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STUDIES ON THE FIELD PERFORMANCE OF *METARHIZIUM ANISOPLIAE* VAR. *ACRIDUM* (GREEN MUSCLE®) AGAINST MIXED POPULATIONS OF GRASSHOPPER IN ETHIOPIA

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ABSTRACT: Two consecutive field studies were carried out on the performance of *Metarhizium anisopliae* var. *acridum* against mixed grasshopper species under field conditions in North East of Ethiopia during the 2003 cropping season. Both the entomopathogen and control (gasoline) were applied against the experimental grasshoppers using a handheld, battery operated ULV+ sprayer. Each treatment was assigned randomly. In both trials, the fungus killed more grasshoppers than the control. The mortality of grasshoppers treated with Green Muscle® per day ranged from 0 to 19.78 % in the first trial and 20 to 70.91% in the second trial. Only 0 to 8.7% and 7.79 to 35.78% of insects in the control were dead, respectively. About 37.91% and 37.71% of the grasshoppers died from the fungus treated plots showed external sporulation of conidia following incubation in the first and second trials, respectively, confirming that death was due to mycosis. The fungus was found to be target specific as none of the non-targets showed sign of infection/fungal external sporulation following routine checkups on cadavers. The present result conform with those findings elsewhere that Green Muscle® can cause infection which could be sufficient to suppress grasshopper populations below that which could cause economic injury level when applied under field conditions in a similar way as chemical pesticides.

Key words/phrases: Entomopathogen, Ethiopia, gasoline, grasshopper sp., *Metarhizium anisopliae*

INTRODUCTION

Insect pests have been implicated as the most important constraints to the subsistence agricultural production system in Ethiopia. Grasshoppers are among the greatest factors contributing to low productivity. For instance, Tadesse Gebremedihin (1988) reported losses of tef (*Eragrostis tef*) due to grasshoppers ranging between 25 to 35% of the expected yield. Losses between 2 and 47% for tef and between 12 and 26% for wheat, with a mean about 20% for each, were recorded by previous work (Tibebu H/Wold and Landin, 1992). Complete crop failures in tef due to grasshoppers occur every year in central Ethiopia (Tibebu H/Wold and Landin, 1992).

Grasshoppers are phytophagous insects, common in a variety of often dry habitats such as semi-deserts, open meadows and grasslands, as well as in disturbed areas such as crop fields and along roadsides (Tibebu H/Wold and Landin, 1992). According to Jago (1977), there are at least two hundred species of grasshoppers in Ethiopia. A few of them constitute a high risk to economic crops in different parts of the country.

Similarly, according to Tibebu and Landin, (1992) twenty nine taxa of short and long-horned grasshoppers grouped into four families and nine subfamilies from central Ethiopia were recorded. Many of the species are of great economic importance. They are either pest or potential pests of cultivated crops in different countries including Ethiopia (Jago, 1977; Stretch-Lilja, 1977; CORP, 1982).

Among economically important species of grasshoppers, *Aiolopus longicornis* is a serious pest of cereals, tef in particular at early seedling stage and highly mobile, suddenly appearing in swarms (Jago, 1977; Tibebu H/Wold and Landin, 1992). *Oedaleus senegalensis* (Krauss), *Diabolocatantops axillaris* (Thunberg) are also reported to cause serious crop losses particularly to late season millet in Africa (Jago, 1984). The later is also characterized by high mobility and very rapid rates of population increase. *A. thalassinus* also causes considerable damage to seedlings of tef and wheat.

This and other *Aiolopus* species are becoming increasingly important as crop pests, being able to adapt from natural grassland habitat to crop areas

(Hollis, 1968). *Acrotylus patruelis* is also one of the most abundant species throughout the year. It is a pest of tef and sorghum at early heading. *E.noxia* too is relatively numerous during the short and long rains, usually on black clay soils, causing considerable damage to seedlings or growing stages of tef and wheat and sorghum (CORP, 1982) The Senegalese grasshopper, *Oedaleus senegalensis* occurs in semi-arid grasslands in Africa, the Middle East and the Indiana sub-continent (Cheke *et al.*, 1990). The species is the most important grasshopper pest in Sahelian zone of West Africa. In 1974 it infested 3500 x 10^3 ha in West Africa and was responsible for the loss of 368000 tones of agricultural production (Batten, 1969; Bernardi, 1986). A suite of other locust and grasshopper species and species assemblages cause much more regular and through their cumulative effects more significant damage (Kooyman *et al.*, 1997).

