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Effect of Vegetation Height and Cover on the Viability of *Metarhizium acridum* Conidia Used in the Control of the Desert Locust Nymphs (*Schistocerca gregaria*) (Forskål)

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Abstract: The effect of plant height and density of vegetation cover (millet) on the efficacy and residual effect of *Metarhizium acridum* conidia (Green Muscle®) in controlling Desert Locusts was evaluated in semi-field conditions inside large cages of 2x2x1 m. A mixed population of third (L3) and fourth (L4) instars of nymphs was used as target. The study was organized in two trials, separately carried out according to the plants height: Trial I, on short vegetation (27-37 cm) and Trial II, on tall vegetation (73-93cm). In both trials, the insects were treated in two different vegetation covers: low (~10 %) and high (~100 %). A dose rate of 2.5 x 10^{12} conidia/ha was used with two different application volumes: 1 and 2 L/ha. The efficacy of the biopesticide was evaluated by mortality rate of treated nymphs followed during two weeks. On the other hand, untreated nymphs released in cages with treated vegetation were used to evaluate the effect of residual conidia in the two different vegetation states. No significant influence of vegetation cover on the efficacy of *Metarhizium* was observed in short vegetation ($p = 0.828$) or in tall vegetation ($p = 0.334$) when a volume rate of 1 lire per hectare was used. In high vegetation cover, the increase of the applied volume rate to 2 liters per hectare significantly improved mortality rate. Also, conidia were alive six days after treatment with good effect on untreated nymphs released on treated vegetation. For the same period, no residual effect was observed in

short vegetation. The results of this study show the importance of vegetation height (important mass of vegetation) for good efficacy and persistence of *M. acridum* conidia. For short vegetation, even with high cover, *Metarhizium* conidia are more exposed to the sun radiation and, therefore, quickly lose viabilities.

Key Word: Desert Locust; nymphs; plant cover, *Metarhizium;* viability.

INTRODUCTION

In the current context of environmental protection, the use of Green Muscle® (GM) in Desert Locust control can reduce the negative impact of conventional chemical pesticides on the environment and non-target species. GM is a mycopesticide consisting of conidia of the fungus *Metarhizium acridum* [=*Metarhizium anisopliae* var. *acridum* (Bischoff *et al*. 2009]. Over the last few years, GM has been particularly promising and has received much attention for the control of Desert Locust. It is regarded as being of low risk to humans and livestock, while having few effects on non-target organisms (FAO 2001). The Food and Agriculture Organization (FAO) listed GM as a biopesticide recommended against the Desert Locust in a dose rate of 50 g/ha, equivalent to 2.5×10^{12} conidia/ha (FAO 2004). Under field conditions, this dose is relatively robust and is expected to provide adequate control under favorable and moderate environmental conditions (Van der Valk 2007).

Despite its slow action in comparison with chemical insecticides, *M. acridum* shows real potential for the control of Desert locust nymphs in the field (Kooyman and Godonou 1997). Three key abiotic factors determine its effectiveness: temperature, amount of free moisture for initial germination of conidia and the rate of solar radiation reaching the conidia (Blanford and Klass 2004). Direct exposure of conidia to solar radiation for one day could affect the viability of conidia (Scherer *et al*. 1992; Moore *et al*. 1993). On the other hand, dry conidia can withstand high temperatures up to 55 C°. With the same dose of solar radiation, the negative effect on the viability of conidia is 2 to 3 times higher with 55 °C than 25 °C (Moore and Morley-Davies 1994). Blanford and Klass (2004) concluded that temperature is the key environmental determinant of control efficacy. According to them, the impact of radiation on conidia

survival in the field does not seem to be an important constraint to the successful use of the pathogen for two reasons: i) the recommended dose provides sufficient amount of conidia to kill in spite of losses due to radiation. ii) Conidia deposited in shaded conditions (as shaded parts of leaves) will be protected. Van der Valk (2007) suggested that, in addition to ambient temperature, thermoregulation by the insect was identified as a key factor influencing the field efficacy of *M. acridum*.

Furthermore, locusts can be exposed to conidia in three ways: direct exposure, secondary pick-up of conidia from treated vegetation and horizontal transmission from infected cadavers. In arid and semi-arid areas, rainfall may be a critical factor for successful horizontal transmission (Arthurs *et al*. 2001).Currently, the targets for the spray droplets are both individual locusts and the vegetation. Secondary pick up from treated vegetation is an important mode of exposure of the insect to conidia (FAO 2007). It is a major if not the most important mode of exposure of locusts and grasshoppers (Van der Valk 2007).Treated vegetation can thus increase the probability of the insect to pick up conidia leading to its death. On the other hand, treated vegetation may infect untreated insects moving into treated vegetation. Under operational conditions, secondary pick-up of conidia from vegetation is essential for enhanced effectiveness of *M. acridum* (Lomer *et al*. 1999). Thomas *et al.* (1997) found that the infection due to residual conidia accounted for 40-50 % of the total infection measured 12 days after application. The importance of secondary pick up is closely related to the length of time that the conidia remain viable (persistence). In this case, it appears that the pathogen must contact a mobile host within 24-48 hours to be effective (Scherer *et al*.1992; Van der Valk 2007). Scanlan *et al*. (2001), modeling the effect of *M. acridum* on migratory locust in Australia, concluded that secondary pick-up from vegetation is important at low to moderate dose rates of the pathogen and at moderate vegetation density. It is likely to become important at lower dose rates and higher vegetation density (Van der Valk 2007).

