Workshop on the use of Green Muscle® (*Metarhizium anisopliae* var. *acridum*) and Desert Locust adult pheromone (Phenylacetonitrile, PAN) to control Desert Locust hopper bands

Organized under the umbrella of the FAO EMPRES/CR Programme in collaboration with GTZ, ICIPE and the PPD Sudan

Port Sudan, 10 - 20 January 2003

Report by
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List of Acronyms

a.i.	Active ingredient
BCP	Biological Control Products Ltd., Pinetown, South Africa
cm	Centimetre
EMPRES	Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (FAO)
FAO	Food and Agriculture Organization of the United Nations
g	Gram
GM	Green Muscle®
GTZ	Deutsche Gesellschaft fuer Technische Zusammenarbeit
ha	Hectare
ICIPE	International Centre for Insect Physiology and Ecology
IITA	International Institute for Tropical Agriculture
km	Kilometre
l	Litre
m	Metre
OF	Oil formulation
PAN	Phenylacetonitrile
PPD	Plant Protection Directorate in Sudan
s	Second
ULV	Ultra Low Volume

1 Introduction

Over the past several years, biological products for the control of locusts and grasshoppers have attracted increasing interest. There is a growing demand for alternatives to chemical control because of stricter regulations regarding pesticide residues, especially in Europe. Two companies are now offering commercial biological products, both based on the fungus *Metarhizium anisopliae* var. *acridum*: Biological Control Products (Pty.) Ltd. (BCP) of Pinetown, South Africa, produces Green Muscle® and Australian Seed Inoculants Ltd. of Wodonga, Australia, produces Green Guard®.

Research on the application of a Desert Locust pheromone for the purpose of control is now also at an advanced stage. These products represent new approaches to locust and grasshopper control. Because of their high specificity, they have very little impact on the environment, especially in comparison with chemical products. In particular, at the recommended dose rates they are not toxic to humans and livestock. When sprayed on crops, they do not create a residue problem, because consumption, even within days of application, does not have any harmful effect. In addition, these products will not pose logistical difficulties when becoming obsolete, and thus do not require expensive disposal, as is the case with most chemical based products.

The FAO has already recognised *Metarhizium* as a viable alternative control option and placed it on the list of recommended products for locust control. The Australian product has been used by the Australian Plague Locust Commission on a large scale for several years, but the South African product has not yet been used on the same scale, because large locust populations have been absent since commercial production started. FAO and GTZ promote the use of *Metarhizium* products by the locust control agencies, especially in Africa and Asia. However, biological products differ substantially from chemical ones regarding their use and mode of action. National Locust Control Units should become more familiar with these products so that, when locust upsurges occur, they will be in the position to conduct large-scale field trials to promote the introduction of such products for Desert Locust control. Unfortunately, due to the absence of Desert Locusts since 1998, no large-scale field trials were conducted. For that reason, a series of workshops was organized to familiarise locust control officers with the new products. The first workshop was held in Mauritania and a second in Sudan, jointly organized under the EMPRES/CR Programme by GTZ, ICIPE and the PPD in Sudan. BCP provided 5 l of an OF formulation of Green Muscle free of charge.

2 Goal and workshop objectives

The goal of the workshop was to promote the use of biological products in locust and grasshopper control by familiarising locust control officers from selected countries with these products, i.e. their handling and use.

The workshop gave the participants the opportunity to gain first-hand experience with two biological products: a formulation of *Metarhizium anisopliae* var. *acridum* and one of the

main components of the adult Desert Locust pheromone (Phenylacetonitrile, PAN), specifically:

- inoculating Petri dishes with *Metarhizium* and observe its growth
- estimating spore viability
- determining spore concentration
- applying *Metarhizium* and PAN to hoppers, both in the laboratory and in the field, and observing their effects

In addition, they attended lectures about the development of the two products and their special characteristics.

3 Organisation of the workshop

Participants from various countries from the Western and Central Region (see annex IV) were accommodated at Al Maysara Hotel in Port Sudan. Eight students from the University of Khartoum also participated in the workshop and assisted in setting up the trial sites and in monitoring the locusts during the trails.

Presentations were given at the ICIPE Research Station, while laboratory work was undertaken at the PPD Plant Quarantine Laboratory and the field trials were carried out near the ICIPE Station in Salloum, 35 km from Port Sudan. The PPD provided six Land Cruiser pick-ups for transport of the participants and materials, while ICIPE offered two Land Cruiser station wagons.

4 Workshop programme

The workshop started with an official opening at the Port Sudan Palace Hotel. The Wali (Head of the Red Sea Governerate) and the Minister of Agriculture of the Red Sea State were invited to attend the opening ceremony. Other officials from the PPD and the Red Sea University were also present. All participants showed high interest in the subject of the workshop. There was a consensus that biological control and IPM should be promoted in the Region. As an example, the exotic tree Mesquite (*Prosopis juliflora*) was mentioned. This tree is causing a serious problem in Red Sea State and a biological control programme had already been proposed. The programme, divided into lectures, laboratory and field work (see annex III), was adopted by the participants without major changes.

4.1 Lectures

Presentations were given by Ir. Christiaan Kooyman, GTZ consultant, Mr. Benedict Banda, marketing manager of BCP, Dr. Ahmed Hassanali and Prof. Magzoub Bashir, both researchers at ICIPE and Mr. Douro Kpindou of the International Institute of Tropical Agriculture (IITA). These lectures, addressed the mode of action of the two bio-control agents (the mycopesticide *Metarhizium* and the Desert Locust pheromone phenylacetonitrile, PAN), and the laboratory and field tests. Because the many questions raised regarding the non-target effects of Green Muscle, an extra presentation on the eco-toxicology of *Metarhizium* and its effects on non-target organisms was given.

4.2 Laboratory work

The organizers were grateful that the PPD in Port Sudan made their Quarantine Unit available to the workshop, since it was better equipped than the ICIPE Research Station. Extra equipment was provided by the organizers. A laminar flow cabinet was unfortunately not available, thus a sterile glass chamber had to be prepared by a craftsman.

Mr. Kpindou (IITA) gave an introduction on the lab work programme. The participants were shown how to estimate spore viability and spore concentration. Mr. Kpindou tested the viability of the spores in the OF formulation provided by BCP and dry spores from IITA that had been kept at ICIPE since 1997. The germination rate was between 77 and 86 % after 20 hours incubation. Participants could practice checking spore viability. But because of lack of experience some Petri dishes were contaminated by other micro-organisms, which made reliable estimates difficult.

Adult locusts were infected with *Metarhizium* spores and kept in cages. The mortality was checked on a daily basis and compared with uninfected locusts. The cumulative number of dead locusts in the cages is shown in Table 1. Figure 1 shows the mean number per treatment.

The difference in mortality between treated and untreated locusts was high. As clear sign of *Metarhizium* infection the majority of the cadavers became red.

