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Effect of afternoon and morning applications of Green Muscle® and phenylacetonitrile on Desert Locust nymphs, *Schistocerca gregaria* (Forskål, 1775)

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ABSTRACT

An experiment was carried in Niger to identify the best time to apply Green Muscle® and Phenylacetonitrile to control nymphs of *Schistocerca gregaria* (Forskål, 1775). Ten treatments (50 g of GM/ha, 25 g of GM/ha, 25 g of GM + 10 ml of PAN per ha, 12,5 g of GM/ha and 12,5 g of GM + 10 ml of PAN per ha, each of them applied at 9:00 am and at 5:00 pm) were tested in a randomized complete block design with 3 replicates. Plots were field enclosures of 20 m^2 (5 x 4 m) containing millet plants to feed nymphs. Two hundred and forty nymphs of desert locust in the third instar were released in each enclosure one day before the application. Observations on mortality were done in the enclosures and in cages for a close survey. Sporulation of the fungi was also followed on dead nymphs. Results were analyzed with Genstat software using ANOVA, Student-Newmann-Keuls test and Standard error of mean. In the field enclosures with afternoon application, nymph mortality with 25 g of $GM.ha^{-1}$ was significantly ($P \leq 0.05$) higher than those with 12,5 g of $GM.ha^{-1}$. Mortalities were significantly ($P \leq 0.05$) higher with afternoon application for any dose and in cages left under the tree shade. There was no significant ($P \leq 0.05$) difference on mortality in cages for a given moment of the day. The fungus sporulated on dead nymphs from field enclosures and cages. So, afternoon applications of Green Muscle may increase mortality rate of Desert locust nymphs.

Keywords: Biopesticide, pheromone, locust control, Niger.

INTRODUCTION

In the Sahel, agricultural production is limited by adverse climatic conditions, soil poverty and crops pests among which is Desert locust *Schistocerca gregaria* (Forskål, 1775). This species remains a major constraint to food security and social stability for many rural populations living from agriculture with climatic elevated risk (Lecoq, 2004). Since the beginning of activities in agriculture, humanity has faced this pest which can lead to enormous swarms in its gregarious form, infesting near to 20% of the world, which means 28 km^2 (Magor, 1993). Desert locust has been regularly controlled by the utilization of enormous quantities of synthetic pesticides which are easy to use and shows a destructive and rapid effect. During the last invasion from 2004 to 2005, 13 million liters of organophosphate pesticide were applied in 22

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countries on about 13 million hectares (FAO, 2007).

For an environmentally and sustainable approach to locust control, the biopesticide Green Muscle®, which is made with spores of the entomopathogenic fungi, *Metarhizium acridum*, was developed and put in the market by the project of Biological control of Locusts and grasshoppers (LUBILOSA, 2004). Later on, Hassanali et al. (2005) discovered that Phenylacetonitrile (PAN), a pheromone produced by the adult male of the desert locust has effects on the behavior of Desert locust and it sensitivity to insecticides. In spite of the advantages of the biopesticide Green Muscle®, environmental factors deeply influence its effectiveness. Temperature and humidity act particularly on the spores' viability and on their capacity to penetrate the host. Taking into account these factors is necessary for an optimal utilization of the biopesticide. The objective of this work is to identify the best time of the day to apply Green Muscle® with and without PAN in order to control Desert Locust nymphs in an ecologically and economically way.

MATERIALS AND METHODS

Experimental design

The trial was conducted in Niger in the irrigated farm of the AGRHYMET Regional Centre (ARC) (13°29'985''N; 2°6'151''E). Temperature, humidity, wind speed and direction and rainfall were monitored with the meteorological station of the Centre.

The experimental design was a Complete Randomized Design with 10 treatments in 3 replicates. An experimental plot consisted of a field enclosure of 20 m^2 (5 x 4 m) with millet plants in the stem elongation stage (Figure 1). Two hundred and forty (240) nymphs of third instar (L_3) were released in each field enclosure before application of the following five treatments that were applied in the morning at 9:00 am and in the afternoon at 5:00 pm, with a MICRON ULVA Plus sprayer with a 20 ml nozzle.

- 50 g of Green Muscle® (GM) per ha which is the standard recommended dose
- 25 g of GM per ha,
- 25 g of GM + 10 ml of Phenylacetonitrile (PAN) per ha
- 12.5 g of GM per ha
- 12.5 g of GM + 10 ml of PAN per ha.

Nymphs were released in the morning for afternoon applications and in the afternoon of the day before for the morning applications. At the time of application, the wind speed was controlled with a wind speed meter and oil sensitive papers were displayed in the enclosures to verify the quality of the droplets distribution and the application. After treatment applications, 40 nymphs were collected from each enclosure and kept in 2 cages (20 for each). Both cages were left in the experimental area, one of them under the sun near the field enclosure and the other one in the shade of a tree. Field enclosures were kept under surveillance from sunrise to sunset in order to minimize predation by birds.