In recent years, the challenge of controlling these ubiquitous pests has fallen largely to synthetic chemical insecticides. Fenitrothion, a short persistent organophosphate with a half life of around 24h (Sekizawa *et al.*, 1992) is one of the most widely used chemical insecticides for locust and grasshopper control (Milner, 1997). It requires repeated applications within a season or large scale blanket sprays to achieve more than temporary relief (Kooyman *et al.*, 1997). Furthermore, the extensive use of even these non-persistent chemicals has led to environmental impacts (to humans and non-target organisms). These shortcomings underscore the need for alternative strategies, such as pest control with biological control agents (Goettel and Johnson, 1997).

Entomopathogenic fungi such as *Metarhizium anisopliae* (Metscnikoff) Sorokin and *Beauveria* spp. are among the biological control agents used against insect pests. Few micro-organisms are available to replace conventional chemical insecticides against different insect pests (Klein and Lacey, 1999). Fungi have the potential as microbial control agents because, they are genetically stable, infect their host through the cuticle; can be mass produced cheaply, exhibit high virulence and relatively rapid action and are target specific (Prior and Greathead; 1989, Lomer and Prior, 1992). The spores can be formulated in oil to overcome high humidity requirement during the infection process and to protect the conidia from soil radiation (Lomer *et al.*, 2001). As well as being environmentally inoffensive, biological control agents are capable of self propagation. Fungal pathogens have been examined as potential control agents for different insect pests (Dowd *et al.*, 1992; Pell *et al.*, 1993; Lacey *et al.*, 1994; Vega *et al.*, 1995; Klein and Lacey, 1999).

Inundative augumenation of entomopathgenic deuteromycete fungi formulated as biopesticides could replace chemical spraying. Thus, the main objective of this study was to evaluate the efficacy of *Metarhizium anisopliae* var. *acridum* (Green Muscle®) against mixed grasshopper populations under field conditions.

MATERIALS AND METHODS

Study sites

Two trial sites (range lands) were selected in the Northeastern part of Ethiopia. The first trial was carried out in a place locally known as Rasa (09° 54' N, 40° 03' E), 255 km north east of Addis Ababa (the capital city of Ethiopia). The second trial was carried out in Shoa-Robit (10° 00' "N and 39° 53' E), 225 Km north east of Addis Ababa. The sites were selected because they are the most frequently affected areas by grasshoppers. The second trial site is typical of open savannah, with mixed grasses and with almost no vegetation. While, the first trial site is composed of different grasses and scaterred acacia trees especially *Acacia seyal* and *Acacia ethabia*.

The total trial field size was 5.46 hectares for each trial including the buffer zones. Each trial field was divided into three blocks (Green Islands). Each block was separated from the surrounding field and block by clearing the vegetation in 50 m radius as a barrier. Both field trials were carried out using a Randomized Complete Block Designs (RCBD). Each block was divided into two treatment plots (sub-green islands) of 600 m^2 each separated by a 50 m buffer zones (Fig. 1).

The product used in these trials was an oil miscible flowable concentrate (OF Formulation) of *Metarhizium anisopliae* var. *acridum* conidia obtained from the Biological Control Products (BCP), South Africa. Gasoline was used as a diluent and control. The viability of the fungus was checked using standard procedures (Lacey et al., 1994), 30 minute before and after and during spray.

The treatments, Green Muscle®, and control (gasoline) were assigned randomly to each plot in each block and replicated three times. The dose/ha of *Metarhizium anisopliae* var. *acridum* (Green Muscle®) was done according to the BCP's recommendation (2.5 l/ha).