This investigation was organized at the beginning of the summer season in the Red Sea coast area. It was conducted at the research station of the International Centre of Insect Physiology and Ecology (ICIPE) at Port

Sudan, Sudan. The various treatments were conducted at a farm in Sallom area (19 38 55 N x 37 15 37 E) 28 km South West of Port Sudan. The farm is part of the ICIPE station in Port Sudan.

The main object of the study was to evaluate the effect of vegetation height and cover (density) on the efficacy and residual effect of *M. acridum* conidia applied on Desert Locust nymphs to determine which states are better for it to act properly.

MATERIALS AND METHODS

Insect rearing

Due to lack of natural locust population during the study period, test insects were obtained from the mass rearing facility of Desert Locust at ICIPE field Station. The colony was developed initially from egg pods collected from the field and the laboratory culture was regularly refreshed with new field collections as described by Bashir and Hassanali (2010). Locusts were crowd-reared in wood frame cages of 1 x 0.5 x 0.5 m with wire mesh of 2 mm^2 on the ceiling and three sides of the cage. The door at the fourth side was made of white cloth with a sleeve to enable inspection, easy cleaning, and food supply. The bottom false floor is made of wood with 10 holes to fit ice cream plastic cups (10 cm in diameter) for egg laying. Cages were placed in a room with open windows to allow temperature and humidity conditions to equilibrate with outside conditions. Insects were fed on natural desert plants collected from the field, mainly millet and *Heliotropium* spp, with some *Dipterygium glaucum* and *Crotolaria microphylla*. During shortage of field plants, fresh alfalfa, millet and wheat bran were provided. For heating, 3 light bulbs of 60-W were placed at the top of each cage and switched on for 6 to 8 h daily.

After laying, the plastic cups (with egg pods) were covered with mosquito wire mesh and placed under light bulb of 60-W for 6 to 8 h to accelerate embryonic development and hatching. After hatching, the 1st instars were transferred to new cages designed in the same way as above but with different dimensions (1x1x0.5 m, LxWxH). In these cages, the insects were kept until they reached the 3rd or 4th instar. At this stage, the insects were used for experiments or transferred into large field cages (2x2x2 m)

placed outside for mass rearing under natural conditions. Locusts stay in these outdoor cages until maturation, and are then transferred to laboratory cages for laying.

Cage experiments

The study was conducted under semi field conditions, in wood frames cages of 2x2x1 m with wire mesh of 2 mm^2. These cages, with the bottoms open, were placed on 2 x 2 m plots cultivated with millet. Control plots were placed upwind from the treated ones to prevent contamination during treatments. Plots were cultivated in accordance to vegetation structure required: high and low vegetation covers. For the high vegetation cover, all the plots were sown to give ~ 100% vegetation cover. With low vegetation cover, only ~ 10 % of each plot surface was sown. The effect of vegetation cover was evaluated in two levels of vegetation growth according to plants height: short vegetation (3 weeks after sowing) and tall vegetation (7 weeks after sowing). The height of vegetation was assessed in three randomly defined plots. The average height of vegetation varied from 27.6 ±7.53cm to 37.6±7.07 cm in short vegetation and between 73.7±6.76 cm to 90.7±7.52cm in tall vegetation. In all cases, each plot was replicated three times. The number of the tested locusts in each treatment was 100 fourth and third instar nymphs. These insects were introduced into the cages 24h before treatment.

For sampling, 25x25x25 cm wood frame cages were used for monitoring. To evaluate residual effect and conidia persistence, cages of 50 x 50 x 50 cm with open bottom were used.

Treatments and sampling

A powder formulation of GM with a minimum concentration of 4 x 10^{10} per gram of *M. acridum* conidia produced by Biological Product Control (BCP, South Africa) was used. Fifty grams (the dose used in the study) of this formulation contains approximately 2.5 x 10^{12} conidia (according to the product label). Twenty four hours before treatment, 100 g was weighted directly from the package in two clean containers (50 g in each one). Germination test carried out on the initial formulation gave 91% of viable conidia.