Table 1: Cumulative numbers of cadavers in the cages.

Day	Untreated			Treated		
	C1	C2	C3	T1	T2	T3
1	0	0	0	0	0	0
2	1	1	0	0	0	0
3	1	2	0	0	0	0
4	1	2	0	0	0	0
5	3	2	0	4	3	1
6	4	3	1	14	11	5

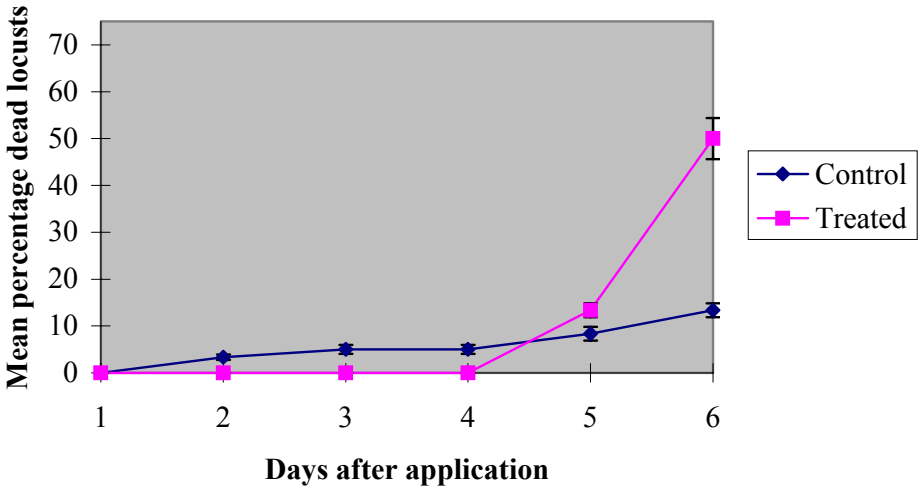

Figure 1: Mean percentage mortality in the cages

PAN was applied to hoppers in cages and their behaviour observed and compared with untreated hoppers. Not all participants found the differences convincing, although there appeared to be reduced feeding of the treated hoppers.

4.3 Field work

4.3.1 Application of Metarhizium

4.3.1.1 Materials and methods

Since natural hopper bands were not available and the already scarce vegetation was already drying up, the trials were carried out under semi-field conditions on an irrigated field planted with wheat, millet, sorghum and soybeans. Twelve enclosures (bomas) were erected in three rows of four bomas each. The 10 x 10 m enclosures were made of plastic sheets of 75 cm

high. Reared hoppers were released into the enclosures when the vegetation was around 20 cm high.

With 10 m spacing between the plots it was not possible to apply the spray as under operational conditions and to build the bomas around the treated hopper bands. In order to avoid contamination of the neighbouring plots the treatment was done directly in the enclosures by using a mistblower.

It had originally been planned to complete the treatments five days before the beginning of the workshop, with the intention that the actual effects of the bio products were visible during the course of the seminar. Unfortunately this was not possible, because the Green Muscle consignment from BCP did not arrive. It was found that the parcel with the spores had been destroyed because of security reasons at London Heathrow Airport. The organizers had therefore to delay the treatments until the BCP marketing manager eventually arrived with a new batch.

Approximately 5000 hoppers were released in each enclosure the evening before the treatment. The following morning, the OF formulation of Green Muscle was diluted with diesel at dose rates of 25, 50 and 75 g a.i./ha in 4 l. This volume was chosen because of the small area and the limited time for spraying to guarantee an equal coverage. Two spray passes of 15 s each were made using a mistblower with Micronair AU8000 attachment. Restrictor no. 2 was used, which gave a flow rate of roughly 80 ml/min. Each dose was applied at random in three enclosures, while three enclosures were left untreated. Wind speed varied from 0-2.5 m/s and the temperature was 25-28° C.

The enclosures were guarded from morning until sunset and regularly examined to avoid escape of the hoppers. Birds were constantly scared away. The mortality was checked twice per day and cadavers incubated to check for infection. The hopper population in the enclosures was counted on day 2, 11 and 15, and their behaviour observed.

Samples of 20 hoppers were taken from each enclosure one day after treatment. These were kept in cages in a room on the farm. Fresh food was provided and the mortality was checked on a daily basis.

4.3.1.2 Results and discussion

During the first three days, the few cadavers found in the enclosures were discarded, because it is was not likely that *Metarhizium* could have acted that fast. Later, the number of dead infected nymphs increased slowly in the treated plots. Unfortunately, the effects of

Metarhizium became only clearly visible after six days. During this period, the mortality was still low, which was disappointing for the participants. Staff from the PPD continued the counting even after the workshop finished to illustrate the results for the workshop document. The cumulative numbers of cadavers collected is shown in Table 2, while Figure 2 shows mean numbers per dose rate. Most cadavers from the treated plots became red as a typical sign of *Metarhizium* infection. The cadavers from the control enclosures showed no sign of infection.

Table 2: Cumulative numbers of cadavers in the enclosures.

	Cumulative numbers of cadavers											
	0 g			25 g			50 g			75 g		
Day	**Boma 9**	**10**	**11**	**4**	**6**	**7**	**1**	**3**	**8**	**2**	**5**	**12**
4	0	0	1	12	93	3	21	1	23	9	52	17
5	0	4	4	26	136	9	29	8	42	10	78	30
6	0	10	7	76	151	13	33	16	59	11	90	54
7	0	10	10	121	165	17	40	29	71	11	101	70
8	0	11	12	162	171	31	43	46	92	13	105	104
9	0	13	21	162	177	35	57	63	101	16	114	119
10	0	16	22	162	193	36	64	86	105	30	124	125
11	0	17	22	-	196	39	-	89	109	37	137	129
12	1	18	23	-	198	39	-	89	114	57	150	131
13	2	20	-	-	201	39	-	89	114	65	154	132
14	4	21	-	-	202	40	-	89	114	68	161	132
15	4	24	-	-	203	41	-	90	114	69	163	136

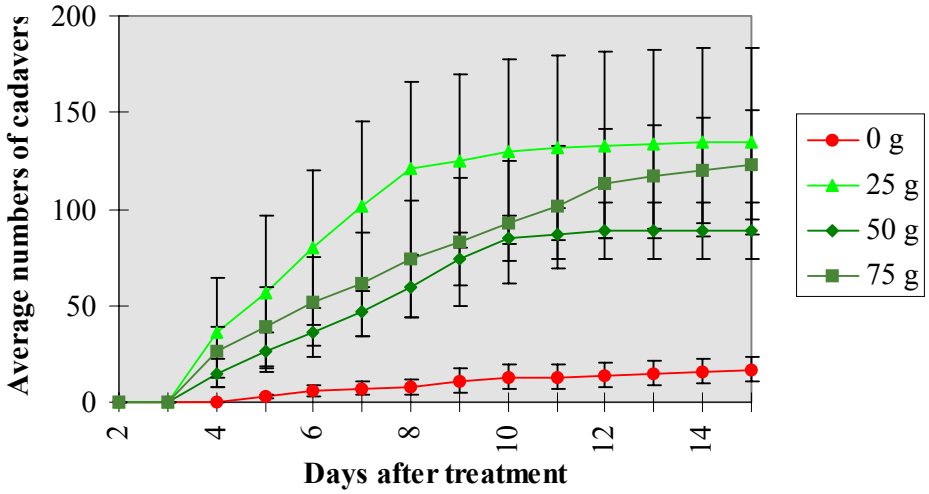

Figure 2: Mean cumulative numbers of cadavers in enclosures per dose rate.