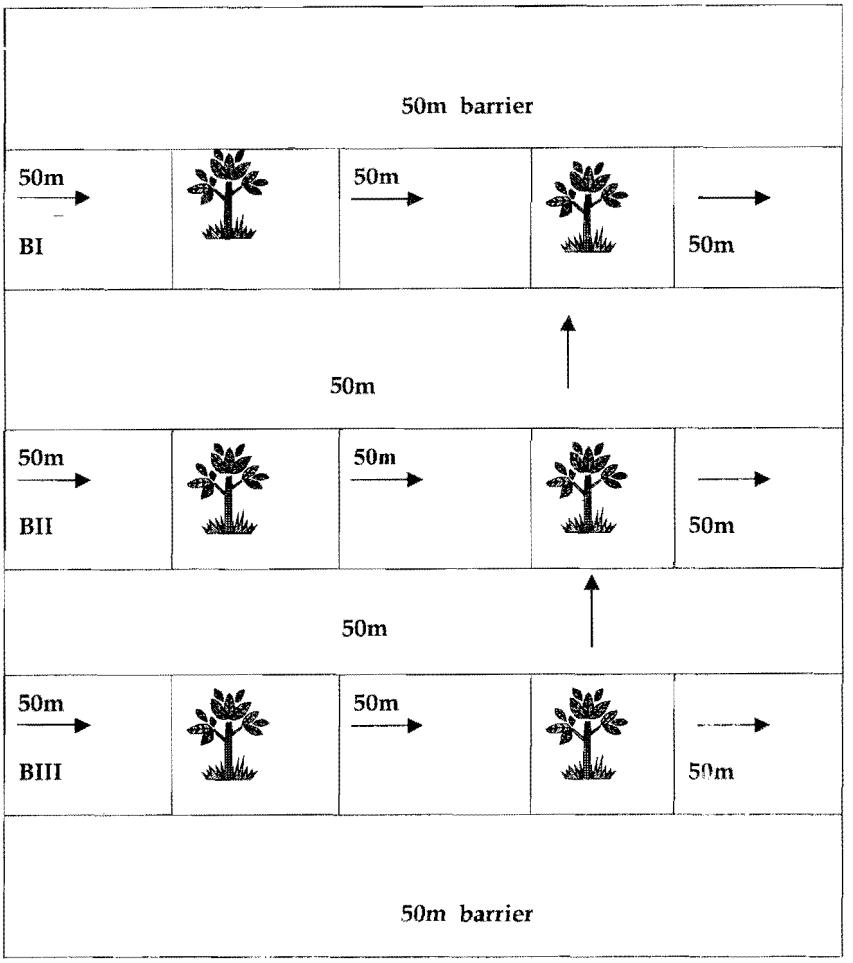

Fig. 1. Field lay out. Indicates the treatment plot. * Clear area indicates buffer zone

Total land used was 210 m x 260 m. The experimental field was divided into three blocks (Green islands), of 20 m x 210 m (w x l). Each block was divided in to two plots for treatments, which are 20 m x 30 m each. Each treated plot is separated from the neighbouring treated plot by 50 m (barrier). Similarly each block is separated from the neighbouring block by 50 m width buffer zone.

Dilution was made in less than 10 minutes before spray. Battery operated hand-held ULV+ sprayers (one for each treatment) were used for the application of the treatments. Eight fresh dry cell batteries were loaded and used that resulted in a disc speed of 10,000 RPM measured using a Vibratak. The average spray height of the nozel disc from the ground was 1.3 m.

The flow rate of the treatments was 40 ml/min. The swath width was 10 m and the average walking speed was 1.2 m/sec (Emiru Seyoum, 1994; Kooyman and Abdalla, 1998). The first trial was carried out during the rainy season (August/September, 2003) while the second trial was carried out at the end of the rainy season (October/November, 2003).

Sampling of experimental insects from each plot was made between 8 am and 9 am commencing 24 hrs after application for a duration of 21 days for the two trials. Sampling was made by using different sweep nets for each treatment to avoid possible contamination between treatments. Ten 180° sweepings were made in each plot. The collected insects were kept in cages of 20 cm x 40 cm x 30 cm (L x H x W) within the experimental field. The insects kept in the cages were fed with untreated fresh grass daily. Mortality was recorded from day two onwards every day at the same time

throughout the whole post treatment monitoring periods of each trial.

The cadavers collected from the cages placed in the field as described above were incubated by sterilizing the surface of the dead insects with 70% ethanol, followed by rinsing with distilled water and then placed on moist filter paper in petridishes to observe the external growth of the fungus (external sporulation of mycelium) under room temperature.

Field observations for 10 minutes/plot were carried out each day to collect dead or moribund target and non-target organisms. Live non-target species were collected from fungus treated plots in the same way as for the target grasshoppers and kept in cages (Table 1). Once dead, the cadavers of the non-target organisms were incubated in the same way as the cadavers of target grasshoppers.

Table 1. Non-target arthropods collected from the Green Muscle®.