The study was organized separately in two trials and carried out according to the height of vegetation: Trial I (on short vegetation) and Trial II (on tall vegetation) (Table 1). In both trials, mixed populations of L3 and L4 nymphs of gregarious Desert Locust were used. For the Trial I, a number of 120 nymphs (60 L_3 and60 L_4) were placed in each large cage to be treated. Twenty four hours after application, a sample of 20 nymphs (10 L_3 and10 L_4) were taken from each cage and transferred to small cages for monitoring. Because of the lack of L_3 nymphs during the Trial II period, mixed population was formed with 40 L_3 and80 L_4. The nymphs' samples taken from this population were randomly picked.

Treatments were made by hand held battery-driven ULVA+sprayer. Before treatments, the sprayer was calibrated to apply the desired correct volume rate. Two nozzles were used: the orange, for a volume rate of 1 L/ha and the black for 2L/ha. To simulate field application conditions, (because it is difficult to apply the exact volume on 4 m^2 plots), all cages were treated in the same procedure adopted under field conditions, i.e. making one pass with the sprayer which covers approximately $2m^s$. Treatments and replications were randomly distributed among cages.

To evaluate residual effect of conidia on vegetation, 20 untreated nymphs were caged in contact with part of the treated vegetation inside the large cages, one hour after treatment. The nymphs were left for 24 h before they were transferred to monitoring cages. Residual effect of the product was compared with its direct effect on treated nymphs picked from large cages 24 h after treatment and placed in monitoring cages. To evaluate the persistence of conidia under the trials conditions, residual effect was evaluated at 0, 3 and 6 days after treatment. During monitoring period, caged nymphs were fed with uncontaminated vegetation.
To assess the efficacy of treatments, all cages (large and small ones) were daily monitored for mortality records. This was done for monitoring the behavior and activity of treated nymphs and the control ones. As meteorological conditions can greatly influence the efficacy of treatments, speed and direction of wind were registered during the treatment. Daily temperature and relative humidity were recorded every two hours, from 7 AM to 9 PM using digital thermometer.

Table 1. Different treatments design conducted for both trials. Each treatment and the control were replicated three times.

Vegetation height	Vegetation cover	Applied volumes		Controls
		1 L/ha	2L/ha	
Short (Trial I)	High	☒	☒	☒
	Low	☒	☒	☒
Tall (Trial II)	High	☒	☒	☒
	Low	☒	☒	☒

Data analysis

All results were analyzed using SPSS 15.0 for Windows. Mean mortality was subjected to logarithmic transformation (log_{10}) to stabilize the variance and analyzed using analysis of variance (ANOVA).

RESULTS

Meteorological data

Figure 1 presents average meteorological data (temperature and relative humidity) taken during the trials period. During treatments, wind speed ranged between 2 and 4.8 m/s in Trial I, and between 2.5 and 4.1 m/s in Trial II. The study was conducted in July at the beginning of the summer season. The weather conditions became more and more warm. Generally, the conditions were more favorable for *M. acridum* efficacy during the Trial I period (on short vegetation). Mean daily temperatures recorded were between 21 and 27°C, with a maximum of 42°C, and a minimum of 21°C. The relative humidity was between 21 and 58%. On the other hand, the weather conditions during Trial I period (on high vegetation) were hotter with 33 to 38°C as mean temperature. During the monitoring period, maximum temperatures exceeded 40°C in almost all days. The relative humidity recorded was between 14 and 30% in average.

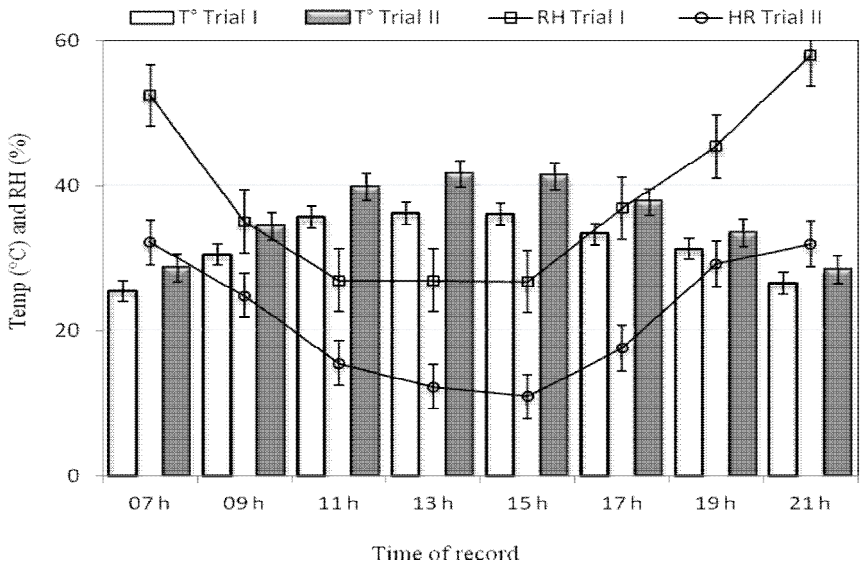

Figure 1. Mean temperature and relative humidity records during trials period in 1 m below soil surface level

Treatment efficacy

Trial I: In short vegetation

No influence of the vegetation cover on the efficacy of *M. acridum* conidia was observed in the short vegetation. With both applied volumes, nymph's mortality was statistically similar after two weeks in low and high vegetation cover (F=0.295; P=0.828). In spite of favorable temperatures, the mortality rate was low with less than 60 % dead nymphs (Figure 2).

