# APPLICATION OF POWDER FORMULATION OF *Metarhizium anisopliae* TO CONTROL *Oryctes rhinoceros* IN ROTTING OIL PALM RESIDUES UNDER LEGUMINOUS COVER CROPS

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**ABSTRACT**

*The powder formulation of* Metarhizium anisopliae *was applied by spraying method onto the rotting heaps of oil palm residues under the leguminous cover crops. The* M. anisopliae *infected all stages of* Oryctes rhinoceros. *At eight months after treatment (MAT), the application of product at rates of T1, 0.2 g (2.2 x $10^7$ spores) and T2, 0.4 g (4.4 x $10^7$ spores) $m^{-2}$ heap was significantly reduced ($P<0.05$) the L2 and L3 larvae, pre-pupae and pupae. Reduction of these four stages has reduced the overall* O. rhinoceros *population in T1 to 30.8 individual per sampling plot (ISP) and T2 to 41.0 ISP, significant lower ($P<0.05$) as compared to population in the control (132.1 ISP).*

*The growing of cover crops delayed the impact time of the fungus, but it provided protection to the fungus from the detrimental factors, giving favourable conditions to the spores to grow and initiate infection. Therefore, at eight MAT, as the population was significantly reduced, the infection levels were increased to 33.3% in T1 and 30.4% in T2, both were significantly higher ($P<0.05$) as compared to control (12.9%). Then it was further increased to 52.0% (control), 67.8% (T1) and 72.9% (T2) at 12 MAT. The use of the product at both rates did not affect the non-target insects. The oil palm pollinating weevil,* Elaeidobius kamerunicus *was not affected as they was no difference in the population density in the treated and control plots. The population density of the stag beetle,* Aegus chelifer *in both treated plots was always not significant ($P>0.05$) with the control at all times of data recording. Although infection can occur on larvae and adults, but the percentage was low, ranging from 0% - 0.6% at five MAT and 1.7% and 2.5% at eight MAT.*

**Keywords:** *Metarhizium anisopliae, Oryctes rhinoceros*, powder formulation, *Elaeidobius kamerunicus,* biological control.

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## INTRODUCTION

The use of *Metarhizium anisopliae* to control the rhinoceros beetle (*Oryctes rhinoceros*) was initiated in the coconut area by Latch (1976). Of 27 isolates tested, it was found only three isolates producing promising results, later identified as *M. anisopliae* var. *major*, having a spore length of 9-18 μm (Tulloch, 1979; Rombach *et al.*, 1986). These isolates were further tested by applying the sporulated growing medium of oat to breeding of the pest (Latch and Fallon, 1976). A laboratory assay on larvae of *O. rhinoceros* collected from oil palm fields showed that the fungus killed 100% of them within 12-14 days (Ramle *et al.*, 1999a). A field test by applying the sporulated medium and spore solution onto the

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breeding sites of the pest was effectively reduced the population of *O. rhinoceros* as early as three months after application (Ramle *et al.*, 1999b).

The toxicity study of the *M. a.* var. *major* on rats, fish and a coleopteran insect was carried out by Ramle *et al.* (2004). On rat, the oral $LD_{50}$ was > 5000 mg $kg^{-1}$ and dermal $LD_{50}$ was > 2000 mg $kg^{-1}$. The fungus also did not affect the freshwater fish, *Tilapia nilotica*, at the treatment concentrations of 0.1, 1.0, 10.0 and 100.0 mg spores $L^{-1}$. To the stag beetle, *Aegus chelifer*, direct application of the spore solution caused mortality of 33.3% - 83.3% on the larvae.

Mass production of spores using solid medium led to the development of powder formulation of the fungus (Ramle *et al.*, 2005a). The developed product extended the life span of the spores to about nine months. It is easy to handle, store and apply, especially in the field that required big quantity of the product. Small scale tests by applying the product onto the artificially plots made from field collected rotting oil palm materials showed that the product killed 100% larvae between three to five weeks after the application (Noor Hisyam *et al.*, 2005).

In this study, the product was further tested in the replanting areas where the rotting of chipped oil palm materials were already fully covered by leguminous cover crops, as one of the control measures of the *O. rhinoceros* (Wood, 1968). The effectiveness of the formulation to control the *O. rhinoceros* and their effects to the oil palm pollinating weevil, *Elaeidobius kamerunicus* and *Aegus chelifer* were evaluated.