Non-target species	Number
Hemipetra	14
Lepidoptera	8
Coleoptera (Beetles)	12
Mantodia (Praying mantids)	5
Acarina (spiders)	9

The identification of grasshopper species encountered in the trial sites was carried out using methods developed or described by previous works (Jago, 1984; Steedman, 1990; Tibebu H/Wold and Landin, 1992; Rentz, 1991).

Statistical analysis

Mortality between fungus treated and control on the same day was compared using independant sample t-test, test of Homogenity of variance was tested using Levene Statistic of SPSS 10.00. Similarly to compare the overall mortality between fungus treated and control t-test was used. Natural mortality in the controls was corrected using Abbot's formula (Abbot, 1925). Percentage mortality was computed by dividing number of grasshoppers died by those alive from the same cage and the same date and multiplied by 100, *i.e*

$$\% \; mortality = \frac{No.\; dead}{No.\; alive} \times 100$$

Pearson correlation was computed for mortality and mycosis.

RESULTS

Trial I

Species composition of target grasshoppers

The first trial site was found to be dominated by the following grasshopper species

Zonocerus variegatus,
Cataloipus tartarica.,
Cyrtaconthacris spp.,
Diablocatatops spp.,
Oedalus spp.

Other grasshoppers belonging to acridinae and catantopinae sub-families were also encountered.

Spore viability

The germination rate of *Metarhizium* was found to be above 90% in all tests indicating high spore viability.

Mortality of target grasshoppers

The percentage mortality of grasshoppers treated with fungus ranges from 0 to 19.78%, and that of control from 0 to 8.77% between days. The variation in the overall target mortality between the fungus treated insects (11.3%) and that of control (6.9%) was significant ($t = 3.607$, $P < 0.001$). Mortality in the fungus treated group was generally low for the first six days following application, and there were no significant differences between fungus treated and the control in mortality during those early days. However, the mortality in the mycosed insects increased from day 7 to day 10 and again between day 16 to day 21 during the monitoring period (21 days) and a significant difference was observed between these days in mortality between treated and the control groups (Fig. 2). However, between days 11 and 15 there was no significant difference between the control and fungus treated, even though mortality in the fungus is greater than that of the control.

The mortality in plots treated with Green Muscle® was confirmed to be due to fungal infections as the external growth of mycelium was apparent for 37.91% of dead grasshoppers showed external sporulation. Moreover, the characteristic red coloration of the dead grasshoppers was also clearly seen from grasshoppers which died from fungus treated plots (Fig. 3). There is a positive correlation between mortality and mycosis in the fungus treated plots ($r = 0.303$, $p = 0.194$) even though the relation was not significant. However, none of the dead grasshoppers from the control treated plots showed sporulation or red coloration after incubation.

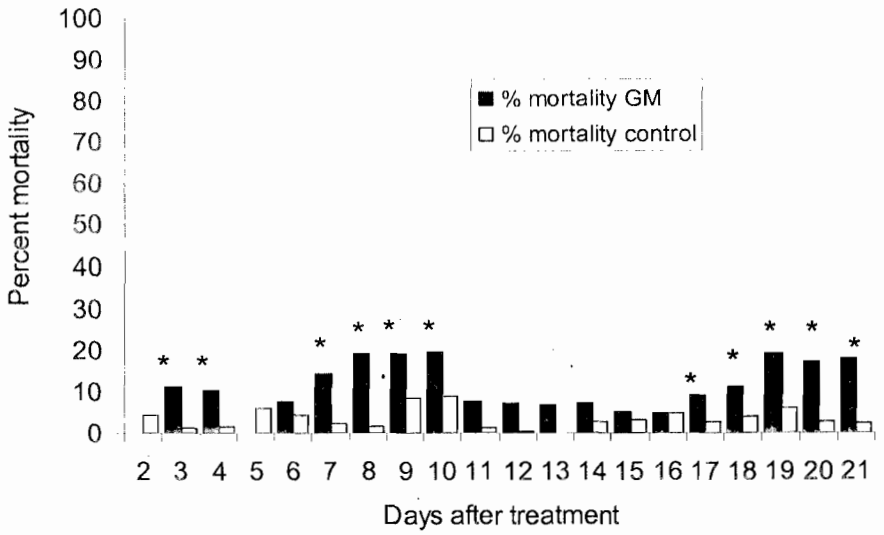

Fig. 2. Percent mortality between fungus treated and control versus time in the first trial.
(* depicts significant difference in mortality between fungus and control at that particular day ($P < 0.05$). The statistical analysis used was independent sample t-test.

