

FINAL REPORT ON FIELD EVALUATION OF GREEN MUSCLE (*Metarhizium anisopliae* var. *acridium*), AGAINST DESERT LOCUST HOPPERS IN EASTERN ETHIOPIA

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1. Introduction

Locusts and grasshoppers are serious agricultural pests that cause considerable damage to food crops and pasture grasses through out the world. At present, chemical pesticide is the main tool used for the control of locust and grasshopper outbreaks. However, the environmental and health effects of chemical pesticides e.g. toxicity to non – target organisms (Tingle, 1996) and humans (Pretty, 1996) have led to the development of environmentally friendly alternatives to locust control.

The uses of bio-control agents are considered suitable alternatives to the use of chemical pesticides for locust control (Prior and Greathead, 1989; van Huis, 1992). Of the various bio-control agents considered, the entomopathogenic fungus, *Metarhizium anisopliae* var. *acridum*, has received considerable attention as a viable alternative to chemical pesticides. The fungus is highly specific to the Acrididae to which the majority of economically important grasshoppers and locusts belong. At field application rates, it is considered safe to non-target Hymenoptera, Coleoptera and Homoptera (Hall et al., 1994; Prior, 1997), and to mammals (El-Kadi et al., 1993; Zimmermann, 1993). The fungus can be mass produced relatively easily on artificial solid substrates and when formulated in oil, can be applied under a wide range of environmental conditions using commonly available pesticide application equipment (Bateman, 1997; Langewald et al., 1999). Insects treated with

Metarhizium bio-pesticide die slowly; 70 – 90 % of the treated insects could die within 14–20 days (Lomer et al., 2001). Moreover, *M. anisopliae* has an advantage of long persistence in the field. Thomas et al (1997) reported that healthy grasshoppers migrating into treated plots can still be infected by the fungus up to three weeks after application.

Metarhizium anisopliae has been registered for use in parts of Africa (Green Muscle), and in Australia (Green Guard) and it is under evaluation in a number of other locust affected countries around the world (Thomas, 2000). Green Muscle is registered for locust control purposes in some countries in southern and western Africa and in Sudan. It is also included in the FAO's Desert Locust Pesticide Referee Group list for the control of locusts and grasshoppers in environmentally sensitive areas. It is produced and marketed by Biological Control Products (BCP) Company of South Africa. (BCP, 2002).

Although the Green Muscle (*M. anisopliae* var. *acridum*) has been tested last year against mixed populations of grasshoppers under natural conditions in eastern Ethiopia in comparison with an organophosphate insecticide (Fenitrothion), the current work is designed to evaluate Green Muscle against Desert Locust hoppers and to fulfill the requirements for the registration of this bio-pesticide for the control of grasshoppers and locusts in the country.

Green Muscle field trial during 2005

This field trial was conducted against mixed populations of grasshoppers in locust habitat in eastern Ethiopia in the middle of the rainy season. The treatments tested were Green Muscle (*Metarhizium anisopliae*), Fenitrothion 96% ULV as reference and control (untreated).

The results of the field trial showed 57.5% reduction in grasshopper populations on day 21 in Green Muscle treated plots. This seems low compared to the results obtained by other researchers. Fenitrothion provided 73% reduction on day 3. The

result also showed significant reduction in grasshopper population in the control plots. Although much effort was made to avoid contamination, some contamination had occurred. The cause of contamination could be partly due to the randomization of the treatments and partly due to the wind speed, which were 5–6 m/s at the time of application. Furthermore, the migration of grasshoppers might also have contributed to the reduction in the control plots. Therefore, the trial was repeated in 2006 season to overcome the cause of high reduction in the control plots and to meet the requirements of the registration authority for season trials.

2. MATERIALS AND METHODS

The study was conducted towards the end of the rainy season from late October to mid November, 2006 in eastern Ethiopia at forest nursery site, some 5 km north west of Dire Dawa. This area is considered favorable for the Desert Locust breeding and development. The coordinates of the study site is 093620N and 041494E and the altitude is 1185 meters above sea level.

The experimental design was a Randomized Complete Block Design (RCBD) with three replications. The plot size for each treatment was 20 m² (4 x 5 m). The spacing between plots were made according to the available area, 15 meters were kept between plots in one replicate and 10 meters spacing for the remaining two replicates. Each plot was enclosed by plastic fence (Boomas) one – meter high and the top was covered by a fabric similar to that used for making a mosquito net, to prevent locusts moving out of the plots and prevent birds from picking locusts.