## MATERIALS AND METHODS

### Experimental Area

The study was conducted in an inland area at Kekayaan Estate, Paloh, Kluang, Johor. The size of the experimental area is 120 ha, located in one end of the replanted block of more than 1000 ha. The area is slightly undulating to hilly. The estate practiced the under-planting replanting technique. The replanted palm is the commercial hybrid *Tenera*. At the commencement of the experiment, the age of palm was about 2-year-old. The age of the rotting oil palm materials is about 15-month after felling. They were chipped, heaped and arranged at every alternate palm rows, and left to rot in the field naturally. The rotting heaps were already covered with leguminous cover crops and grasses, at the start of the experiment.

### Preparation of Powder Formulation

The spores of *M. anisopliae* were produced on solid medium of broken maize following the method developed by Ramle *et al.* (2005a). Production was first made by propagating the fungal mycelia in a liquid media containing sucrose (1.0 %), yeast extract (0.25 %) and peptone (1.0 %). After agitation of the liquid media at 28°C, 166 rpm for four days, the mycelia was then used to inoculate 250 g (wet weight) of autoclaved maize that was placed in the autoclavable plastic bags. The bags were then fermented in the dark at 28°C until maturity of 30 days.

The spores were separated from the media by washing them with water containing a weak solution (0.0002% v/v) of wetting agent (Triton 20). To separate the media, the mixture was then filtered via 200 µm mesh. The collected spore solutions were further filtered through a filter paper (No. 1, double ring) using vacuum filtration machine. The wet spore cakes were dried in a refrigerator at 10°C for about 9-12 hr and another hour in a circulating air chamber at 28°C. The dried spore cakes were then ground to powder. The viability of the dry spores was measured by counting the germinated spores on potato dextrose agar (PDA) plus 0.02% w/v anti-bacterial, chloramphenicol. Spores with viability of more than 80% were used to prepare the powder formulation.

The spores were homogenously mixed at a ratio of 20% spores and 80% kaolin (moisture content < 2%, particle size < 40 µm). The product (1 kg) was packed in a 2.8-litre plastic bottle and stored at 25°C prior the commencement of the experiment.

### Experimental Design and Application Rate

The experiment was conducted in a randomized complete block design (RCBD). Two application rates were tested, which were Treatment 1 (T1) 0.2 g (2.2 x $10^7$ spores) and Treatment 2 (T2) 0.4 g (4.4 x $10^7$ spores) product per square metre ($m^2$) of rotting materials, respectively. Plot in control (Ctr) were untreated. Each treatment was replicated four times in blocks that consisted of three 10-ha plots. Each plot contained 40 palms x 40 palms and consisted 20 rows of rotting heaps. The row dimension of the rotting heaps is 240 m (length) x 2.0 m (width) x 0.3 to 0.5 m (height) (*Figure 1*). There are two rows of rotting heap in 1 ha. The rotting heap in a hectare is therefore estimated about 960 $m^2$ (2 rows x 240 m length x 2.0 m width).

### Delivery Method

The product was first pre-mixed in a 50-litre container which contained about 30-40 litre of water added with weak solution (0.0002%) of wetting agent. The mixture was thoroughly mixed until homogenous, then poured into a 6000-litre sprayer tank. The solution was then sprayed directly onto the heaps using a 1 bar pressure sprayer that is

attached to a tractor. The sprayer has two nozzles, each one placed at opposite directions. As the tractor moved, two rows of rotting heaps were sprayed simultaneously. The rate of water used was about 0.75 litre $m^{-2}$ of rotting heap, which was about 720 litre $ha^{-1}$ (0.750 litre x 960 $m^2$).

**Parameters and Methods of Recording**

The following parameters were determined:

- the populations of larval stages, first instars (L1), second instars (L2) and third instars (L3), pre-pupae, pupae and adults in the breeding sites;
- the dead and infected stages of *O. rhinoceros* by *M. anisopliae*;
- the population and infection of *M. anisopliae* on the stag beetle, *Aegus chelifer*;
- the population of oil palm pollinating weevil, *Elaeidobius kamerunicus;* and
- the population of adults of *O. rhinoceros* sampled from the pheromone trap.