Data collection and analysis

Daily observations were made at 08:00 am, 12:00 and 05:00 pm. Mortality of nymphs in the field enclosures and in the cages were noted. Observations in the enclosures were done until fledging start, 21 days after application of treatments. At that time, remaining nymphs were transferred to cages where observations were continued. But this is not considered in this study due to its objective. Dead nymphs were collected and dried for 3 days and then kept for observation for sporulation in Petri dishes on a moisturized filter paper. Sporulation was monitored 4 days later.

Statistical analysis was performed with Genstat software, using analysis of variance (ANOVA), with the Student-Newman-Keuls test at $\alpha = 0.05$ for means separations. Standard error of mean was used for error bars in the figures.

RESULTS

Temperature and humidity

Mean temperature were between 25 °C and 31 °C while mean relative humidity was between 52% and 84%.

Droplets counting on oil-sensitive papers

Mean number of droplets on the oil-sensitive papers varied from 10 to 16 per cm^2 according to treatments and they were not significantly different (Table 1).

Mortality of Desert Locust nymphs

Even though nymph mortality was monitored three times per day, cumulative mortality for some days were reported and analyzed.

Mortality in the field enclosures

Mortality started in the field enclosures 3 days after treatment application. But the mortality rates were less than 10% before Day_9 for most of the treatments. Mortality in the field enclosures were reported till Day_{21} when fledging starts (Table 2).

For morning applications, mortalities varied between 21.1%, for 12.5 g of $GM.ha^{-1}$ and 34.5% for 50 g of $GM.ha^{-1}$. No significant difference was pointed out between mortalities according to treatments.

For afternoon applications, the highest cumulative mortality at Day_{21} was 60.5% for 25 g of $GM.ha^{-1}$. The smallest cumulative mortality was 17.3% and it was observed with 12, 5 g of $GM.ha^{-1}$. Significant differences were noted from Day_{12} to Day_{21}. Mortalities with 12.5 g of $GM.ha^{-1}$ were significantly lower than others. The highest mortalities with 25 g of $GM.ha^{-1}$ from Day_{12} to Day_{21} were not statistically different from these of 50 g of GM. ha^{-1} and also from these of 25 g of GM + 10 ml of PAN per ha ; they were nevertheless higher than these with 12.5g of $GM.ha^{-1}$. Also nymph mortality is statistically higher when applications of 25 g of $GM.ha^{-1}$ were made in the afternoon in comparison with these made in the morning (Figure 1).

Mortality in the cages

Mortality of nymphs in the cages started also 3 days after application but it was very low till $Day_{12.}$ In Table 2 and Table 3 mortalities in cages under the sun and these in cages in the tree shade are presented.

There is no significant ($P \leq 0.05$) difference in the mortalities of nymphs in the cages according to treatments. So neither GM doses nor PAN has significant effect on the mortality in cages when applications were done in the morning or in the afternoon. But mortalities in cages under the sun are significantly lower than these in cages under the tree shadow from Day_{12} to Day_{21} (Figure 2)

At day_{21}, in cages kept under the sun, mortality for morning application varied between 26.7% with 25 g of GM. ha^{-1} + 10 ml of PAN per ha and 43.3% with 12.5 g of GM. ha^{-1} + 10 ml of PAN per ha. But if applications were made in the afternoon, mortalities in these cages varied between 33.3% with 12.5 of $GM.ha^{-1}$ and 56.7% with 50 g of $GM.ha^{-1.}$ No significant difference was pointed out in mortalities from different treatments in cages kept under the sun whenever applications were made in the morning or in the afternoon (Figure 3). In cages kept under the tree shadow, cumulative mortality for morning applications on Day_{21} varied between 26.7% with 25 of GM + 10 ml of PAN per ha and 43.3% with 12.5 g of GM + 10 ml of PAN per ha. If applications were made in the afternoon, mortalities varied between 46.7% with 25 g $GM.ha^{-1}$ and 66.7% with 50 g of $GM.ha^{-1}$. In these cages, mortalities of DL nymphs from Day_{12} to Day_{21} are significantly lower when application is done in the morning in comparison with these for application done in the afternoon (Figure 4).

Sporulation from dead nymphs

Results on percentage of sporulation for morning and afternoon applications are shown in Figure 5, Figure 6 and Figure 7 for dead nymphs from the field enclosures, cages under the sun and cages under the tree shade respectively.

On dead nymphs from the field enclosures, sporulation rate is higher when application is done in the afternoon than when it is done in the morning for treatments with 25 g GM, 12.5 g GM and 12.5 g GM + PAN (Figure 5). For dead nymphs from cages under the sun, sporulation is significantly higher in dead nymphs from afternoon applications than these in dead nymph from morning applications except for the treatment with 25 g of GM.ha^{-1} (Figure 6). In cages under the tree shade it is only for the treatment with 25 g GM + PAN that there is no significant difference between sporulation rates on dead nymphs from morning and afternoon applications (Figure 7).

Photo 1: Field enclosures to run experiments with Desert Locust nymphs.

Table 1: Droplets quality control on oil sensitive papers.