The estimated numbers of hoppers in the enclosures are shown in Table 3. Figure 3 shows the mean percentage reduction in numbers per dose rate.

Table 3: Estimated numbers of hoppers in the enclosures.

	Estimated numbers of hoppers											
	0 g			25 g			50 g			75 g		
Day	**Boma 9**	**10**	**11**	**4**	**6**	**7**	**1**	**3**	**8**	**2**	**5**	**12**
2	3000	4000	5000	5000	6000	2000	2000	4000	3000	5000	3000	3000
11	2000	3000	3500	0	3500	600	0	800	1000	3000	2000	1000
15	1500	2600	2700	0	3000	100	0	100	200	2800	1500	100

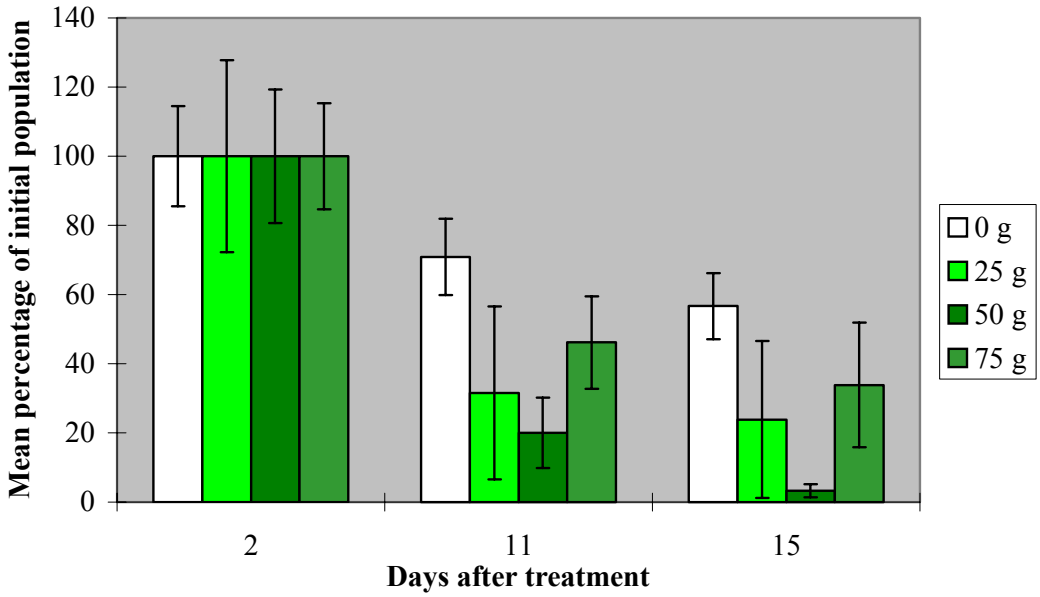

Figure 3: Mean percentage reduction in hopper numbers per dose rate.

When comparing Tables 2 and 3, it was clear that more hoppers disappeared from the enclosures than could be accounted for by the recovered dead nymphs. Some may have escaped from the enclosures, but most probably the missing hoppers were removed by predators. For example, it was observed that wasps were carrying nymphs away to their nest chambers. Others might have been eaten by ants, beetles or birds. The predation rate was higher in the treated enclosures, most probably because of the infection by the fungus which made the hoppers a more easy prey.

There was a difference in the behaviour of the locusts in most treated enclosures as compared to the control. Treated hoppers spent more time huddling together and basking in the sun. This behaviour was a reaction on the infection to increase the body temperature.

The mortality of the hoppers in the cages is shown in Table 4, while in Figure 4 illustrates the mean mortality per dose rate. More than half of the dead nymphs in treated cages changed colour to red as an indication of the effects of the fungus. None of the cadavers found in the control cages showed this phenomenon.

Table 4: Cumulative numbers of cadavers in the cages.

	Cumulative numbers of cadavers											
	0 g			25 g			50 g			75 g		
Day	**Boma 9**	**10**	**11**	**4**	**6**	**7**	**1**	**3**	**8**	**2**	**5**	**12**
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	1	0	0	0	0	0	3	0	1	0
4	0	1	1	0	0	1	1	1	3	0	1	1
5	0	1	1	0	1	1	2	1	4	0	1	1
6	0	1	1	0	1	1	5	1	4	1	1	1
7	0	1	1	0	2	3	11	5	8	4	5	8
8	0	1	2	1	5	7	14	8	11	8	15	11
9	0	1	2	1	6	10	16	9	11	12	20	16
10	0	1	2	2	6	10	16	11	13	14	20	17

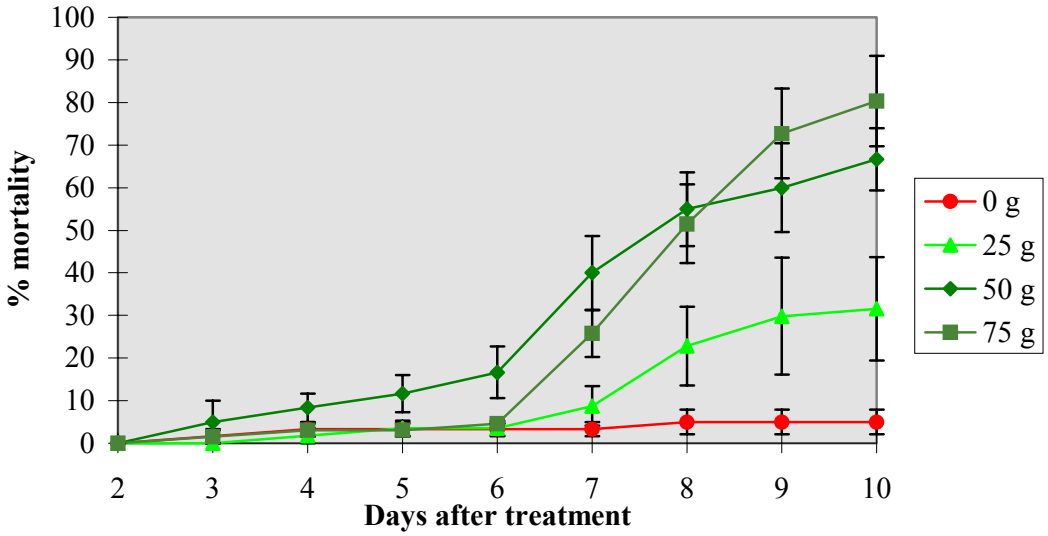

Figure 4: Mean percentage mortality in cages per dose rate.