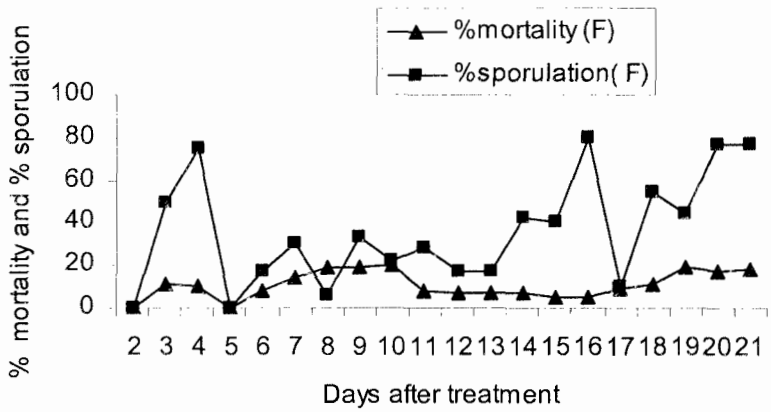

Fig. 3. Percent mortality and external growth of the fungus (sporulation) in grasshoppers from fungus treated plots in days after treatment.

Non-target insects' mortality assessment

During the assessment period, no dead non-target insects were found in both the control plots and in plots treated with Green Muscle®

Trial-II

Species composition of grasshoppers

The second trial site also consisted of similar grasshopper species

Oedalus spp,
Cyrtaconthacris spp,
Zonocerus variegatus.,
Cataloipus tartarica spp.,
Diablocatatops spp.

Other unidentified grasshoppers belonging to acridinae and catantopinae sub-families were also found.

Similar to the first trial, the germination rate of *Metarhizium* was found to be above 90 percent.

Mortality of target grasshoppers

The percent mortality/day of grasshoppers due to fungal infection in this trial ranged from 20 to 70.91% while that for the control ranged from 7.79 to 35.78% (Fig. 4). More number of grasshoppers were dead in the fungus treated plots than the control up to eleven days and the variation was statistically significant ($P<0.05$). However, there is

no significant difference between the control and fungus treated insects after eleven days. Similarly, overall comparison of mortality between fungus treated 44.46% and control 19.22% was statistically significant (t=2.321, P=0.021).

Insects from the Green Muscle® treated plots were infected with *Metarhizium* as external growth of mycelium and red coloration became apparent following incubation in 37.71% of dead insects (Fig. 5) confirming that death was due to mycosis. Moreover, there was a positive correlation between mortality and mycosis and the relation was significant (r = 0.0.528, P= 0.017).

However, none of grasshoppers incubated similarly from the control group showed external growth or other signs of mycosis. Following routine post treatment field assessments, 10 cadavers of target insects were found in the field from fungus treated plots from 5-10 days post-treatment.

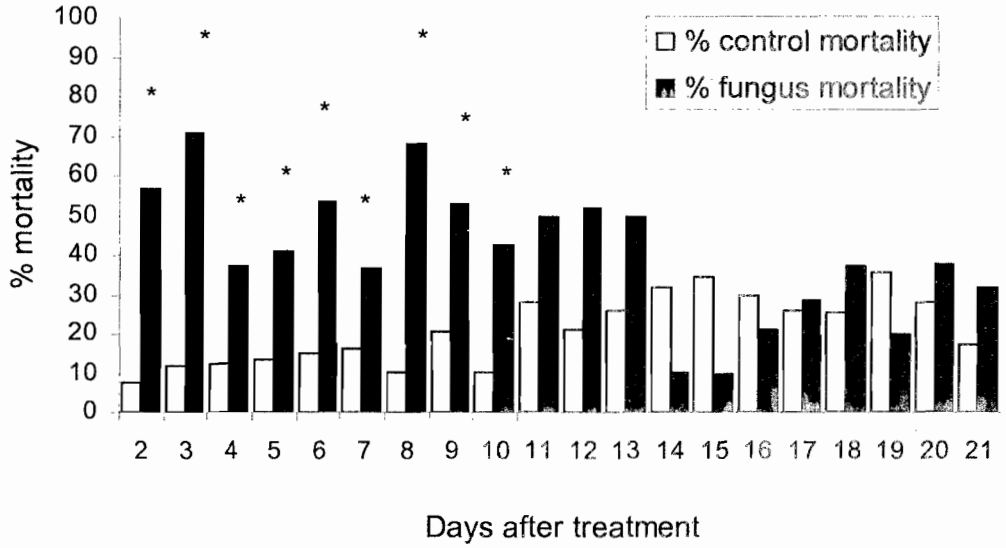

Fig. 4. Percentage mortality of grasshoppers versus different treatments during the monitoring period.
(* depicts significant difference in mortality between fungus and control at that particular day (P < 0.05). The statistical analysis used was independent sample t-test).