Treatments	Number of droplets.cm^{-2}
T_1 : 50 g GM/ha (morning)	14.25
T_2 : 50 g GM/ha (afternoon)	12.33
T_3 : 25 g GM/ha (morning)	10.01
T_4 : 25 g GM/ha (afternoon)	11.14
T_5 : 25 g GM+10ml PAN/ha (morning)	12.86
T_6 : 25 g GM+ 10 ml PAN/ha (afternoon)	15.66
T_7 : 12.5 g GM/ha (morning)	11.72
T_8 : 12.5 g GM/ha (afternoon)	12.29
T_9 : 12.5 g GM+10 ml PAN/ha (morning)	10.01
T_{10} : 12.5 g GM+ 10 ml PAN/ha (afternoon)	13.83
F pr value for treatment effect	0.321
F pr value for time effect	0.269
F pr value for treatment*time effect	0.555

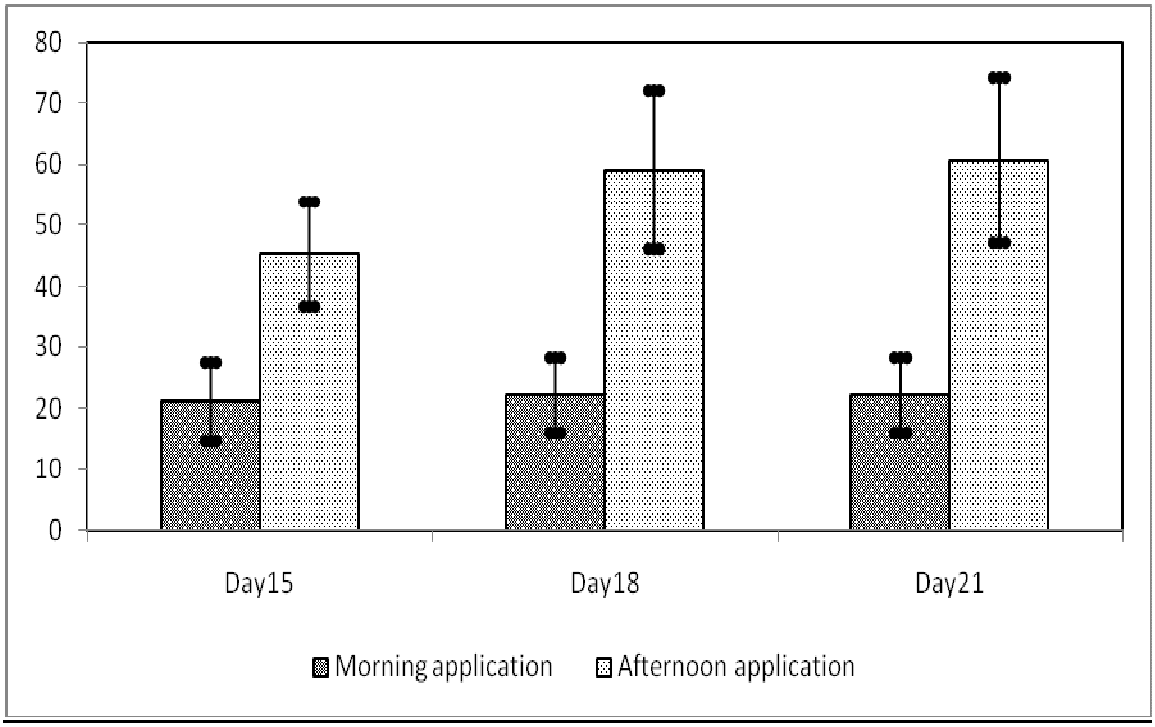

Figure 1: Mortality of desert locust nymphs in the field enclosures with 25 g of GM.ha^{-1}.

Table 2: Cumulative mortality of Desert locust nymphs (%) in the Field enclosures.

Treatments	Day and application time									
	Day_9		Day_{12}		Day_{15}		Day_{18}		Day_{21}	
	morning	afternoon	morning	afternoon	morning	afternoon	morning	afternoon	morning	afternoon
50 g GM/ha	10,4 a	6,7 a	24,6 a	19,0 ab	31,7 a	38,8 a	34,5 a	44,1 ab	34,5 a	48,3 ab
25 g GM/ha	2,9 a	5,3 a	8,6 a	21,8 a	21,1 a	45,3 a	22,1 a	59,1 a	22,1 a	60,5 a
(25 g GM + 10 ml PAN)/ha	6,6 a	10,4 a	16,2 a	16,5 ab	21,1 a	22,5 ab	21,1 a	26,8 ab	21,1 a	26,8 ab
12,5 g GM /ha	4,8 a	2,4 a	6,9 a	6,0 b	17,0 a	13,4 b	22,3 a	17,3 b	22,3 a	17,3 b
(12,5 g GM + 10 ml PAN)/ha	7,6 a	10,8 a	15,8 a	18,1 ab	21,2 a	25,3 ab	22,3 a	38,3 ab	22,3 a	40,4 ab

In a column, means followed by the same letter are not significantly different at P = 0.05 (SNK test).

Table 3: Cumulative mortality of Desert locust nymphs (%) in the cages under the sun.