On the other hand, samples collected from large cages and placed in shade showed a mortality rate of 100 % within one week. No significant difference was observed between treatments on both vegetation covers (Table 2).

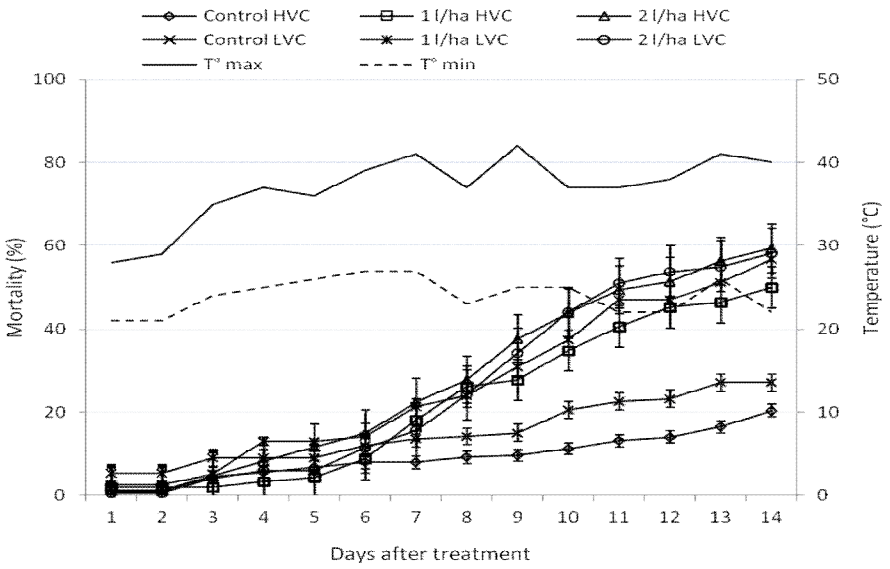

Figure 2. Cumulative mortality of Desert Locust nymphs on short vegetation with maximum and minimum temperatures recorded during the trial period. HVC and LVC: high and low vegetation covers

Table 2. Mean percent mortality ±SD of samples of nymphs collected from large cages 24 h after treatment on short vegetation and kept in monitoring cages under shade

		Mean mortality (%)±SD						
Days		1	2	3	4	5	6	7
Control		0±0[a]	0±0[a]	6.8±5.9[a]	10±0.3[a]	14±6.4[a]	15±5.5[a]	20±5.7[a]
		1 liter/ha						
Vegetation cover	high	0±0[a]	1.8±3.2[a]	5.8±5.6[a]	33±13[a]	88±0.7[c]	100±0[b]	-
	low	0±0[a]	0±0[a]	5.3±5.1[a]	36±9.1[a]	96±3[d]	98±3[b]	100±0[b]
		2 liters/ha						
	high	0±0[a]	2.1±3.6[a]	4.2±7.2[a]	29±18[a]	80±6.6[b]	100±0[b]	-
	low	0±0[a]	3.9±6.4[a]	7.8±6.1[a]	39±11[a]	100±22[d]	-	-

Means followed by the same letter within the same column are not significantly different ($p > 0.05$).

The rate of mortality observed in the large cages cannot be entirely attributed to the product, because almost all nymphs died without clear sign of infection by the fungus. Furthermore, it was noted that many nymphs were cannibalized. Also, the remaining parts of carcass were taken by ants. On the other hand, the high efficacy observed in nymph samples kept in shade shows that nymphs irrespective of the dose of infection, were invaded by the fungus. In the large cages, erected under natural conditions, there must be enough inoculum that the infected nymphs cannot get rid of through basking under the sun rays (Figure 2).

Residual effect and persistence of conidia

Figure 3 presents mortality rate of untreated nymphs kept on treated vegetation for 24 h at 1 hour, 3 and 6 days after treatment. In trial condition, no difference in mortality rate was observed between treated and untreated nymphs kept on treated vegetation during the first 24 h after treatment. Until 3 days after treatment, conidia did persist and have great ability of infection. On the other hand, no residual effect was observed at day 6 after treatments. Increase of applied volume did not improve residual effect of conidia (Figure 4).