The overall low mean mortality observed in the 25 g variable was the result of only one sample which showed almost no mortality. The spray coverage in this enclosure was probably too patchy resulting in a reduced infection rate. In the other two replicates however a similar mortality as in the 50 g trials was observed.

The results showed contamination of the nymphs could be achieved, but the infection rate in individual trials was very variable, perhaps because of differences in the spray coverage

caused by varying wind speeds during the treatments. Another source of error probably was due to predators. The infection may have made the hoppers more susceptible to predators, because the hopper population in these trials decreased much faster than in the untreated ones.

Although the differences in the mortality between treated and untreated enclosures were high, there were no clear differences in mortality as a result of the different dose rates. This is not unusual, because it is known that the dose/response curve of *Metarhizium* is not steep. On the basis of these results, no recommendation could be made on the application rate (dose in g per ha) to be used for control operations.

Because of the different application techniques used during the workshop conducted in Mauritania, it was not possible to compare the present results with the findings from the workshop in Mauritania. In the latter, the hoppers were treated in open field under natural conditions before being placed in enclosures. However, these tests on free-moving hopper bands were affected by other difficulties, as it was not easy to keep track of the movements of the hopper bands and to estimate their exact sizes. A comparatively more significant effect of Green Muscle was observed in some of these trials. Predation on the treated bands was higher than on the untreated ones. In some cases some hopper bands were completely wiped out by predators, in particular by birds.

4.3.2 Application of *Metarhizium* and PAN

4.3.2.1 Materials and methods

Four 10 x 10 m enclosures were set up in a row and hoppers released into each of the enclosures in the evening before applications. Two of these bomas were treated with *Metarhizium* at 25 g a.i./ha and the other two with *Metarhizium* at 25 g/ha and 0,05 % PAN at 1 ml/ha, using an ULVA^{+} sprayer.

4.3.2.2 Results and discussion

The effects of PAN, increased stress and less coherent behaviour of the nymphs, were observed during the first days after treatment. These effects were less clear after several days.

The results showed that the average number of dead nymphs in the enclosures treated with PAN + Green Muscle was higher than that in the Green Muscle only enclosures (see Table 5, Figure 5).

Table 5: Cumulative numbers of cadavers in enclosures treated with *Metarhizium* and PAN.

	Cumulative numbers of cadavers			
	25 g		25 g + PAN	
Day	Boma 3P	4P	1P	2P
4	8	5	8	10
5	16	10	17	31
6	27	15	27	58
7	41	22	39	92
8	58	30	51	128
9	76	39	66	164
10	98	49	90	202
11	124	61	120	240
12	156	80	154	282
13	188	101	188	324
14	231	139	227	371
15	275	178	269	421

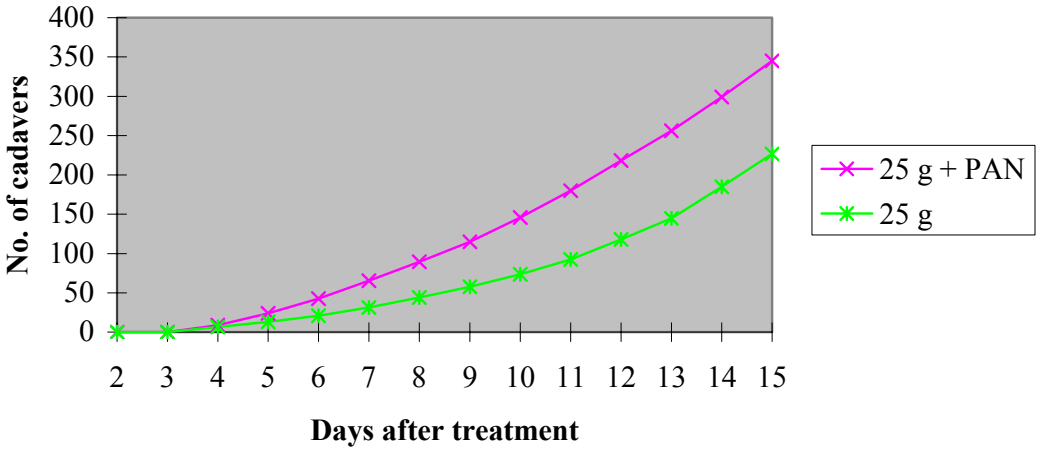

Figure 5: Mean cumulative numbers of cadavers per treatment in enclosures treated with *Metarhizium* and PAN.

The trials confirmed that the presence of PAN apparently makes Desert Locust hoppers more susceptible to *Metarhizium*. As the behaviour of the hoppers may have been influenced by the confined plots, the effects of PAN were not as clear as they might have been under natural conditions.

4.3.3 Application of Dursban and PAN

4.3.3.1 Materials and methods

Eight 10 x 10 m enclosures were set up and hoppers released. Two enclosures each were treated as follows using an ULVA+ sprayer:

(a) Dursban 45 % ULV at 250ml/ha (112.5g a.i./ha)

(b) Dursban 45 % ULV at 250 ml/ha (112.5g a.i./ha) + PAN 0,05 % at 1 ml/ha

(c) Dursban 45 % ULV at 125 ml/ha (56.25g a.i./ha)

(d) Dursban 45 % ULV at 125 ml/ha (56.25g a.i./ha) + PAN 0,05 % at 1 ml/ha

4.3.3.2 Results and discussion

There was no clear difference between Dursban and Dursban mixed with PAN for the 250 ml dose. In the case of Dursban at 125 ml plus PAN, 100 % mortality was achieved by day three, while Dursban alone realized only about 60% kill (see Fig. 6). These results demonstrated the potential of PAN to reduce the dose of conventional insecticides for Desert Locust control.