Treatments	Day and application time									
	Day_9		Day_{12}		Day_{15}		Day_{18}		Day_{21}	
	morning	afternoon	morning	afternoon	morning	afternoon	morning	afternoon	morning	afternoon
50 g GM/ha	3,3 a	5,0 a	8,3 a	23,3 a	26,7 a	35,0 a	30,0 a	51,7 a	30,0 a	56,7 a
25 g GM/ha	8,3 a	5,0 a	16,7 a	8,3 a	25,0 a	20,0 a	26,7 a	35,0 a	35,0 a	38,3 a
(25 g GM + 10 ml PAN)/ha	6,7 a	11,7 a	8,3 a	21,7 a	20,0 a	33,3 a	23,3 a	40,0 a	26,7 a	50,0 a
12,5 g GM /ha	3,3 a	0,0 a	15,0 a	3,3 a	21,7 a	10,0 a	30,0 a	16,7 a	30,0 a	33,3 a
(12,5 g GM + 10 ml PAN)/ha	1,7 a	1,7 a	10,0 a	16,7 a	21,7 a	26,7 a	25,0 a	28,3 a	43,3 a	35,0 a

In a column, means followed by the same letter are not significantly different at P = 0.05 (SNK test).

Table 4: Cumulative mortality of Desert locust nymphs (%) in the cages under the tree shade.

Treatments	Day and application time									
	Day_9		Day_{12}		Day_{15}		Day_{18}		Day_{21}	
	morning	afternoon	morning	afternoon	morning	afternoon	morning	afternoon	morning	afternoon
50 g GM/ha	18,3 a	26,7 a	23,3 a	50,0 a	36,7 a	61,7 a	38,3 a	66,7 a	38,3 a	66,7 a
25 g GM/ha	10,0 a	16,7 a	21,7 a	35,0 a	38,3 a	46,7 a	43,3 a	46,7 a	45,0 a	46,7 a
(25 g GM + 10 ml PAN)/ha	28,3 a	38,3 a	33,3 a	53,3 a	36,7 a	58,3 a	36,7 a	60,0 a	38,3 a	60,0 a
12,5 g GM /ha	10,0 a	13,3 a	20,0 a	31,7 a	33,3 a	41,7 a	38,3 a	56,7 a	45,0 a	56,7 a
(12,5 g GM + 10 ml PAN)/ha	10,0 a	18,3 a	16,7 a	30,0 a	20,0 a	43,3 a	21,7 a	46,7 a	25,0 a	48,3 a

In a column, means followed by the same letter are not significantly different at P = 0.05 (SNK test).