Sorghum was planted in each plot three weeks before applying the treatments. A day before applying the treatments the sorghum was thinned and trimmed in order to get good coverage of the treatments.

Just before treatment, 425 3rd – 4th instars Desert Locust hoppers reared in the Desert Locust Control Organization for Eastern

Africa (DLCO–EA) field station in Dire Dawa from egg pods and mature adults obtained from Sudan were released in each plot.

The treatments tested were Green Muscle, *Metarhizium anisopliae* var. *acridum*, IMI 330189 and Fenitrothion 96% ULV as a reference material and control (untreated). The OF formulation of Green Muscle was obtained from the Biological Control Products of South Africa (Pty) Ltd. The OF formulation of Green Muscle was diluted with diesel oil to achieve dose rate of 50 gm a.i./ha, a volume application rate of 2 lt per hectare. The flow rate was 90 ml/minute and walking speed was 0.75 m/s. Germination test was done one day before application and was found to be over 80%. Fenitrothion 96% ULV was applied at the rate of 1 lt/ ha. The flow rate was 60 ml/ and walking speed was 1m/s. Both products were applied at track spacing of 6m and emission height of 1 m between 10:00–11:00 am using ULVA+ hand held spinning disc sprayer.

Before spraying the boomas were opened in one side and closed after spraying. To avoid contamination during the spray time, although appropriated distances were kept between plots, more precautionary measure was taken by spreading a plastic sheet measuring 4x5 meters held vertically by two persons at the wind direction to prevent the spray drift from contaminating the nearest plot.

To check droplet density three oil sensitive papers were placed horizontally in each Green Muscle and Fenitrothion treated plot at equidistance (1m spacing). The droplet density for fenitrothion 96% ULV was 68/m², 66/m² and 82/m² for blocks 1, 2 and 3 respectively, while the droplet density for Green Muscle treated plot was difficult to count because of the diffusion and accumulation of droplets on the edges of the oil sensitive paper. This might be due to the diesel oil, which can not stick to oil sensitive papers.

At the time of application, the dry temperature was 26 °C, the wet temperature 23 °C and RH was 78%. Sling Psychrometer (BACHARCH, INC, PGH, PA) was used for measuring the dry and wet air temperature from which percentage relative humidity

was calculated. The wind speed ranged from 2–3 m/s. which were measured using wind meter (Anemometer). Immediately after treatment, samples of 25 locust hoppers were collected from each plot and kept in aluminum cages of 25x25x30 cm, and kept under shade. Daily the cages were cleaned and the locusts were fed with untreated grass. Dead locusts were also collected daily and incubated for 24 hrs in petri dishes with a moist filter paper to check for mycosis.

Assessment of mortality in the field was carried out at three days interval for 21 days by counting dead and live hoppers. Dead hoppers in *Metarhizium* treated plots and control were incubated in moist chamber checking for mycosis to find out the cause of mortality. Dead non–target organisms were also collected to find out the effect of *Metarhizium* on non- target organisms. The data collected both in field and in the cages were transformed using square root transformation and analyzed by SAS statistical program.

The weather conditions during the trial period were mostly clear sky and sunny days except the first two days, where some overcast and small showers were recorded. The RH and the dry air temperature recorded three times per day, in the morning, mid-day and afternoon. Table 1 shows dry air temperature and relative humidity during the trial period.

Table 1: Mean dry air temperature and relative humidity during the trial period

9:00 AM		12:00 PM		03:00 PM	
D. Air Temp °C	RH %	D. Air Temp °C	RH %	D. Air Temp. °C	RH %
26	62	28	51	30	50

3. RESULTS AND DISCUSSION

Boomas populations:

During the first three days obvious reduction in treated and untreated boomas was recorded. Table 2 shows the mean survival numbers in the treated and untreated boomas. Although during these periods the reduction in the boomas treated with the Green Muscle were a slightly higher than the untreated boomas, it is unlikely that *Metarhizium* can act as fast. However, this reduction could be due to the environmental factors and to escaping. The population counts after day 3 in the untreated boomas remained almost constant over the whole observations period, while the counts in the boomas treated with *Metarhizium anisopliae* (GM) declined with time and differed significantly from the control boomas, GLM = 1543.16 (2df) $P < 0.0001$. A clear reduction was observed on the boomas treated with the GM from day 9 onwards, while the highest percentage reduction was recorded on day 12 and day 15. Figure 1 shows the average percentage reduction in the treated and untreated boomas, while figure 2 shows the cumulative numbers of cadavers in the treated and untreated boomas. The number of cadavers from the GM treated boomas peaked on day 15 (Table 3). When comparing table 2 & 3, it is obvious that more cadavers disappeared from the boomas. Although boomas were carefully watched, those are likely to have been taken by ants, cricket, beetles etc. The first mycosis mortality was recorded on day 8 in all boomas. Moreover, the numbers of the dead infected hoppers increased slowly. At that time, the majorities of the treated hoppers were much less active than the untreated ones and could easily be caught. They were found in patches on the ground, while the untreated hoppers were almost observed on the vegetation. This may have been a behavioural fever response (Kooyman & Godonou, 1997; Blandford et al., 1998), accounting for the delay in mortality compared with that in cages. At the end of the trial period, over 84 % reduction is recorded on the hoppers density treated with the GM and only 26% reduction was recorded on the untreated hoppers (Figure 1). Cadavers collected from boomas treated with the GM showed over 75% mycosis (Figure 3). It is worth to mention that no mycosis appeared on the dead insects

collected from the control and incubated under humid conditions, which indicates that contamination during the spray time was almost zero. Fresh cadavers and dying of hoppers were observed until the last day of the trial.

Fenitrothion is known as a quick acting pesticides and over 65% mortality was recorded on day zero and reached 100% by day 3 (Figure 1).

Table 2: Mean numbers of survival hoppers in the treated and untreated boomas population

Days	GM	CONT	FEN
0	400.0	400.0	139.3
3	255.0	322.7	0
6	241.7	302.0	0
9	211.3	301.0	0
12	168.7	301.0	0
15	145.3	301.0	0
18	103.7	298.3	0
21	63.7	294.7	0

Table 3: Numbers of cadavers collected the treated and untreated Boomas

Days	GM	CONT	FEN
0	0	0	782
3	7	0	0
6	13	3	0
9	32	4	0
12	28	3	0
15	43	2	0
18	38	3	0
21	26	0	0

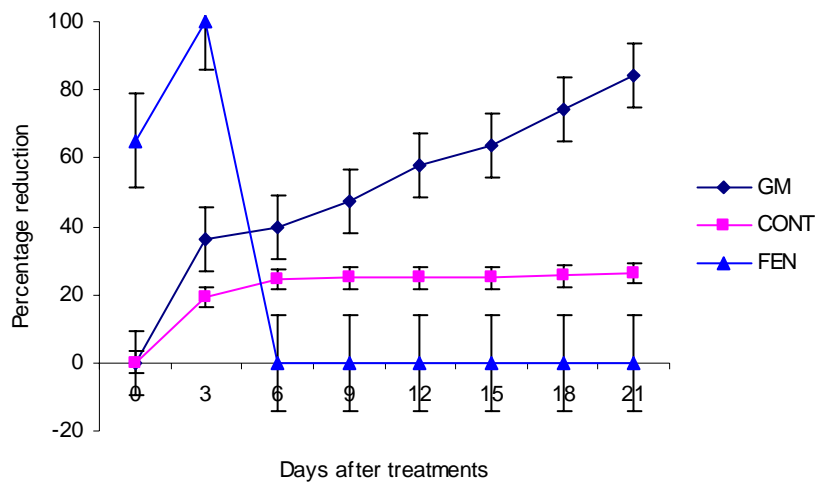


Figure 1: Average percentage reduction of hoppers in the treated and untreated boomas

