative mean of millet dry weight eaten per nymph after inoculation with different concentrations of *Metarhizium anisopliae*.

Days after inoculation	Treatment			
	Control	1.3x0^6 spores/ml	1.3x0^7 spores/ml	1.3x0^8 spores/ml
1	3.58± 7.9	6.09±20.4	3.71±8.01	0.64±0.6
2	4.95± 20.0	7.39±28.8	5.51±20.5	4.03±14.5
3	5.51± 2.4	8.68±38.1	6.26±24.4	9.12±15.3
4	13.11± 59.8	8.68±38.1	6.26±24.4	9.12±15.3
5	14.24± 73.6	8.68±38.1	6.26±24.4	
6	18.16±99.9	8.68±38.1		

Note: Cumulative means measured in milligrams dry weight ±SE. ±SE calculated as the square root of the sum of sample variances.

DISCUSSION

This study has demonstrated that oil formulation of different concentrations of *Metarhizium anisopliae* var. *acridium* causes a significant reduction of feeding by *Schistocerca gregaria* before death and killed after 5-7 days. However field experimental leads to an estimation that 10.000 per locust

applied by ultra –low – volume spinning disc technology will lead to death within 9 days (Bateman unpublished data). All treatments showed the trend of a reduction in food consumed even at a dose rate as low as 1.3 x0^6 spores /ml.

The reduction in feeding, as indicated by food consumption was significant by the second and third days after inoculation for all doses. Thus, it is likely that even the lowest dose, at least when applied as a single source of inoculum under the pronotum, is higher than would be acquired in the field following a spray application. Infection by *M. anisopliae* resulted in significant reduction in food consumed in the first 24hr at highest dose. The quantity of food consumption in five days (insect of death) was equivalent to the quality consumed in 2 days by the control group.

These finding are in agreement with those obtained by *Moor, et al. (1992)* when *M. flavoviride* was used against adult of desert locust. Also reduction in feeding has been shown with the grass hopper *Melanoplu sanguinipes* infected with *Nosema locustae (Johnson and Pavlikova, 1986)*. Infection by *Nomureae rileyi* may also reduce feeding by other hosts *(Igroffo, 1981 and Seyoum et al., 1994)* reported that significant reduction in both feeding and flight occurred by the 4th day application of *M. flavoviride* against the Desert Locust .

The result in this study agrees with work conducted on *S. gergaria* and *Zonocerus variegates*, by *Thomas, et al. (1997)* using *M. flavoviride*. The study differs from the result obtained with the *Lepidoptera Choristoneura fumilerna* and *Malacosoma disstria* infected with *Entomophaga aulicae*. These species appeared to assimilate food in a manner similar to non infected larvae until 24 hr before death *(Tyrrel, 1990)*.

Also Fagade and others found in microbial control of caged populations *of Zonocerus variegatus* using indigenous fungal entomopathogens (*Beauveria bassiana* and *Metarhizium* sp. isolated from the grasshopper's cadaver. Bioassay response indicated a dose-dependent mortality coupled with drastic reduction in food consumption among spores infected grasshoppers *(Fagade et al., 2005)*.

Recent field trail in *(Victor et al., 2007)* have shown that reduction of feeding by *Schistocerca piceifrons* piceifrons (Orthoptera: Acrididae), Following Infection by *Metarhizium anisopliae* var. *acridum*.

Other studies have indicated that reduction in feeding may be attributed to degradation of tissues in combination with the production of secondary metabolites. *Vey, et al. (1985)*; *Samuals, et al. (1989) and Vey and Quiot (1989)* suggested that secondary metabolites produced by *M. anisopliae* act on insect tissues including the midgut. The production of these metabolites in combination with utilization of glycogen and lipid reserves *(Zacharuk, 1971)* and possible mechanical disruption of tissues by mycelial growth may be responsible for loss of appetite. Result studies on *Oedalens snegalensis and S. gergaria* (unpublished data) have shown evidence for behavioral fever in other species; therefore, it is possible that *S. gergaria* adopted a behavioral fever response when infected with *M. anisopliae*.

The time taken to kill the locust is one disadvantage of control strategy on fungal biopesticides, although chemical pesticides such as Fenitrothion, Dieldrin and Malathion may take a similar number of days to achieve desirable kill under certain situations *(Bateman, 1992)*. Adult locusts eat equivalent of their weight of food every day and a square kilometer of swarming locust can weigh 80.000 tones *(Steedman, 1990)*. So reduction in food consumption is of value.

The reduction in food consumed makes the delay in kill with biological less significant (although the inset of kill is some times faster than with some conventional pesticides *(Haynes, 1988)*.

If crawling, moving and flying ability are also adversely influenced by infection, then the damage caused by locust following infection could be even less than anticipated of present. Such reduction (in conjunction with other pre lethal effects such as, moulting inhibition and loss of reproductive potential) are an obvious benefit to the overall picture of sub- and pre-lethal control effects of *Metarhizium* sp. Conveying and image of control that combines such effect with mortality is an important factor when explain the mode of action and other obvious environmental benefits of employing green muscle as mycopesticides. *David (2005)* suggested that the mycopesticides should be a part of integrated pest management of locusts and grasshoppers.

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