Trial II: High vegetation

No significant effect of the vegetation cover was observed in tall vegetation ($F = 1.320$; $p = 0.334$).Despite the hot conditions in Trial II period, the effect of products was high. In all treatments, mortality rate of treated nymphs reached 80% to 95 % after two weeks. Almost all dead nymphs in treated cages revealed clear and typical symptoms of infection by *Metarhizium* (Figure 5). The speed and level of mortality was more obvious with the volume rate of 2 L/ha on high vegetation cover. Ten days after treatment, mortality rate reached 81 % and58 % in 2 and 1 L/ha applied volume rate ($p < 0.05$). Untreated nymphs remained very active until the end of the monitoring period.

Regardless of vegetation cover, samples collected from large cages and placed in shade showed similar effect after 8 days (97% to 100% of mortality ($P>0.05$))(Table 3).

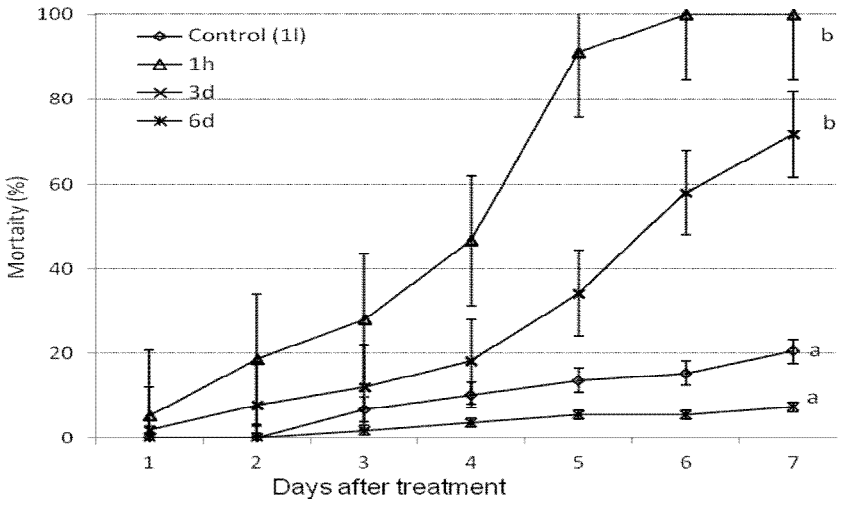

Figure 3. Mean mortality of untreated Desert Locust nymphs kept on vegetation treated with 1 L/ha volume rate at different times after treatment. Curves with same letter are not significantly different ($p < 0.05$)

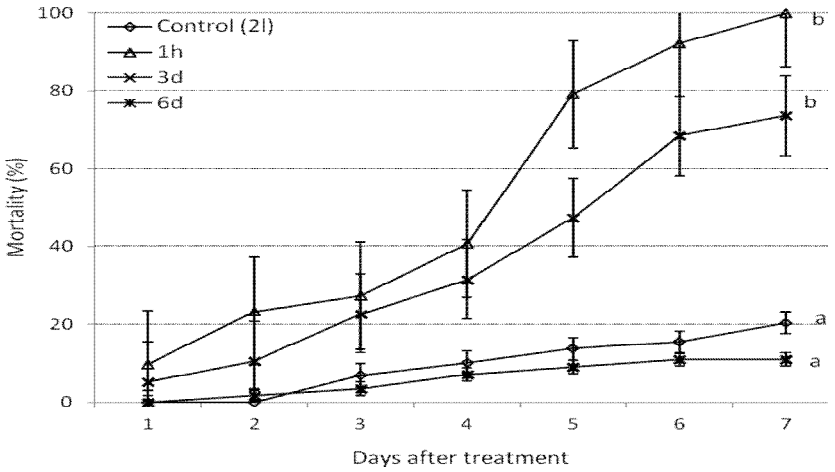

Figure 4. Mean mortality of untreated Desert Locust nymphs kept on vegetation treated with 2 L/ha volume rate at different times after treatment. Curves with same letter are not significantly different ($p < 0.05$)

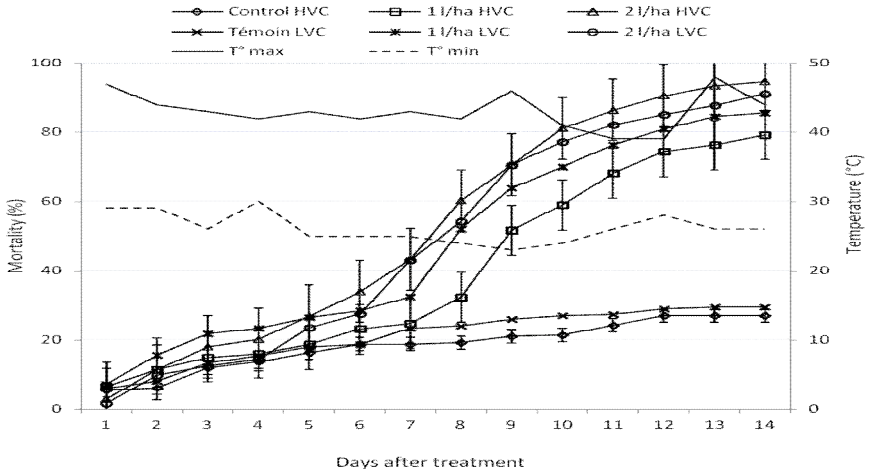

Figure 5. Cumulative mortality of Desert Locust nymphs on tall vegetation with maximum and minimum temperatures recorded during the trial period. HVC and LVC: high and low vegetation covers