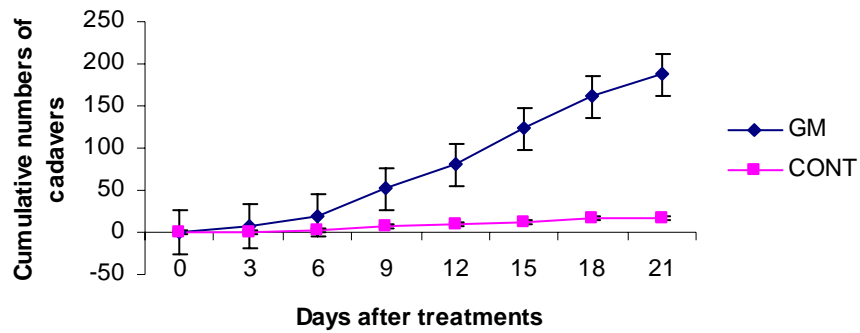
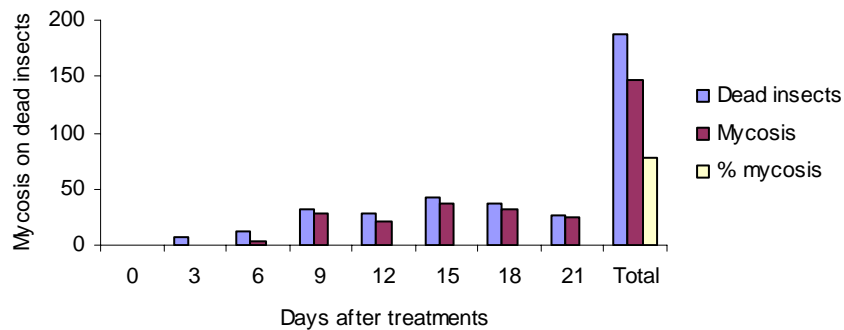


Figure 2: Cumulative numbers of cadavers in the GM and untreated boomas



**figure 3: Mycosis on dead insect collected from GM boomas
Cages:**

The daily mortality of the hoppers in cages is shown in table 4, while figure 4 shows survival percentage of the treated and untreated hoppers in cages. It is obvious that the highest mortality in GM cages occurred on day 9 and 100% mortality was recorded by day 14, while during these period only 13.3% was recorded in control cages.

Table 4: Total daily mortality in cages

Days	GM	Cont	Fen
0	0	0	74
1	1	1	74
2	1	0	75
3	0	1	0
4	0	0	0
5	3	1	0
6	1	1	0
7	7	0	0
8	14	1	0
9	26	1	0
10	9	0	0
11	6	1	0
12	4	0	0
13	2	1	0
14	1	2	0
Total	75	10	75

Most of the cadavers from the treated cage turned red and the cause of the death was confirmed after plotting them in Petri Dishes containing moist filter papers. The calculated percentage mycosis was 93% (Figure 5), which indicated that the spray pick-up had been satisfactory. The high mortality in the cages indicates that spray cover in the boomas was appropriate.