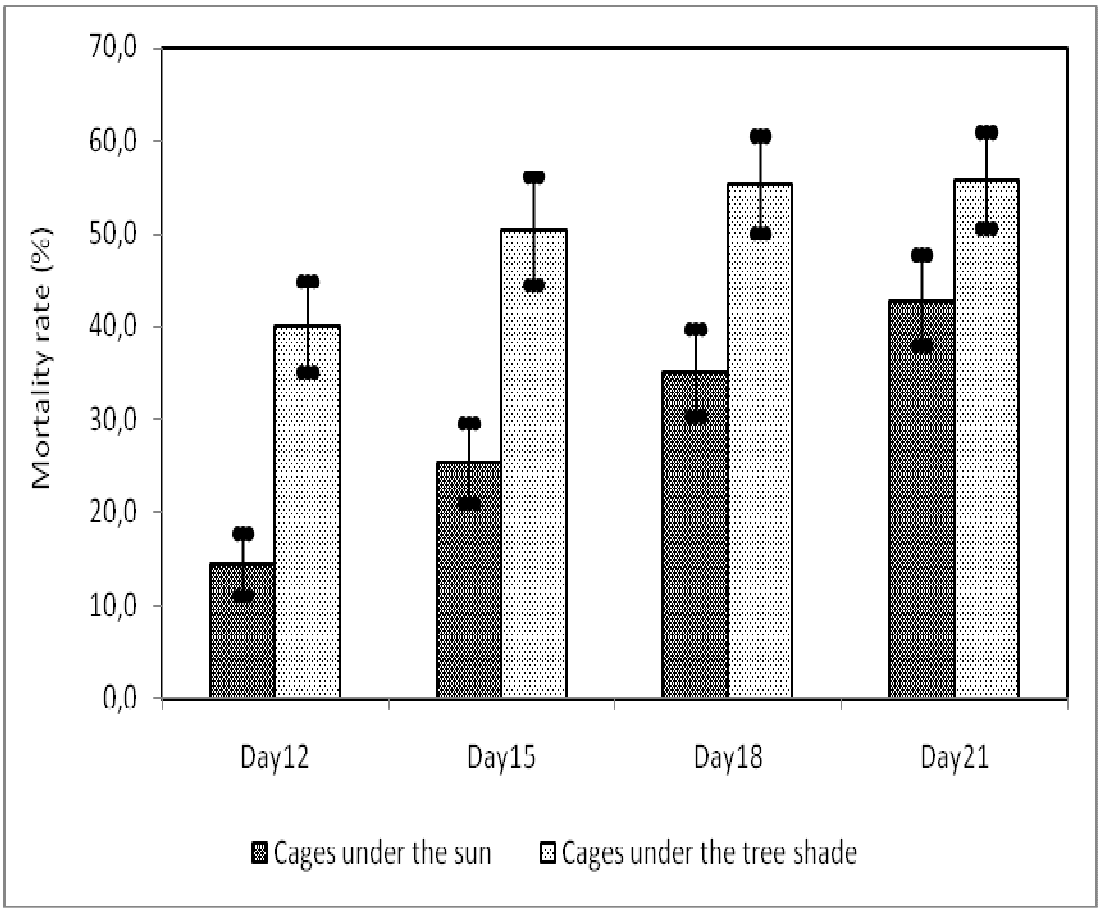

Figure 2: Cumulative mortality of Desert Locust nymphs in cages.

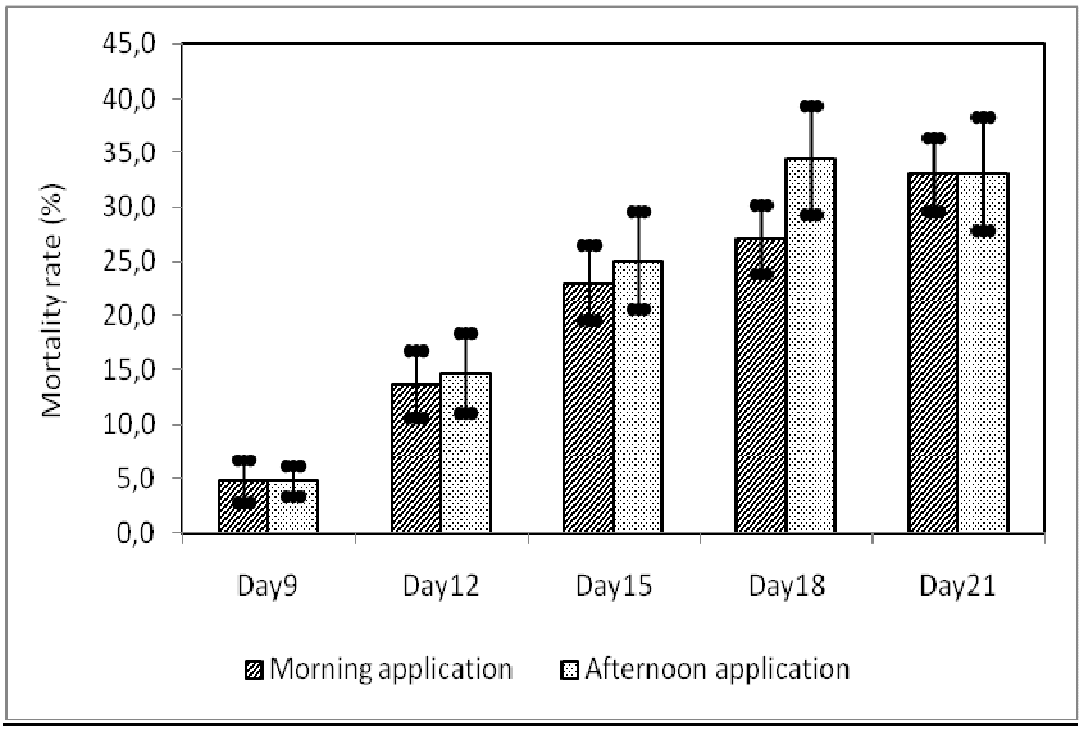

Figure 3: Mortality of DL nymphs in cages under the sun.