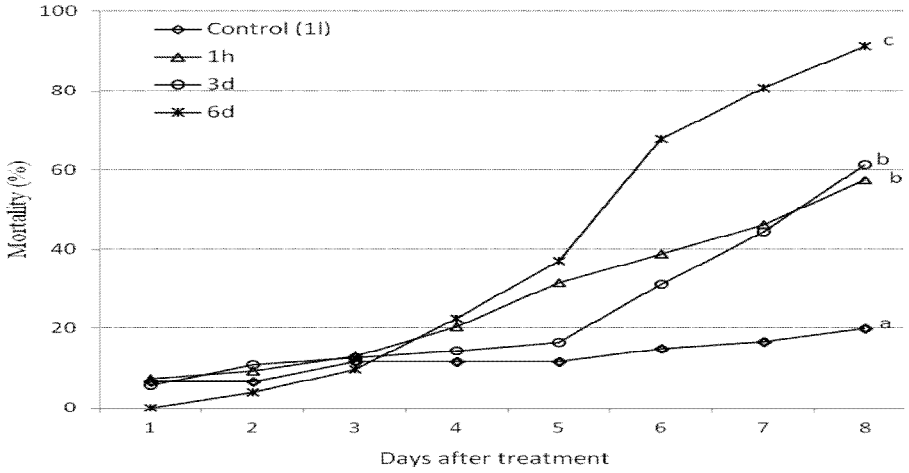

Figure 6. Mean mortality of untreated Desert Locust nymphs kept on vegetation treated with 1 L/ha volume rate at different times after treatment. Curves with same letter are not significantly different ($p \geq 0.05$).

Table 3. Mean% mortality ±SD of samples of nymphs collected from large cages 24 h after treatment on tall vegetation and kept in small cages under shade

		Mean mortality (%)±SD							
Days		1	2	3	4	5	6	7	8
Control		6.7±2.9[a]	6.7±2.9[a]	12±5.8[a]	12±5.8[a]	12±5.8[a]	15±5[a]	17±5.7[a]	20±5.7[a]
Vegetation cover	**1 liter/ha**								
	high	9.4±3.4[a]	11.2±1.1[a]	16±4.3[a]	27±13[a]	36±14[b]	75±14[b]	91±8.3[b]	98±3.5[b]
	low	7±2.6[a]	11±4.7[a]	11±4.7[a]	14±7.6[a]	44±11[b]	81±3.8[b]	96±3.1[b]	98±2.9[b]
	2 liters/ha								
	high	10.5±13[a]	12.5±14[a]	17±8.4[a]	26±13[a]	51±17[b]	81±12[b]	95±4.8[b]	97±4.8[b]
	low	10±6.7[a]	12±3.6[a]	14±3.6[a]	29±7.2[a]	60±14[b]	82±16[b]	100±11[b]	100±0[b]

Means followed by the same letter within the same column are not significantly different ($p < 0.05$).

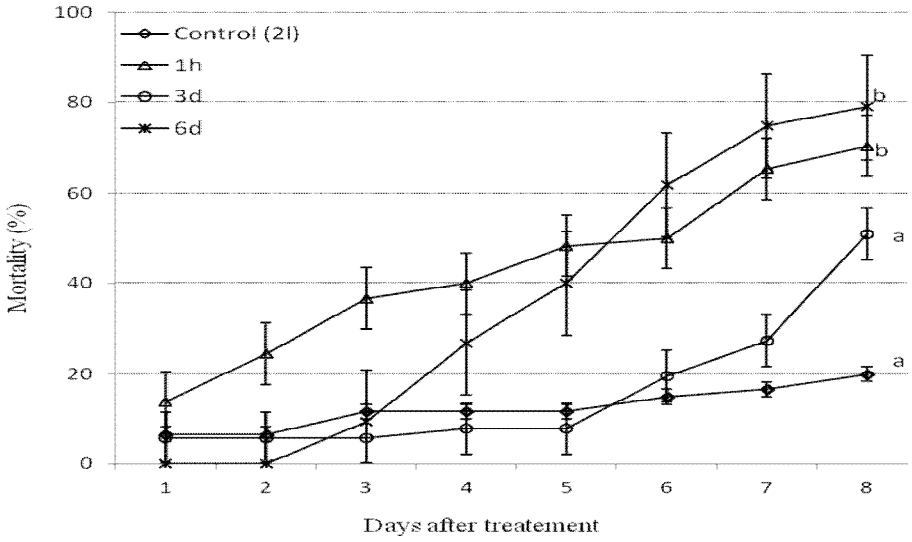

Figure 7. Mean mortality of untreated Desert Locust nymphs kept on vegetation treated with 2 L/ha volume rate at different times after treatment. Curves with same letter are not significantly different ($p > 0.05$)

The effect of *Metarhizium* on behavior and activity of treated nymphs was clearly observed from day 5. The time spent by the insect in basking increased in the treated nymphs compared with untreated control. A reduction in nymphs' activity and feeding resulted in low vegetation consumption clearly observed in the treated plots.