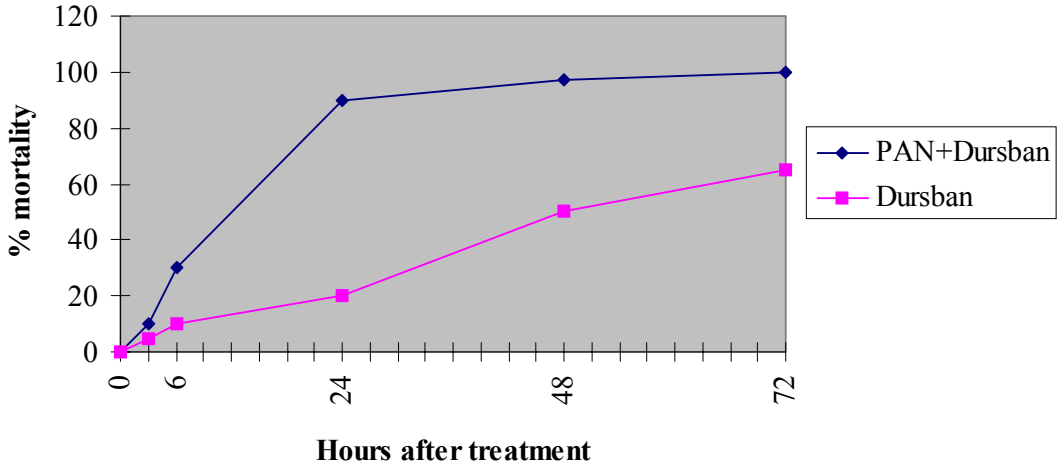

Figure 6: Mean percent mortality in enclosures treated with Durban and Dursban + PAN.

5 Group discussions and report writing

The workshop was evaluated by the participants at the end of every day. During the last two days, the opportunity was given to the participants to discuss the results in three groups. The comments made by the participants were discussed briefly on the last day as a basis for participants' report for reaching a consensus on the potential of the produces for locust control. The workshop closed after a short ceremony on 20 January 2003.

6 Conclusions and recommendations

Because of the destruction of the first shipment of Green Muscle, it was not possible for the participants to observe the full effect of *Metarhizium* in the field. The problem was compounded by the fact that its effect could not be demonstrated on natural hopper bands. Results from enclosures are never very clear and were even less so, because the products had to be sprayed inside the enclosures. The workshop did not succeed in its objective of demonstrating clearly to the participants that *Metarhizium* and PAN would be efficacious under field conditions. PAN's effect is most striking on the behaviour of natural hopper bands, but less so in enclosures. The only convincing result was the enhancement of mortality by PAN combined with low doses of pesticide.

The lectures and the laboratory experiments answered many of the questions on the minds of the participants on most aspects of *Metarhizium*, on the commercial product Green Muscle and on PAN. There were two main issues that many participants saw as problematic in the case of *Metarhizium*. The first one was its slow action. Many chemicals also take days to achieve 90% kill (e.g. Fipronil and IGRs), but *Metarhizium* typically takes two weeks or more. There was concern that hopper bands may enter crop fields before mortality sets in. It was surprising that most participants had an exaggerated idea of the effect that hopper bands have on crops, especially bands of young hoppers. Even when it was pointed out that crop loss caused by hopper bands is often relatively little, especially when treated with *Metarhizium*, they maintained that farmers do not accept any damage. If it proves difficult to change this attitude in locust control officers, it will not be easy to persuade them to use Green Muscle, except maybe far away from crops and in areas where chemicals cannot be used. It is recommended to undertake more trials on the combination of *Metarhizium* with PAN, since there are clear indications that PAN will stop the movement of hopper bands until *Metarhizium* starts to take effect.

The second issue was the impact of Green Muscle on non-target organisms. Although the product was classified as having very low impact on NTOs by the FAO expert consultation on mycopesticides against locusts, and ample documentation was available on its safety, participants felt that more safety testing should be done, especially on organisms that are indigenous to the various countries where Green Muscle is to be introduced. They did concede that most chemical insecticides have a wider and more serious impact, but they seem to fear certain insidious effects of the pathogen, which is considered erroneously as "exotic" in certain countries. Some registration authorities share this point of view. These fears can be reduced, after locust control officers have gained more experience in the use of *Metarhizium*, and for that matter, other mycopesticides.

A slightly different issue was the current price of Green Muscle, which was considered too high by the participants, if the product has to be used at 50 g/ha. This could be a serious obstacle to its adoption in most countries, unless a lower dose can be used, or some form of subsidy provided until the price is lower. As the price of Green Muscle becomes competitive at 25 g/ha, this dose should be tested as soon as natural populations are available. The present trial did not prove that this dose is effective, but the indications were encouraging.

After this workshop and that held in Mauritania, the most important countries that are affected by the Desert Locust have one or more persons who have an understanding of *Metarhizium* for locust control. There seems to be little reason, therefore, to organize further workshops, as it appears that they do not contribute to demonstrating the efficacy of Green Muscle under field conditions. Convincing results can only be obtained on natural populations, but conducting a workshop around an outbreak is difficult to organize given the logistical implication that might disrupt a locust control campaign. The best way forward was judged to be to use available funds to supporting registration trials whenever and wherever outbreaks occur.

Registration dossiers should be deposited in those countries where such outbreaks are most likely to occur. In CILSS countries, where Green Muscle has already been registered, locust control agencies should be encouraged to use the product when locusts need controlling, so as to generate acceptable data. Observers from other countries could be invited, who would then assist in the assessments, thereby helping to keep costs down. It would be advisable to hire an expert on handling and use of Green Muscle when undertaking registration trials and some of the early control operations using Green Muscle.

It may take a long time before suitable outbreaks of Desert Locusts occur. However, grasshoppers pose problems almost on a yearly basis in most locust-affected countries. Examples are *Oedaleus senegalensis* and *Zonocerus variegatus*. These grasshoppers are regularly controlled in countries like Chad, Mali, Niger, Senegal and Sudan. The West African countries already benefit from a project to promote the use of Green Muscle for grasshoppers control. Similar activities should be financed in Egypt, Ethiopia and Sudan where grasshoppers are recognised as problem pests. If registration authorities accept a single registration for both grasshoppers and locusts, since they belong to the same order, work on grasshoppers would speed up registration. It might even eliminate the need for separate registration trials on locusts, which are more difficult to carry out and are therefore more costly. In Sudan, tree locusts (*Anacridium spp*.) also deserve attention, because large-scale control operations are often mounted against this pest in gum arabic plantations. It has already been shown that tree locusts are easily controlled using Green Muscle.

Annex I: Participants' report

1. Introduction

This workshop on the biological control of the Desert Locust, held in Port Sudan from 11-20 January 2003, was financed and organized by FAO/EMPRES/CR and GTZ in collaboration with the Plant Protection Directorate (PPD) of Sudan and the International Centre for Insect Physiology and Ecology (ICIPE).

The products to be tested in this field-based workshop were a component of the locust pheromone (Phenylacetonitrile or PAN), provided by Sigma-Aldrich of the UK, and the insect pathogenic fungus *Metarhizium anisopliae* var. *acridum* (Green Muscle® or GM) provided by Biological Control Products (BCP) of Pinetown, South Africa. These products form a new approach to locust and grasshopper control, which has very little impact on the environment.

2. Objectives

The workshop aimed at familiarising locust officers from selected countries with the new biological products, i.e. their handling and use. The workshop gave participants the opportunity to work with these products and to learn how to:

- inoculate Petri dishes with *Metarhizium* and observe its growth,
- estimate spore viability,
- determine spore concentration,
- apply *Metarhizium*, and PAN to hoppers, both in the laboratory and in the field, and observe their effects.