Parameters 1, 2 and 3 were collected from three identified rows of A, B and C (*Figure 1*). Row A was located at row No. 5, B at row No. 10 and C at row No. 16. In each row, the samples were collected from three sampling plots (SP) of 2 palms x 2 palms of dimension 8.8 m x 2.0 m (17.6 $m^2$). This gave a total of nine SP in each treatment plot. The first SP (A1) was located 10 palms from the first palm in the front row, the second SP (A2) was located next 10 palms from A1 and the third SP (A3) was at another 10 palms from the A2. Samples of the rhinoceros beetle and stag beetle were collected by manually digging and removing the heaps. All live and dead insects were then sorted according to their developmental stages. The next sampling was done on a new SP of 2 palms x 2 palms next to the previous SP.

Assessment of populations was conducted before the treatment, 1, 3, 5, 8 and 12 months after treatment (MAT).

**Population of Pollinating Weevil**

The population of pollinating weevil was collected at least from nine palms that bore one to three days post-anthesized male inflorescences. Nine spikelets were samples from the inflorescence (three spikelets each from upper, middle and bottom regions) and must be done before 12 am while the weevil was still in the spikelet. The average number of adults on each spikelet was counted and compared.

**Population of Adults of *O. rhinoceros* Captured by Trapping**

Five traps were placed in each of the treatment plots (*Figure 1*). Traps 1 and 2 were placed at the sampling row A at SP A1 and SP A3. Trap 3 was placed at the centre of treatment plot at row B at SP B2. Traps 4 and 5 were placed at row C at SP C1 and SP C3. The traps were monitored for a period of two weeks, started from the first day of recording activities of the above parameters. The total numbers of captured adults from all traps were compared among treatments.

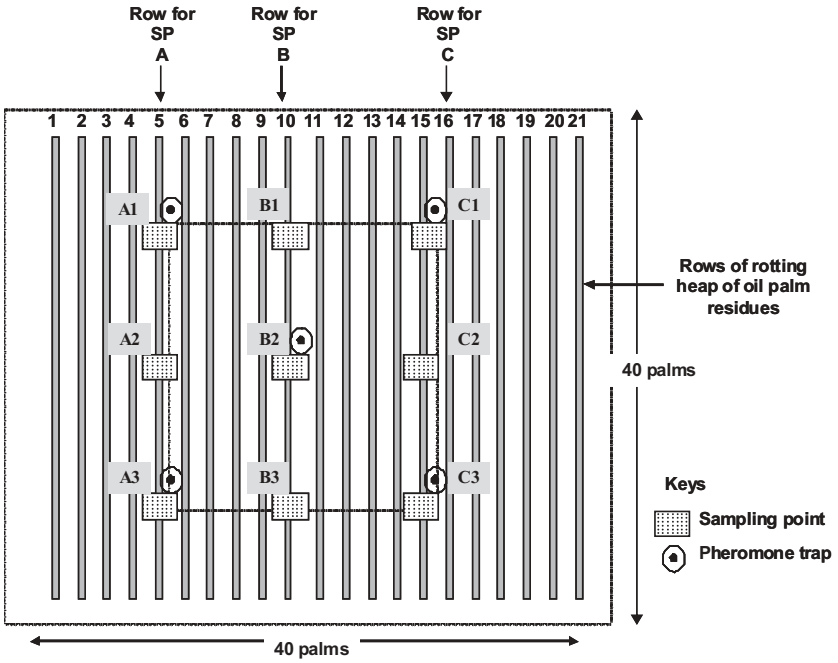


*Figure 1. The layout of a treatment plot showing the location rows of rotting heaps, the sampling plots and the pheromone traps.*

**Statistical Analysis**

The mean numbers of immature stages, adults of *O. rhinoceros* and the stag beetle collected from the breeding sites, the adults of *O. rhinoceros* captured from pheromone traps and the pollinating weevil were analysed using analysis of variance (ANOVA) and the means were separated by the least significant difference (LSD) at P = 0.05 (SAS Institute, 1990). The percentage infection of *M. anisopliae* on *O. rhinoceros* and stag beetle were angularly transformed prior to ANOVA and the means were also separated by LSD test at P = 0.05. Presented figures with bars in groups with the same letters are not significantly different (P>0.05) after the LSD test.