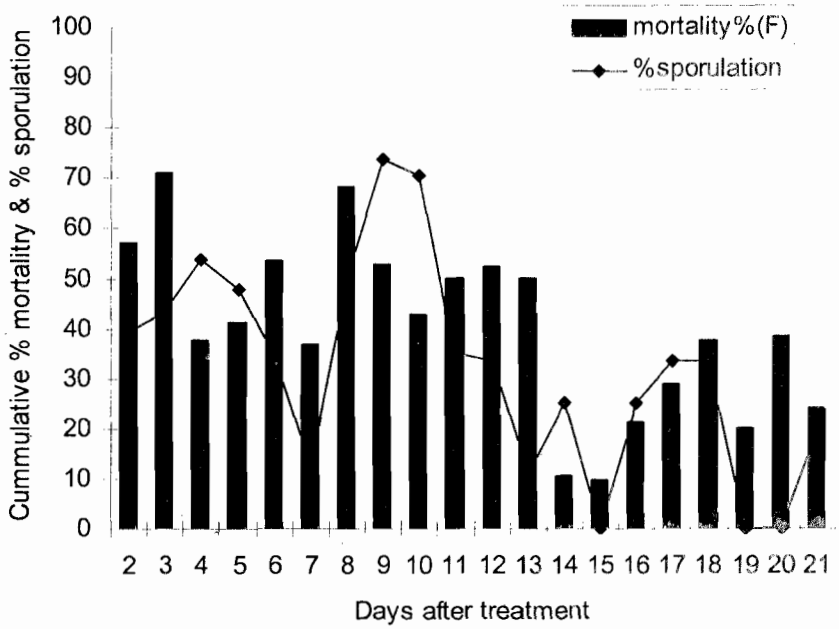

Fig. 5. Percentage mortality and % sporulation in the Green Muscle® plots during the monitoring period.

Mortality of non-target organisms

No non-target insects were found dead or moribund in the field from fungus treated plots. From 48 non-target insects and other arthropod belonging to orders (Hemiptera, Coleoptera, Lepidoptera, Mantodia, and Acarina (spiders) collected from the Green Muscle® plots and incubated and checked for external growth of the fungus, none showed external sporulation.

DISCUSSION

The significantly high percentage mortality in the fungus treated plots in the present study suggests that the performance of the pathogen was up to expectation in terms of its virulence.

The possible reason for the high mortality in the fungus treated targets specially during the early stage of the monitoring period after treatment could be due to both stress and fungal infection (Beenakkers *et al.*, 1984) (for external growth of mycelium was apparent in incubated mycosed cadavers). The absence of significant difference after eleven days in the second trial and first trial may be associated with the half-life of the pathogen. Contacts with spores from the spray residue provide an important route of infection. Spores can persist for several days after spraying and half-lives of spray residues of 5-7 days are typical (Thomas *et al.*, 1996, 1997; Milner and Staples, 1998). Thus it appears that not all insects were contacted directly by the spray to get infected. The reason for this is unclear but it is likely that a threshold number of spores is required to cause infection (Kooyman *et al.*, 1997). It is also possible that some individuals can escape infection through moulting before spores have had the chance to penetrate the cuticle. Further more, inclusion and migration contribute to new individuals to the field and join the existing populations at various times after spraying, which may dilute the treated populations (Kooyman *et al.*, 1997). These individuals are exposed to different levels of infectivity as the spray residue decays and may take several longer days or may escape infection altogether.

The continued high mortality in the control group could have been attributed to different biotic and environmental factors. High control mortality of upto 30-40% was also observed in control treated plots in similar study by Milner and Staples (1998). According to their explanation, the very hot and sunny weather might have contributed for high control mortality.

The principal constraint affecting operational use and limiting the field efficacy of Metarhizium appears to lie in the capacity of grasshoppers and locusts to thermoregulate above the permissive temperature for fungal growth (Blanford *et al.*, 1998). High day temperature combined with the capacity of grasshoppers and locust to elevate its body temperature to several degrees above ambient, which can prolong incubation times to more than 20 day this might have contributed for low mortality in the fungus treated plots in some of the days (Goettel *et al.*, 1995; Thomas *et al.*, 1996).