3. Lectures

Lectures on different topics were given by different speakers, including Mr. Christiaan Kooyman, Mr. Benedict Banda, Prof. Ahmed Hassanali, Prof. Magzoub Bashir and Mr. Douro Kpindou.

3.1. Lectures by Mr. Kooyman

Mr. Kooyman gave three lectures. The first lecture was on the use of pathogens as control agents for locusts and grasshoppers. He went into the potential of the different pathogens, namely viruses, bacteria, fungi and protozoa, for the control of these target pests. He illustrated the weak and strong aspects of each group with more emphasis on entomopathogenic fungi as the most promising agents for control of the Desert Locust. Fungi are so effective mainly due to their mode of penetration through the cuticle without the need to be ingested as the case is for other pathogens. Kooyman further discussed the potential of the different entompathogenic fungus genera, especially *Metarhizium* and *Beauveria*. From his lectures, it was made clear that so far, *Metarhizium* has proved superior to the other genera tested in terms of virulence to the Desert Locust and other characteristics.

In his second lecture, Mr. Kooyman described the various trials that the LUBILOSA programme has conducted, including trial set-up and results. Trials against grasshoppers,

like *Oedaleus senegalensis*, have been very successful giving control for up to six weeks, while a chemical pesticide suppressed populations for only a week or so. Work on locusts proved more difficult because of the logistics of following hopper bands for several weeks. However, all indications are that hopper bands eventually disappear. Though the direct cause is often predation, it appears that *Metarhizium* makes it easier for predators to catch the hoppers. Thermoregulation behaviour is a problem that causes death to be delayed by many days. Fledglings do still carry the fungus, which makes them mature faster and lay eggs at an inappropriate time.

The subject of the third lecture was the effect of GM on non-target organisms, such as insects of different orders (including honeybees), reptiles, birds and fishes. He discussed the 3 tier testing method elaborating each aspect as follows:

- **1st tier:** This approach involves the use of ever higher doses of the control agent to determine the dose that kills 50% to the experimental animals (LD50) under laboratory conditions.
- **2nd tier:** This refers to the application of the control agent at more realistic doses to the target animals in captivity but kept under more natural conditions.
- **3rd tier:** At this stage, the experimental organisms receive the recommended dose and double that under fully natural conditions.

Kooyman explained that so far, no negative effects of *Metarhizium* have been recorded on non-target organisms under natural conditions.

3.2. Lecture by Mr. Banda

Mr. Banda explained the different bioproducts produced by his company, including Green Muscle. He further explained the target beneficiaries (potential buyers) of Green Muscle. These include conservation areas (e.g. game parks and reserves), locust authorities and organic farmers.

From his discussion it was made clear that Green Muscle has been registered in Zambia, Malawi, South Africa and Namibia. Other products containing *Metarhizium anisopliae* var. *acridum* have been registered in the Sahelian countries and in Australia. It was learned that *Metarhizium* has not yet been registered in any country of the Central Region. His discussions included the price of Green Muscle in comparison with chemical pesticides. At the present recommended dose of 50 g a.i./ha, the wholesale price of $347/kg or $17/ha is high. Fortunately, it looks increasingly likely that the dose can be dropped to 25 g/ha, making the product competitive with commonly used chemicals, especially when taking into account the benefits of *Metarhizium* as an environmentally friendly safe biopesticide. The product has practically no external costs, like the medical bills for poisoning and the costs of disposing of obsolete stocks that are associated with chemical products.

3.3. Lecture by Prof. Hassanali

This lecture focused on how the research on PAN started and the long process the research has gone through. He explained that the chemical structure and the impact of PAN at nymphal and adult stages of the Desert Locust have been studied both in the laboratory and at semi-field conditions. It was made clear from his lecture that PAN causes confusion and segregation in target hoppers leading to higher susceptibility to chemical and biological control agents even at low doses. It was also explained that the influence of PAN on

nymphal stages results in various behavioural changes. Since this topic was new to most participants, more time should have been given for this lecture.

3.4. Lecture by Prof. Magzoub

Prof. Magzoub focused on the influence of PAN on different activities of the Desert Locust. The activities studied included the effect of PAN on feeding preference, fecundity, segregation, movement, oviposition and maturation using different doses of PAN at different stages of the Desert Locust. Graduate students under his supervision carried out these experiments. It was also made clear that there are some aspects of PAN which need to be looked into before contemplating large-scale application of PAN. More time should have been allocated to this lecture.

4. Laboratory experiments

The laboratory experiments were introduced and coordinated by Mr. Douro Kpindou and included the following topics:

- Fungal growth
- Germination of spores
- Determination of spore concentrations
- Infection of locusts with *Metarhizium*
- Application of PAN to locust hoppers

4.1. Fungal growth and spore germination

4.1.1. Materials and methods

Metarhizium spores (conidia) were suspended in diesel and diluted to give a concentration of 10^5 spores/ml. One µl of this suspension was dropped into Petri dishes containing Sabouraud Dextrose Agar. The inoculated Petri dishes were kept in an incubator set at 26°C. After 24 and 48 hr, the percentage of germinating spores was estimated. The growth of colonies was examined after 48 hr.

4.1.2. Results and discussion

The plates turned out to have been contaminated by bacteria during the inoculation process. It was therefore not possible to properly see the *Metarhizium* colonies. Some germinating spores were observed, but it was impossible to estimate the germination rate. This shows the importance of working under sterile conditions. Although a sterile chamber was used (a laminar flow cabinet was not available) and all tools were sterilised, the number of people crowding in front of the chamber caused contaminated air to flow inside.

Mr. Kpindou made separate estimates of the germination rates of the OF formulation supplied by BCP and dry spores produced by IITA in 1997 and kept in the fridge at the ICIPE station. The rates were 86% and 77% respectively after 20 hr. This shows that dry spores can be kept under cool conditions for years with little loss in viability.

4.2. Spore concentration

4.2.1. Materials and methods

The same spore suspension as was used in the previous experiments. A small quantity was used to fill the counting space on a Neubauer haemocytometer. The smallest squares visible under the microscope measure 1/400 mm^2. Sixteen of these make up larger squares, which therefore have an area of 0.04 mm^2. These squares form even larger ones of 5x5 squares. The spores present in either 5 or 25 medium-sized squares are counted depending on the concentration. The number of spores in 1 ml is derived with the following formula:

$$C=25*X*10^4$$

where C is the concentration per ml and X the average number of spores in the medium-sized squares. This procedure is repeated at least three times and the average taken as the final concentration.