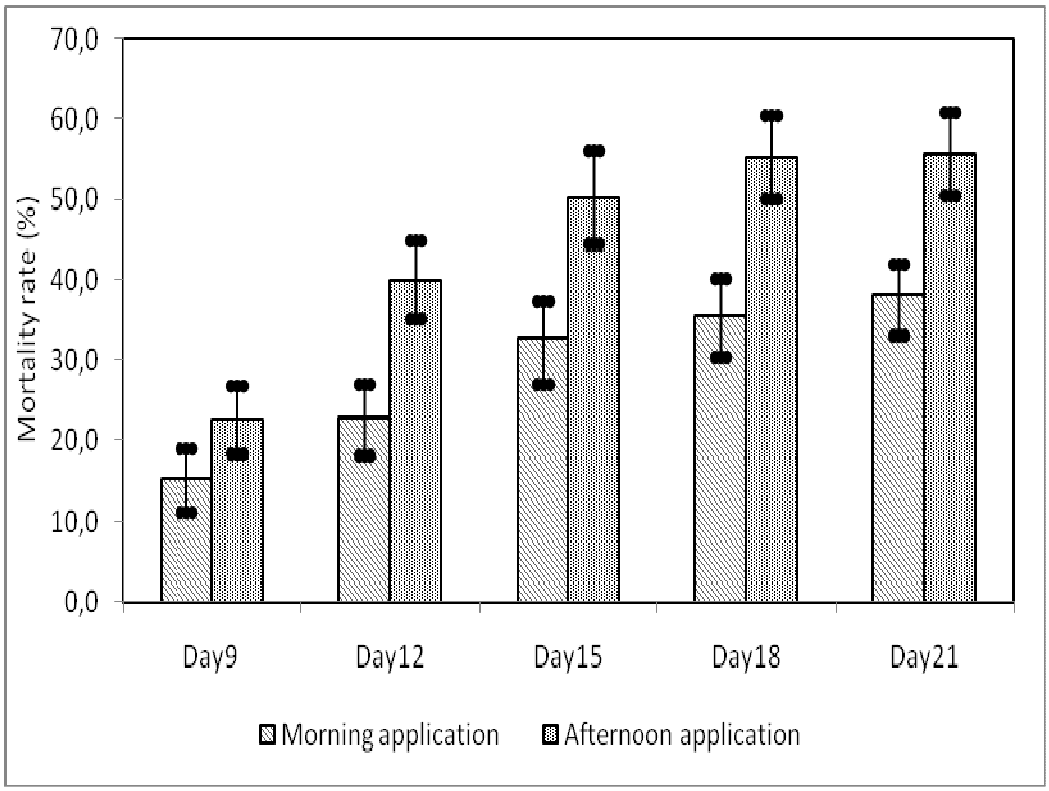

Figure 4: Mortality of DL nymphs in cages under the tree shade.

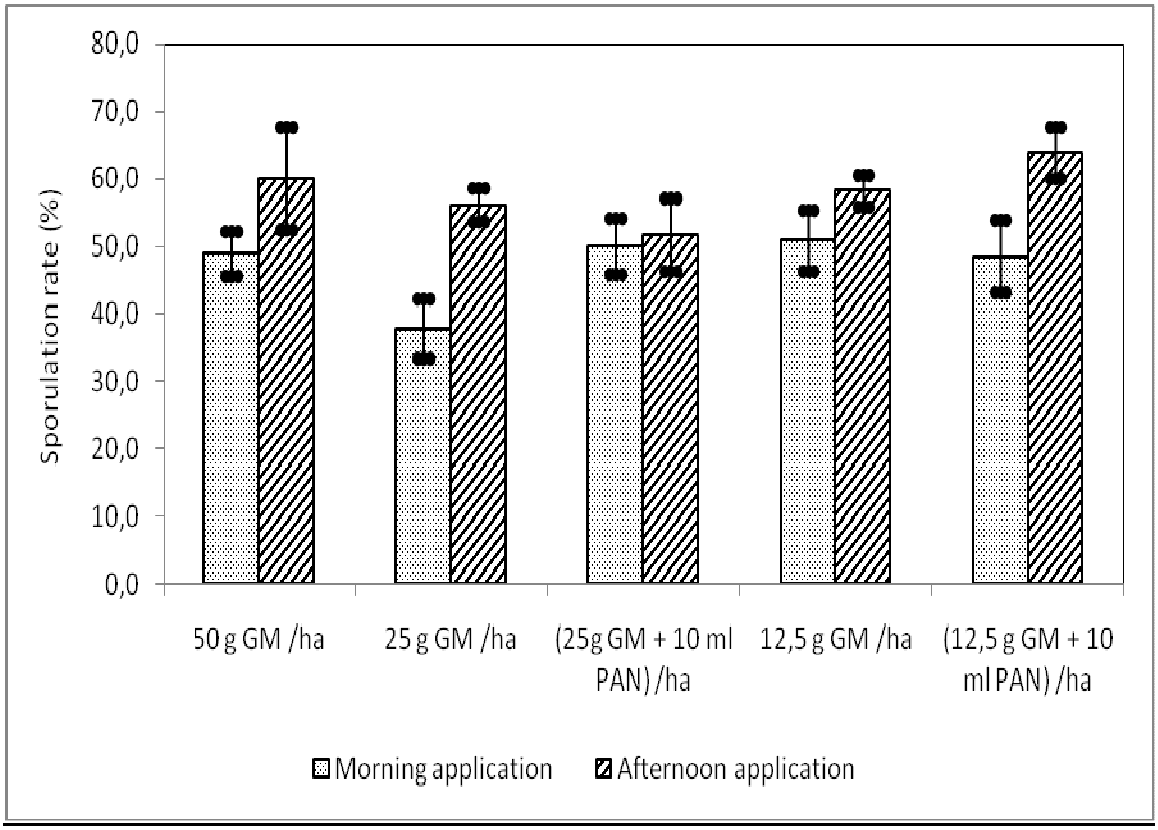

Figure 5: Sporulation on dead nymph of desert locust from field enclosures.