Similarly Carruthers *et al.* (1992) demonstrated that the rangeland grasshoppers, *Camnula pellucida* can slow the development and actually cause mortality of the fungal pathogen by raising their body temperature during active thermoregulation in the field. Boorstein and Ewald (1987) reported a similar finding for another rangeland grasshopper, *Melanopus sanguinipes*. In this case infected grasshoppers were shown to actually prefer higher temperatures than uninfected controls. These higher temperatures were found to be detrimental to pathogen development and survival.

According to Milner and Staples (1998) pathogenicity of fungus also depends on the type of formulation used. Similarly Barson *et al.* (1994) demonstrated that the type of oil used affects the pathogenicity of Metarhizium for house flies. Both these studies suggested that vegetable oils might be better than mineral oils. Thus the formulation used might also had effect on the pathogenicity of the fungus as we have used diesel oil.

Fewer than expected cadavers were recorded from fungus treated plots from day 5 - 10 in the second trial and non in the 1st trial. The possible reason could be that many might have been predated or scavengered. This loss of sources of inoculums is likely to have a marked effect on the extent of horizontal transmission although pathogen cycling cannot be ruled out altogether. Recycling requires conditions of high humidity and reduced scavenger activity (Lomer and Lnagewald, 2001). This is because infection levels are determined by both the functional and numerical responses of the pathogen and the spatial and temporal dynamics of the host (Kooyman *et al.*, 1997). *M. anisopliae* var. *acridum* probably recycles at a low level in susceptible host species and is able to survive from one season to the next in favourable microhabitats particularly in the cadavers of infected insects (Shah *et al.*, 1994; Thomas *et al.*, 1996). This might have also contributed for low mortality in some of the days.

The absence of significant difference in mortality between fungus and control in the middle of the monitoring period may also be related with sampling bias as stated by (Thomas *et al.*, 1996). The possible changes in behaviour in treated insects also may make these insects more prone to capture by sweep net sampling.

The absence of the sign of fungal infection in the non-target insects indicates that Green Muscle is host specific. This is in agreement with Milner and Hunter (2001) where they tested the efficacy of Metarhizium to ten different non target insects and they found that the common var. anisopliae isolates are quite polyphagous while the var. acridum isolates are host specific. This is an added advantage because the absence of effects on non - target insects may promote future mortality of the target pest insect due to predation by insect predators such as mantids.

In conclusion biological and physical properties make Green Muscle® an ideal candidate for augmentative biological control. The satisfactory results from the present studies i.e. high percentage of germination rate, percent mortality and external growth of *Metarhizium* spores, host specificity and its simple application technique i.e. using the existing technology make Green Muscle® potentially useful entomopathogen (mycopesticide) in Ethiopia for the control of grasshoppers as a component of an Integrated Pest Management (IPM) strategy. The results of the present two consecutive field trials have confirmed that Green Muscle® could cause infection sufficient to contain grasshopper population and prevent populations build up from reaching economic injury level when applied under field conditions. Moreover, the availability of biological control, such as entomopathogenic fungi would give growers more pest management options under subsistance farming systems like in Ethiopia.

One apparent problem with the biopesticides revealed is that unlike a fast acting chemical, the pathogen does not cause significant mortality until after day 6 (Kooyman *et al.*, 1997). Thus the pathogen could be interpreted as having only limited efficacy. However, it is a misconception that the slow action of microbials makes them ineffective for pest control as not all control scenarios demand instant knock down. Much of the grasshopper and locust control can be done in non-crop habitats where control is preventive rather than curative (Prior and Streett, 1997). Under these circumstances slow speed of kill need not be a limitation, especially as feeding may be reduced some time before death (Moore *et al.*, 1992). Probably, chemical pesticides would be used only in cases of major emergency and serious large scale outbreaks as a weapon of last resort, Green Muscle would be used in situation of less urgency.

The successful implementation of such an IPM approach will require training at all levels. A good understanding of biological control is necessary to convince plant protection officers and farmers that quick kill is only necessary when using curative control strategies. As such this is the first study to evaluate the efficacy of Green Muscle under Ethiopian condition the authors believe that further similar research covering wider ecological zones in the country will strengthen the research findings.

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