## RESULTS

The rotting heaps in the experiment contained all developmental stages of the *O. rhinoceros*, as estimated before the study (*Figure 2*). The mean population density of *O. rhinoceros* was 66.94 individual per sampling plot (ISP). The L3 larvae contributed the highest numbers (41.6 ISP or 62.2%) followed by L2 larvae (12.4 ISP or 18.5%), adults (4.4 ISP or 6.5%), L1 larvae (4.1 ISP or 6.1%), pupae (3.2 ISP or 4.8%) and pre-pupae (1.2 ISP or 1.9%).

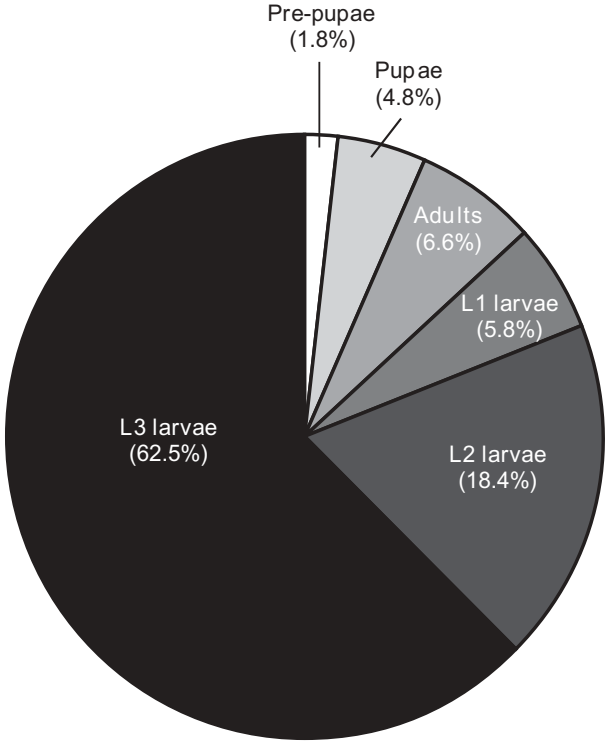


*Figure 2. The population density and percent distribution of each developmental stage of* O. rhinoceros *in the experimental area, as estimated before the commencement of experiment.*

**Effects of *M. anisopliae* on the Larvae of *O. rhinoceros***

For the whole period of 12 months, no difference in the number of surviving L1 larvae in the treated and control plots was observed, except in the treatment T1 at eight MAT (*Figure 3a*). The population of L1 larvae fluctuated between two and six ISP, then reduced to below than one ISP at 12 MAT.

At three MAT onward, the numbers of surviving L2 (*Figure 3b*) and L3 larvae (*Figure 3c*) in the plots T1 and T2 gradually declined. The significant decline of L2 larvae was only recorded in the T1 (four ISP) at eight MAT. The declining trend was also observed on the L3 larvae. At eight MAT, in both plots T1 and T2, the number of L3 larvae were about the same (20 ISP), significantly lower (P<0.05) than the control (80 ISP). At 12 MAT, in the treated plots, the populations of the L2 and L3 larvae were reduced to less than two and five ISP, but not different (P<0.05) than the control.

**Effect of *M. anisopliae* on Pre-Pupae and Pupae of *O. rhinoceros***

Significant reduction of population density of the pre-pupae and pupae was only observed at eight MAT. The numbers of surviving pre-pupae in both plots T1 and T2 were below than one ISP, while the pupae was below than two ISP (*Figures 4a* and *b*), significantly lower (P<0.05) than the control (1.7 ISP for pre-pupae and 3.22 ISP for pupae).

**Effect of *M. anisopliae* on Adults of *O. rhinoceros***

The number of adults in both treatment plots was gradually increased and peaked at three MAT for T1 and at five MAT for T2 (*Figure 5a*). At eight MAT, the population of adults only in the T1 was reduced significantly (P<0.05) to 1.6 ISP as compared to control (5.9 ISP), while the population of adults in the T2 (3.3 ISP) was not significant. Afterward at 12 MAT, the population of adults in both treatments was significantly lower (P>0.05) than control.

The number of adults captured by the pheromone trap in a week is showed in *Figure 5b*. The population of adults in the treatment plots was not significantly different (P>0.05) than the control plot.

The reductions in number of L2 and L3 larvae, pre-pupae and pupae in the treatment plots had contributed to a significant reduction in the overall *O. rhinoceros* populations at eight MAT (*Figure 6*). The population density of *O. rhinoceros* in the respective T1 and T2 was 30.8 and 41.0 ISP, significantly lower (P<0.05) as compared to control (132.1 ISP).