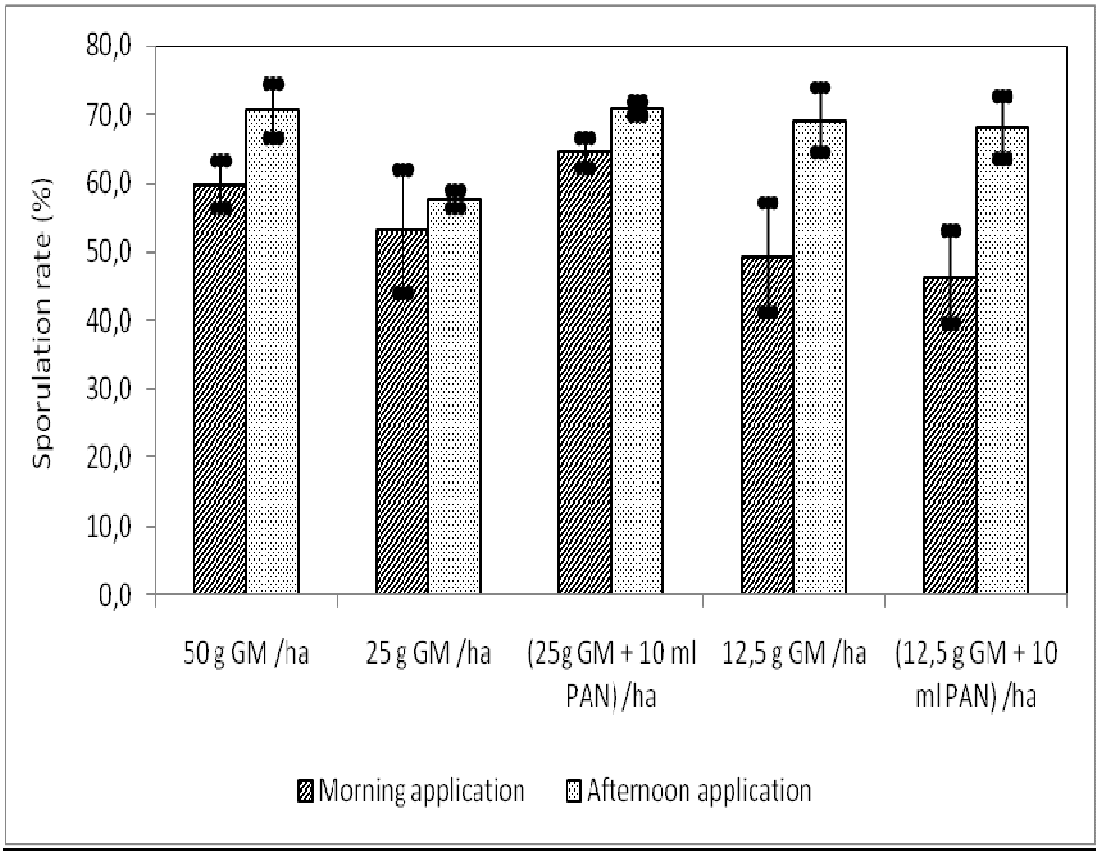

Figure 6: Sporulation on dead nymphs of Desert Locust from cages kept under the sun.

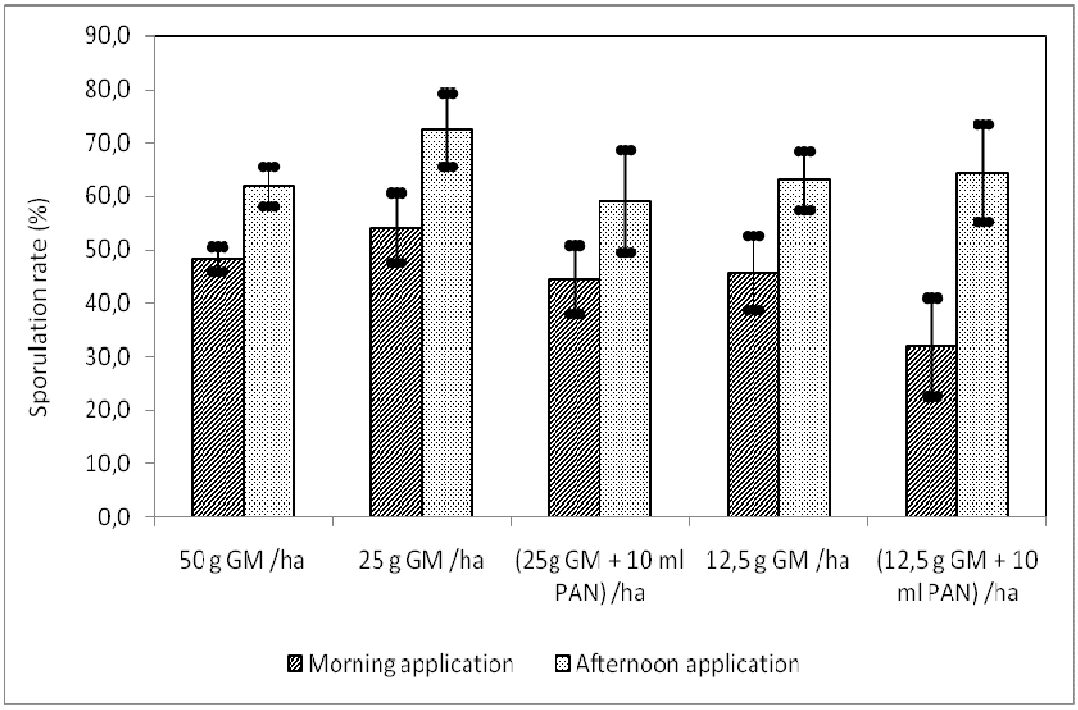

Figure 7: Sporulation on dead nymphs of Desert Locust from cages kept under the tree shade.