Residual effect and persistance of conidia

Dispite the hot conditions,conidia remained active with good efficacy 6 days after treatment. Untreated nymps kept in treated vegetation after this periode showd clear *M. acridum* symptoms with mortality rate of more than 80 %.This clearly indicates that conidia could remain viable under these conditions (Figures 6 and 7).

DISCUSSION

The results of this study indicate that good efficacy of *M. acridum* conidia requires an appropriate vegetation mass (tall vegetation). In this case conidia are more protected against direct exposure to solar radiation. The conidia persist and remain viable to infect new nymphs and increase the dose received by those already infected. On the other hand, vegetation cover has no effect on the efficacy of the conidia. If the nymphs stay for a long time in contact with treated vegetation, even with low vegetation cover (~10 %), the insects could accumulate sufficient amount of conidia by secondary pick-up from contaminated vegetation. In this case, the efficacy of treatment is determined by other factors such as weather conditions.

Despite favorable conditions, the effectiveness of the product was less evident on short vegetation (Trial I). That can be explained only by the damage caused by direct exposition of conidia to solar radiation. In short vegetation, conidia will not be protected against solar radiation; consequently, they rapidly lose viability. During the Trial I period, temperature was more favorable to the fungus than in Trial II (on high vegetation). Average temperatures recorded every two hours from 7 AM to 9 PM were 25 to 36°C in Trial I and 29 to 42 °C in Trial II. Under laboratory conditions, 37 to 39°C were the highest temperature limits for the fungal growth (Ouedraogo *et al.* 1997). Blanford and Klass (2004) concluded that, under field conditions, the optimum temperatures for the

fungal growth are between 27 and 30°C, with the most rapid growth across a temperatures range of 25-32°C.

In short vegetation, the effect of secondary pick-up during the first 24 h, revealed the same effect as direct application with regard to mortality due to *Metarhizium*. Neither the vegetation cover nor the applied volume has a significant effect on mortality level and speed. Caged nymphs in a shade taken from the large cages and the ones kept on treated vegetation the days of treatment, showed high mortality caused by *Metarhizium*. This means that the conidia have reached the target in insufficient amount to cause an effect equivalent to the effect on nymphs exposed under natural conditions. Because in field and semi-field conditions not all the targeted nymphs will come in direct contact with product drift during spraying, secondary pick-up of conidia from treated vegetation is the only way by which conidia reach the nymphs. In this case conidia should persist for appreciable times in treated areas.

In high vegetation (vegetation mass more important) the amount of conidia attached to the vegetation would be more important and conidia are more protected against direct solar radiation. Furthermore, in case of tall vegetation cover, the increase of applied volume rate has improved the speed of mortality and residual effect of conidia during the first 24 h after treatment. Under natural field conditions, conidia appear to remain active in protected locations in the environment as vegetation canopy (Thomas *et al*. 1997). Van der Valk (2007) concluded in his review of the efficacy of *M. acridum* against Desert Locust that the high vegetation cover may reduce direct impact of the spray droplets on the insects. Secondary pickup of conidia from vegetation may also be reduced because the product is diluted over a high vegetation volume. On the other side, in dense vegetation, the insects stay for a longer time in contact with vegetation, thus they accumulate more inoculum. In this study, the nymphs stayed for enough time in contact with the vegetation (two weeks as monitoring period). Wilps (2004) observed that movement of insects decreases in dense vegetation.

In systems where direct contact between spray and insect is limited, such as tall and dense grass vegetation, contact with residual conidia is essential for effective control (Thomas *et al.* 1997). There was no significant difference in the average survival period between locusts exposed directly and those that picked up conidia exclusively from the vegetation (Bateman *et al.* 1998). Thomas *et al.* (1998) showed that in short mixed grass vegetation the dose of conidia acquired through contact with the spray residue exceeds that from direct contact with airborne spray droplets alone. Van der Valk (2007) had concluded that direct and residual effect is similar on the day of treatment. In addition, in short vegetation, if the nymphs did not take a sufficient amount of conidia, they have more chance to control fungus growth within their body by basking under the sun (behavioral fever).Treated nymphs are observed to bask for long time under the sun. This behaviour can help the locust to get rid of infection caused by *Metarhizium* (Bashir, 2004). This behaviour was shown to affect the rate of mortality following infection (Blanford *et al.* 1998).