4.3. Infection of locusts with *Metarhizium*

4.3.1. Materials and methods

For infection of locusts with *Metarhizium*, 6 cages were prepared and 20 adult locusts were kept in each cage. Three of the cages were for treatment with the fungus while the remaining 3 were for the controls. Each locust received 4 µl of diesel containing 10^5 spores under the pronotum while the controls were treated with 4 µl of diesel without spores. Each cage was checked daily for mortality.

4.3.2. Results and discussion

The observed cumulative percent mortality was 50% 6 days after treatment while mortality in the controls was 13.3%. The majority of cadavers from the treated hoppers turned red, which is a clear sign of infection. This result shows that the fungus has good potential to cause death in the target insects in a reasonable time span. The fact that cadavers of infected insects become red, is useful for showing that mortality is caused by the fungus.

4.4. Application of PAN to locust hoppers

4.4.1. Materials and methods

For the application of PAN, four cages containing 150 nymphs were prepared, two for PAN treated and two for control insects. PAN was applied in diesel at a concentration of 0.05%. The control insects received only diesel. Each cage was checked daily for mortality and observations were made on the behaviour of the treated nymphs and compared with the behaviour of the control nymphs.

4.4.2. Results and discussion

With regard to the effect of PAN, segregation of locusts was more pronounced in the treated locusts as compared to the controls. It was also observed that PAN affected the feeding behaviour, since during 1 hour of observation, 10.8 % of the hoppers were observed feeding in the control while only 5.42 % in the treated cages. These results show that PAN has the potential to influence the feeding behaviour of the Desert Locust leading

to stress and susceptibility to pesticides. Reduction in feeding is useful when PAN is combined with slow-acting pesticides.

5. Field work

The field experiments included the following sub-topics:

- Application of *Metarhizium* to hoppers in enclosures
- Application of *Metarhizium* plus PAN to hoppers in enclosures
- Application of Dursban plus PAN to hoppers in enclosures

5.1. Application of *Metarhizium*

5.1.1. Materials and methods

Twelve enclosures (bomas) of 100 m^2 (10x10 m) each were set up in three rows of four and between 5000 and 10,000 hoppers were released into each enclosure. The OF formulation of Green Muscle was diluted with diesel to arrive at dose rates of 25, 50 and 75 g a.i./ha in 4 l. The high volume was chosen because the area to be treated was very small and the time for spraying would have been very short (15 s). With the higher volume, two spray passes of 15 s each were made using a mistblower with Micronair AU8000 attachment. The restrictor no. 2 was used, which gave a flow rate of roughly 80 ml/min. Each dose was sprayed at random in three enclosures, while three enclosures were left untreated.

Mortality was checked twice daily and cadavers incubated to check for the development of red colouration, the sign of infection. Observations were also made on the behaviour of the hoppers.

Samples of 20 hoppers were taken from each enclosure on the day after treatment. These were kept in cages in a room on the farm. Mortality was checked and fresh food provided on a daily basis.

5.1.2. Results

During the first two days, few cadavers were found in the enclosures and these were discarded, as *Metarhizium* cannot kill that quickly. Thereafter, the numbers of cadavers increased, especially in the treated enclosures. After 7 days, the average cumulative number of cadavers was as follows:

0 g	25 g	50 g	75 g
7	101	47	61

Most of the cadavers became red indicating that *Metarhizium* was the cause of death. No cadavers from the control enclosures became red.

There was a difference of behaviour in most treated enclosures as compared with the controls. Treated hoppers spent more time huddling together and basking in the sun. As was explained in the lecture, this raises the body temperature to inhibit growth of the fungus inside the body.

Mortality of treated hoppers in the cages was between 5 and 60% after 7 days with lowest mortality in the 25 g samples. Control mortality was between 0 and 5%. Mean percentage mortality per dose was as follows:

0 g	**25 g**	**50 g**	**75 g**
3.3	8.8	40.0	25.8

The results show that infection took place in the enclosures, but infection rates varied because of differences in spray coverage caused by varying wind strength during spraying. Stronger wind blew more of the spray cloud out of the enclosure. There may also have been an effect of dose, since the 25 g enclosures had lowest infection rates. *Metarhizium* has potential for the control of Desert Locusts, but future trials should be carried out on natural hopper bands.

5.2. Application of *Metarhizium* and PAN

5.2.1. Materials and methods

Four enclosures were set up in a row and hoppers released. Two enclosures were treated with *Metarhizium* alone at 25 g a.i./ha and the other two with *Metarhizium* at 25 g/ha and PAN at 1 ml/ha using an ULVA^{+} handheld sprayer.

5.2.2. Results and discussion

The hoppers exposed to PAN became more dispersed. However, this effect became less obvious later on. The average number of cadavers in the enclosures with PAN was double that in the other enclosures after 7 days:

Metarhizium	***Metarhizium* + PAN**
31.5	65.5

Though there was higher mortality in the combination treatment, the time was insufficient to properly evaluate the effect of PAN on the speed of kill by *Metarhizium*. PAN's effect on behaviour was not as clear as expected. This trial should be repeated on natural hopper bands.

5.3. Application of Dursban and PAN

5.3.1. Materials and methods

Eight enclosures were set up and hoppers released. Two enclosures each were treated as follows:

(a) Dursban alone at 250ml/ ha

(b) Dursban at 250 ml/ha +PAN at 0.05%/ha

(c) Dursban at 125 ml/ha

(d) Dursban at 125 ml/ha +PAN at 0.05%/ha

5.3.2. Results and discussion

There was much variability in mortality in the 250 ml treatments, which was probably due to problems during application. No conclusion was therefore drawn. However, in the case of 125 ml Dursban plus PAN caused >90% mortality by day three, while Dursban alone had killed less than 50%.

These results show the potential of PAN to reduce the dose of conventional pesticides.

6. Evaluation of the tested products

6.1. *Metarhizium*

Metarhizium is considered as a safe biological control agent for Desert Locust and other locusts and grasshoppers. *Metarhizium* has been shown not to have negative impacts on most non-target organisms. *Metarhizium*, like most insect pathogens, acts slowly and in the present trial, mortality occurred after 7-10 days, so it will be difficult to use *Metarhizium* during locust outbreaks; although it could be used when the number of individuals is less than the economic threshold. More research must be done on the possibility of mixing *Metarhizium* with low doses of chemical insecticides to accelerate its action, as well as research on damage assessment during the first week after treatment with *Metarhizium*.

6.2. PAN

When used with Dursban at low dose, PAN gave a very significant mortality to hoppers within 24 h. In combination with *Metarhizium*, it also increased mortality compared with *Metarhizium* alone. Its effect on behaviour was not easily to be observed due to the low numbers of individuals in the cages. More research should be done on the effect of PAN with other insecticides and on its toxicity and non-target effects. An interesting possibility of preventing hopper bands from entering farmers' fields by spraying with PAN should be investigated in combination with *Metarhizium* treatment.