DISCUSSION

Temperature and humidity

Climatic conditions such as temperature and relative humidity during the experiment were in favor of the fungi development. The best temperature for *M. anisopliae* varies between 25 °C and 35 °C according to Welling et al. (1994), and between 15 °C and 35 °C, according to FAO (2005). Temperature at 9:00 am the day of treatment applications was 25 °C ; it increases till 33 °C at 5:00 pm before it shut down to 20 °C at 8:00 am the day after.

Droplets counting on oil-sensitive papers

The lack of difference between mean number of droplets on the oil-sensitive papers according to treatments and time of application means that applications were done in a good way. Also densities of these droplets are acceptable for insects of large size and mobility such as locusts. In fact, according to Dobson (2001), droplets density on oil-sensitive papers during locust control operation varies between 5 and 50 per cm.

Mortalities in the field enclosure and in the cages

The delay on the mortality is conforming to what was found before by Lomer et al. (1997). According to these authors, the first observable mortality in the field generally occurs 7-10 days after application, and the full effects are seen 14-18 days after application. Trials run in Sudan in 2003 by ICIPE under temperature between 25 and 28 °C gave similar results for mortality. Rates of 25 g of GM.ha-1 and 50 g of GM.ha^{-1} have given 30% and 60% mortality respectively (FAO, 2003). But rates of mortality found by Bashir and Hassanali (2000 and Bashir et al. (2010) in Boma and field trials were higher. The reason for the relatively weak mortalities in the field enclosure and cages under the sun for some treatments may be the delay in the disease apparition on the nymphs exposed to the sun. In order to protect themselves from infection, such nymphs typically lay down under the sun (Skovmand et al., 2004).

The reason for differences between morning and afternoon mortalities may be the effect of temperature which can delay or make impossible the development of the fungi when applications are done in the morning. Such situation illustrates the hypothesis from which *M. anisopliae* can take advantage of the favorable temperature to germinate and to develop when it is applied in the afternoon. Data in temperature from the meteorological station have shown a decrease of these temperatures from 5:00 pm; they can reaches 20 °C in the morning of the following day.

Experiments run in Mauritania in 1997 on Desert Locust have revealed similar mortalities in cages in conditions where temperature was between 27 and 32 °C and relative humidity of 22% (Langewald et al., 1997).

Statistical analysis revealed significant differences between mortality in cages under the sun and these under the tree shade. Similar results were also obtained by Ouedraogo (1996) who has pointed out the action of sun rays on *Locusta migratoria migratorioïdes* sprayed with *Metarhizium* and kept in cages in the shade and under the sun. At the rate of 50 g of GM.ha^{-1}, he has obtained 78% mortality of adults in the shadow whereas under the sun the mortality was less than 21%. Exposure to the sun is therefore an important factor. It can stop the expression of the fungi which need moderate temperature (25 °C–32 °C) to develop in an infested host. Putting nymphs in the shade will then enhance the development of the fungi which may be an explanation for the mortality differences observed in cages kept under the tree shade and under the sun.

In general, incubation duration of the fungi is the same no matter the application time of Green Muscle® (morning or afternoon). This duration varies from 5 to 7 days in cages in shade and from 7 to 9 days in cages under the sun. Experiments run in Niger in 1998 by Crop Protection Direction and IITA have pointed out the long duration of incubation of the fungi (7 to 12 days according to the species of acridid); this is not in favor to the biopesticide adoption. According to the fact that rapid effect is a fundamental criterion to choose an insecticide, it will be advisable to work on how to increase the virulence and reduce the incubation duration by selecting strains so adapted (Maïga et al., 1998).

Sporulation

Results on sporulation showed that in most cases nymphs were killed by Green Muscle®. Sporulation evidence is taken as a proof that mortality is due to *Metarhizium* (Kooyman et al., 1997). Sporulation was observed in dead nymphs. Reasons for the lack of sporulation on some dead nymphs may be because they are killed by predators (ants and birds) which were abundant in the locality or because of the relative humidity in the incubating Petri dishes. Some dead

nymphs revealed signs of the fungi attack (red color of the tegument) without sporulation during incubation. The other reason most probably is due to the fact that the infected nymphs were feeding less than usual (Manuel et al., 2007; Ishraga and Magzoub, 2009). It may also be due to that they are able to get rid of the fungus through phenomenon known as behavior fever which was demonstrated for the first time by Elliot et al. (2002). In this case, infected locust can raise their body temperatures to fever levels. This helps in killing the fungus inside their body. Bacterial gut infection is also reported to inhibit *Metarhizium* and gut-passage through conventional insects can reduce viability of conidia of *M. anisopliae* by over 50% (Dillon and Charnley, 1986).

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