Despite the hot conditions during Trial II period, conidia have persisted until the six days after treatment with a good effect on untreated nymphs exposed to contaminated vegetation. For the same period, no residual effect of the product was observed. In field trial at the Red Sea coast (Bashir *et al.* 2007) noticed that conidia of *Metarhizium* remained viable up to day 6-9 when GM at 50 g/ha was used, and up to day 3-6 when GM at 25 g/ha was used. In other previous studies on *Oedeleus senegalensis* (Langewald *et al.* 1999) and *Zonocerus variegatus* (Douro-Kpindou *et al.* 2005) long persistence of *M. acridum* conidia on the vegetation was observed. Evaluation of residual effect during the Sahelian rainy season led to an estimated half-life of roughly one week.

During the treatment (in the large cages) most of the target nymphs were not exposed directly to the spray. Naturally, the nymphs have a tendency to climb the cage and remain perched on the mesh. Furthermore, in dense vegetation plants can affect direct contact of nymphs with conidia during treatment. Despite that, the nymphs were left in cage until death. In the two trials, monitoring period was two weeks; so, the nymphs stay for

enough time to collect a sufficient amount of conidia. Van der Valk (2007) suggested that with a half-life of 4 to 8 days, the insects should remain in the treated area for at least two days after treatment for the fungus to attain maximum efficacy.

The results of this study showed the robust efficacy of *M. acridum* conidia as a biopesticide against the Desert Locust nymphs. Even with favourable temperature, direct exposure of conidia to solar radiation can greatly influence conidial efficacy. In these conditions, height of vegetation plays an essential role to increase conidia viability to be able to reinfect treated nymphs and infect untreated ones that venture into treated zones. On the other hand, vegetation creates a microclimate more favorable for the fungus growth. The nymphs will have less chance of basking.

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أثر كثافة وارتفاع الغطاء النباتي علي حيوية فطر الميتاريزيم في مكافحة الجراد الصحراوي

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المستخلص: تم تقييم تأثير إرتفاع وكثافة الغطاء النباتي (الدخن) على فعالية وتأثير المتبقي من أبواغ الفطر *Metarhizium acridum* في تجربة شبه حقلية داخل أقفاص كبيرة (2 × 2 × 1 م) . تم إستخدام مجموعات مختلطة من الطور الثالث (L3) والرابع (L4) من حوريات الجراد الصحراوي كهدف . وقد تم تنظيم الدراسة في تجربتين ، أقيمت كل على حدة وفقاً لارتفاع النباتات: الأولى على غطاء من نباتات قصيرة (27–37 سم) والثانية علي نباتات طويلة (73– 93 سم). في كلتا التجربتين تمت معاملة الحشرات في إثنين من الأغطية النباتية المختلفة: غطاء ذو كثافة منخفضة (~10٪) وغطاء ذو كثافة عالية (~100٪) باستخدام معدل جرعة يحتوي علي 2.5×10^{12} أبواغ للهكتار في جرعتين مختلفتين هما: 1 أو 2 لتر/ هكتار . تم تقييم فعالية هذا المبيد الحيوي بمتابعة معدل وفيات الحوريات المعاملة خلال أسبوعين . من ناحية أخرى ، استخدمت الحوريات غير المعاملة بعد إطلاقها في أقفاص تحتوي على نباتات معالجة لتقييم أثر الابواغ المتبقية على النباتات المختلفة . لم يلاحظ أي تأثير كبير ناجم عن الغطاء النباتي على فعالية الفطر في حالة الغطاء النباتي القصير ($P = 0.828$) أو في الغطاء النباتي طويل القامة ($P=0.334$) عند تطبيق الجرعة 1 لتر/ هكتار. في الغطاء النباتي عالي الكثافة ، تحسنت فعالية الفطر وارتفع معدل الوفيات بشكل ملحوظ عند تطبيق الجرعة 2 لتر/ هكتار . أيضاً ، ظلت الابواغ على قيد الحياة على مدي ستة أيام بعد المعاملة مع

تأثير جيد على الحوريات غير المعالجة التي تم إطلاقها على النباتات المعالجة . على مدي نفس الفترة ، لم يلاحظ أي أثر متبقي للابواغ في الغطاء النباتي القصير . أبانت نتائج هذه الدراسة أهمية الغطاء النباتي العالي (كتلة عالية من الغطاء النباتي) في رفع فعالية الفطر *M. acridum* وإستمرار أبواغه على قيد الحياة . أما في حال النباتات القصيرة ، حتى مع غطاء عالي الكثافة ، تكون الأبواغ أكثر عرضة لأشعة الشمس ، وبالتالي سرعان ما تفقد حيويتها .