7. Organisation of the workshop

The organisation of the workshop was in general rated as good in terms of bringing different participants together to demonstrate the research status of the bioproducts. However, there were clear weaknesses, arranging for accommodation and transport to and from the field. These need to be taken into account for future workshops

8. Conclusions and recommendations:

- The workshop achieved its objectives by familiarising the participants with the use and handling of *Metarhizium* and PAN.
- Since this workshop concentrated more on field work than on presentations, it should help the participants to carry out similar trials independently in their respective countries.
- The workshop was too short to clearly show the effects of *Metarhizium*.
- It was recommended to send the final results to all participants after the end of field experiments.
- The time it takes for *Metarhizium* to kill a significant number of hoppers was considered as too long. The participants were unable to give strong recommendation for its introduction in their countries. They therefore recommended that more field trials are undertaken to speed up the mode of action of *Metarhizium*.
- No documents were provided about the effect of *Metarhizium* on non-target organisms.

Annex II: Comments and recommendations by the workshop participants

The participants considered that the lectures had given a good picture of the two biological products and their potential in locust control, though more information on PAN would have been welcome, since this was a totally new subject to most of them. Not everyone found the laboratory work relevant, especially spore germination and concentration. It was explained to them that it was necessary to get some idea of these things to fully appreciate the differences between Green Muscle and chemical pesticides.

As for the field work, the participants found it disappointing that they had not seen much effect in the *Metarhizium* treatments, though they understood that the destruction of the first shipment of Green Muscle had forced the organizers to delay application. It had also not been possible to see PAN stopping hopper bands in their tracks. The dispersing effect had not been very clear in the enclosures. For these reasons, future demonstrations should be performed on free moving hopper bands to give a clear picture of the effects of *Metarhizium* and PAN.

On the prospects of integrating the two products in the national locust control strategy, many participants voiced their concern about the slow mode of action of Green Muscle. They called for more research into speeding up it effect on mortality.

Though it had been stressed repeatedly that Green Muscle should be used a good distance away from cropping areas, the participants continued to bring up the possible damage to crops after treatment. Even the observation that the thousands of hoppers in the enclosures had not managed to deplete the vegetation after more than a week, did not make a sufficient impression. Some argued that farmers would not accept even the slightest damage. It was agreed that *Metarhizium* could be used in areas with many bee keepers, who normally resist the use of chemicals for locust control. Another comment was that more research should be done on the effect of *Metarhizium* on non-target organisms, especially species indigenous to the respective countries. Finally, the price of Green Muscle was considered to be on the high side. It was hoped that the product would prove to be effective at 25 g/ha, because then, at a cost of less than US$ 10/ha, it would become affordable.

The participants saw possibilities of using PAN in combination with low doses of chemical pesticides. They also saw the potential of PAN in stopping bands from entering farmers' fields when used in combination with *Metarhizium*. However, they felt that more research should be done on natural hopper bands and on its toxicity and non-target effects. Steps should soon be taken to register PAN.

Annex III: Workshop agenda

S a t u r d a y 1 1 J a n u a r y

OFFICIAL OPENING
Palace Hotel

09.00	Opening remarks by Ir. Christiaan Kooyman
09.15	Welcome by the host, Dr. Magzoub Bashir
09.30	Address by Mr. Rabie Khalil of PPD
09.45	Address by Dr. Hans Wilps of GTZ/EMPRES
10.00	Opening of the workshop by his Excellency the Minister of Agriculture
	Breakfast
11.00	Departure for the ICIPE Research Station
11.30	Introduction to workshop by Mr. Kooyman
11.45	Lecture by Mr. Kooyman
12.30	Lecture by Mr. Banda
	Break for prayer
14.00	Lecture by Prof. Hassanali
	Lunch break
16.30	Departure for Salloum
17.00	Introduction of locusts into enclosures

S u n d a y 1 2 J a n u a r y

08.00	Departure for Salloum
08.30	Preparation of equipment and spray formulations Application of Green Muscle and PAN to enclosures
	Breakfast
12.00	Checking the enclosures, observe the locusts and keep birds away
12.30	Departure for PPD Quarantine Laboratory in Port Sudan
	Break for prayer
14.00	Introduction to lab work by Mr. Kpindou
14.30	Inoculation of Petri dishes for fungal colony growth and germination counts by participants
	Lunch break

M o n d a y 1 3 J a n u a r y

08.00	Departure for Quarantine Laboratory
08.30	Infection of locusts with *Metarhizium*
09.30	Estimation of concentrations of spores
	Breakfast
12.30	Estimation of 24 h germination rates of spores
	Break for prayer
14.00	Departure for Salloum
14.30	Taking samples from enclosures
	Lunch break
17.30	Checking cages in the laboratory

Tuesday 14 January

08.00 Departure for Quarantine Laboratory
08.30 Determining mortality in cages, cleaning and feeding
09.30 Examining fungal growth in Petri dishes

Breakfast

11.00 Treatment of locust hoppers with PAN and low doses of Dursban
12.00 Observing behaviour of treated and untreated hoppers
12.30 Estimating 48 h germination rates of spores

Break for prayer

14.00 Departure for ICIPE
14.30 Lecture by Mr. Kooyman

Lunch break

16.30 Departure for Salloum
17.00 Introduction of locusts into enclosures for PAN treatments
17.30 Determining mortality in cages (3 participants)

Wednesday 15 January

08.00 Departure for Salloum
08.30 Preparation of equipment and product formulations
Application of PAN and Dursban

Breakfast

11.00 Monitoring of PAN and Dursban treated hoppers
12.30 Departure for ICIPE

Break for prayer

14.00 Lecture by Prof. Bashir

Lunch break

17.30 Determining mortality in cages

Thursday 16 January

08.30 Determining mortality in cages, cleaning and feeding
Second application of PAN and Dursban at lower dose

Breakfast

Monitoring of enclosures

Free afternoon

17.30 Determining mortality in cages

Friday 17 January

08.30 Determining mortality in cages, cleaning and feeding
Monitoring of enclosures

Breakfast

Monitoring of enclosures

Break for prayer

Lunch break

17.30 Determining mortality in cages

Saturday 18 January

08.30 Determining mortality in cages, cleaning and feeding
Monitoring of enclosures

Breakfast

Monitoring of enclosures

Break for prayer

14.00 Lecture by Mr. Kooyman on toxicology and non-target effects of Green Muscle

Lunch break

16.00 Group discussions and writing of reports
17.30 Determining mortality in cages

Sunday 19 January

08.30 Determining mortality in cages, cleaning and feeding
Monitoring of enclosures

Breakfast

Group report writing

Break for prayer

Group report writing

Lunch break

Participants finalise their group reports

Monday 20 January

08.30 Plenary discussion of group reports
09.30 Discussion of organisation and content of workshop

Breakfast

11.00 Writing of combined report
12.00 Closure of workshop

Annex IV: Participants and Organizers

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