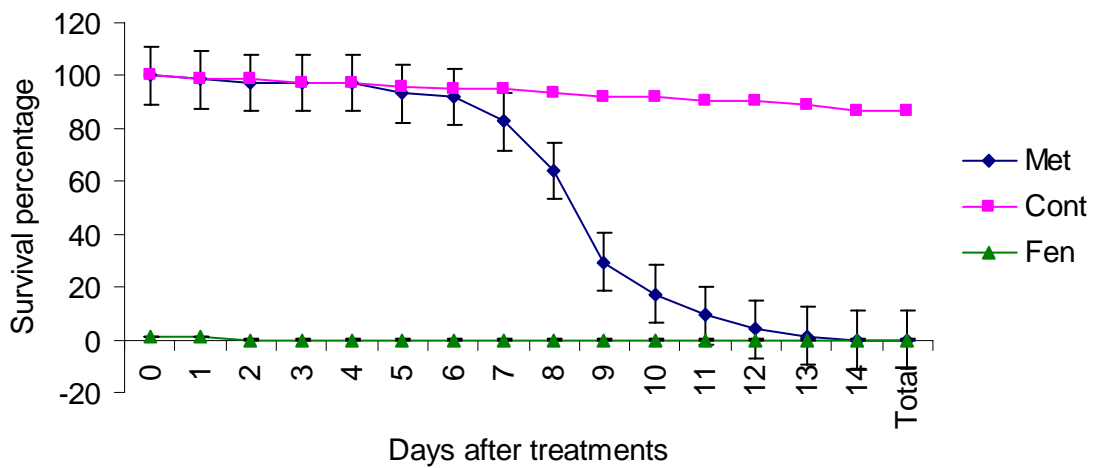


Figure 4: Survival percentage of hoppers in the treated and untreated cages

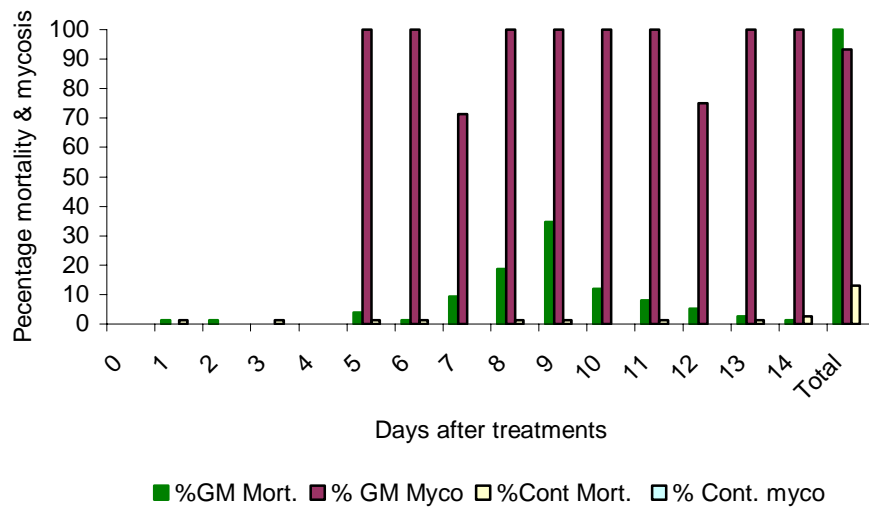


Figure 5: Percentage mortality & mycosis on dead insect collected from GM and control cages

RECOMMENDATION

The overall population reduction achieved with the application of *Metarhizium* (GM) against Desert Locust hoppers instars over a period of 21 days in this trial are among the best results achieved with Green Muscle against Desert Locust. A result obtained from the recommended doses at the rate of 50 g a.i./ha (5×10^{12} spores) in 2 L formulated with diesel oil, under field conditions have significant potential for development as myco-pesticide for bio-control of locusts. This result confirms the findings of other researchers who explored the efficacy of Green Muscle against Locust and grasshoppers. Now, the result of this trial is valid for recommendation and registration of Green Muscle (*M. anisopliae*) against Desert Locust in Ethiopia

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References

- Bateman, R.P. (1997). Methods of application of microbial pesticide formulations for the control of locusts and grasshoppers. *Nemoris of Entomological Society of Canada BCP* (Biological Control Products). (2002). Green Muscle Handbook for Central and Southern Africa. LUBILOSA. **1171**, 69-81.
- Blanford, S. Thomas, M. B. & Langewald, J. (1998). Behavioural fever in the Senegalese Grasshopper, *Oedaleus senegalenses*, and implication for biological control using pathogens. *Ecological Entomology* **23**, 9-14.
5pp
- El- M. Kadi., L. S. Xara, P.R. De Matos, J. V. N. Da Rocha & D. P. de Oliveira, Kadi, 1993. Effects of the entomopathogen *Metarhizium anisopliae* on guinea pigs and mice. *Environmental Entomology* **12**: 37-42.
- Hall. F.R., A. C. Chapple, R. A.J. Taylor, and R.A. Downer. 1994. Dose transfer of *Bacillus thuringiensis* from cabbage to the diamond back moth: A graphical simulator. *Journal of Environmental Science and Health* **B29** (4): 661-678.
- Kooyman, C. & Gondonou, I. (1997). Infection of *Schistocerca gregaria* (Orthoptera: Acrididae) hoppers by *Metarhizium flavoviridae* (Deuteromycotina: Hyphomycetes) conidia in an oil formulation applied under desert locust conditions. *Bulletin of Entomological Research* **87**, 105-107.

- Langewald, J., Ouambama, Z., Mamadou, A., Peveling, R., Stolz, I., Bateman, R., Attignon, S., Blandford, S., Arthurs, S., Lomer, C. (1999). Comparison of organophosphate insecticide with a mycoinsecticide for the control of *Oedaleus senegalensis* Krauss (Orthoptera: Acrididae) and other Sahelian grasshoppers in the field at operational scale. *Biocontrol Science and Technology* **9**, 199-214.
- Lomer, C.J., Bateman, R.P., Johnson, D.L., Langewald, J. and Thomas, M.B. (2001). Biological control of locust and grasshoppers. *Annual Review of Entomology*, **46**, 667-702.
- Pretty, J. N (1996). Regenerating Agriculture: Policies for Sustainability and Self-reliance. Earthscan Publications Ltd., London, England. 320 pp.
- Prior, C and Streett, D. (1997). Strategies for the use of entomopathogenic agents in the biological control of locusts and grasshoppers. *Memoirs of the Entomological Society of Canada* **171**, 5-25.
- Prior, C. and Greathead, D.J. (1989). Biological control of locusts: the potential for exploitation of pathogens. *FAO Plant Protection Bulletin* **37**, 37-48.
- Thomas, M.B., Blandford, S., and Lomer, C.J. (1997). Reduction of feeding by the variegated grasshopper, *Zonoceros variegatus*, following infection by the fungal pathogen, *Metarhizium flavoviride*. *Biocontrol Science and Technology*, **7**, 327-334.
- Thomas, M.B. (2000). Development of mycopesticide for biological control of locusts in Southern Africa. In: R.A. Cheke, L.J. Rosenberg, Kieser, M.E (eds.) *Research Priorities for Migrant Pests of Agriculture in Southern Africa* Proceedings of a DFID/NRI/ARC-PPRI workshop, Pretoria, South Africa, 24-26 March 1999. Natural Resources Institute, Chatham, UK, pp. 173-182.
- Tingle, C.C.D (1996). Sprayed barriers of diflubenzuron for control of migratory locust (*Locusta migratoria* capito (Sauss)) [Orthoptera: Acrididae] Madagascar: Short term impact on relative abundance of terrestrial non-target invertebrates. *Crop Protection*, 15(6): 579-592.
- Van Huis, A. 1992. New developments in desert locust management and control. *Proceedings of Experimental and Applied Entomology* N.E.V. Amsterdam 3: 2-18.

Zimmermann, G. (1993). The entomopathogenic fungus
Metarhizium anisopliae and its potential as biological
control agent. *Pesticides Science*. 37, 375-379.

**Desert Locust Control Organization for Eastern Africa (DLCO – EA)
Financial Report for Green Muscle Biocontrol Project**

Expenditure for the Period November 2005 – November 2006

No.	Description	Amount in US \$
1.	Laboratory Equipment and Materials	
	(a) Petri dishes, test tubes, ethanol Denatured alcohol, cotton	363.86
	(b) Other Materials Plastic sheets, timber and wooden materials, Plastic caps, flag cloth, dry cell batteries, wire mesh, powder soap, measuring tape, rope	125.28
	(c) Labor for Tailoring plastic sheets, sewing boomas, Removing boomas Guarding field site Field Assistant	166.43 25.67 146.40 315.50
	(d) Stationery Note book, glue, clip board, brush, Computer paper, metal tray, stapler, Classer	156.48
	Total	1,299.62
2.	Operational Expenses	
	(a) Motor vehicle fuel, oil & lubricants	171.35
	(b) Motor vehicle tires & tubes	185.49
	(c) Vehicle spare parts	1054.11
	Total operational expenses	1,410.95

3. Travel Expenses

Senior Research Officer 14 days x US 20	280.00
Research Officer 45 days x US 20	900.00
Dire Dawa Base Manager 20 days x 17.50	350.00
Ministry of Agriculture Expert 14 days x US20	280.00
Driver 14 days x US 20	280.00
Air Tickets for the Research Officer	298.98

Total travel expenses	2,388.98
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Expenditure for March 2005 – October, 2005

1. Operational expenses & travel	326.90
1. Laboratory equipment and materials	918.63
2. Operational expenses	682.28
3. Travel expenses	2,032.26

Total	3,960.07
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Total Expenditure for the period March 2005 – November 2006

1. Laboratory equipment & materials	2,218.25
2. Operational expenses	2,093.23
3. Travel expenses	4,748.14

Grand Total	9,059.62
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In the Letter of Agreement signed between FAO/EMPRES – CRC and DLCO – EA, the budget approved for this research project is US \$ 15, 000. Two payments were received amounting US\$ 9,500.00
First expenditure US\$ 3,960.07
Second expenditure US\$ 5099.55
Total expenditure US\$ 9,059.62
Balance unspent US\$ 440.38

After deducting the unspent amount of the first two payments, US\$ 440.38, the remaining amount of the final payment would be **US\$ 5,059.62**, which will be used for strengthening DLCO–EA’s bio-pesticides research programme by procuring laptop computer and statistical package soft ware and for training staff on data analysis.