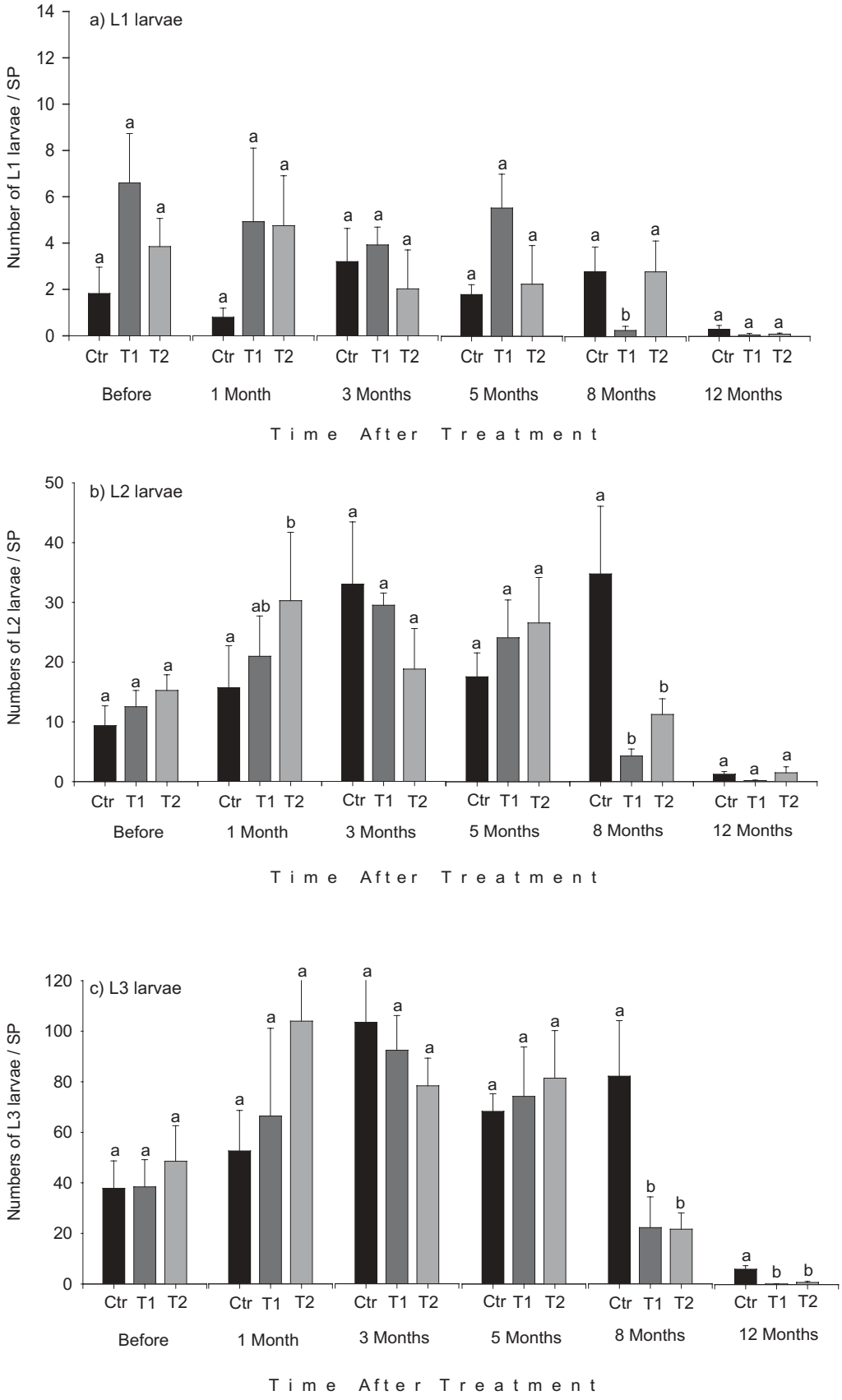


*Figure 3. The effects of* M. anisopliae *applied as powder formulation on the populations of (a) L1 , (b) L2 and (c) L3 larvae of* O. rhinoceros *before and after the treatment. Ctr: untreated control, T1: 0.2 g m$^{-2}$ rotting heap, T2: 0.4 g m$^{-2}$ rotting heap.*

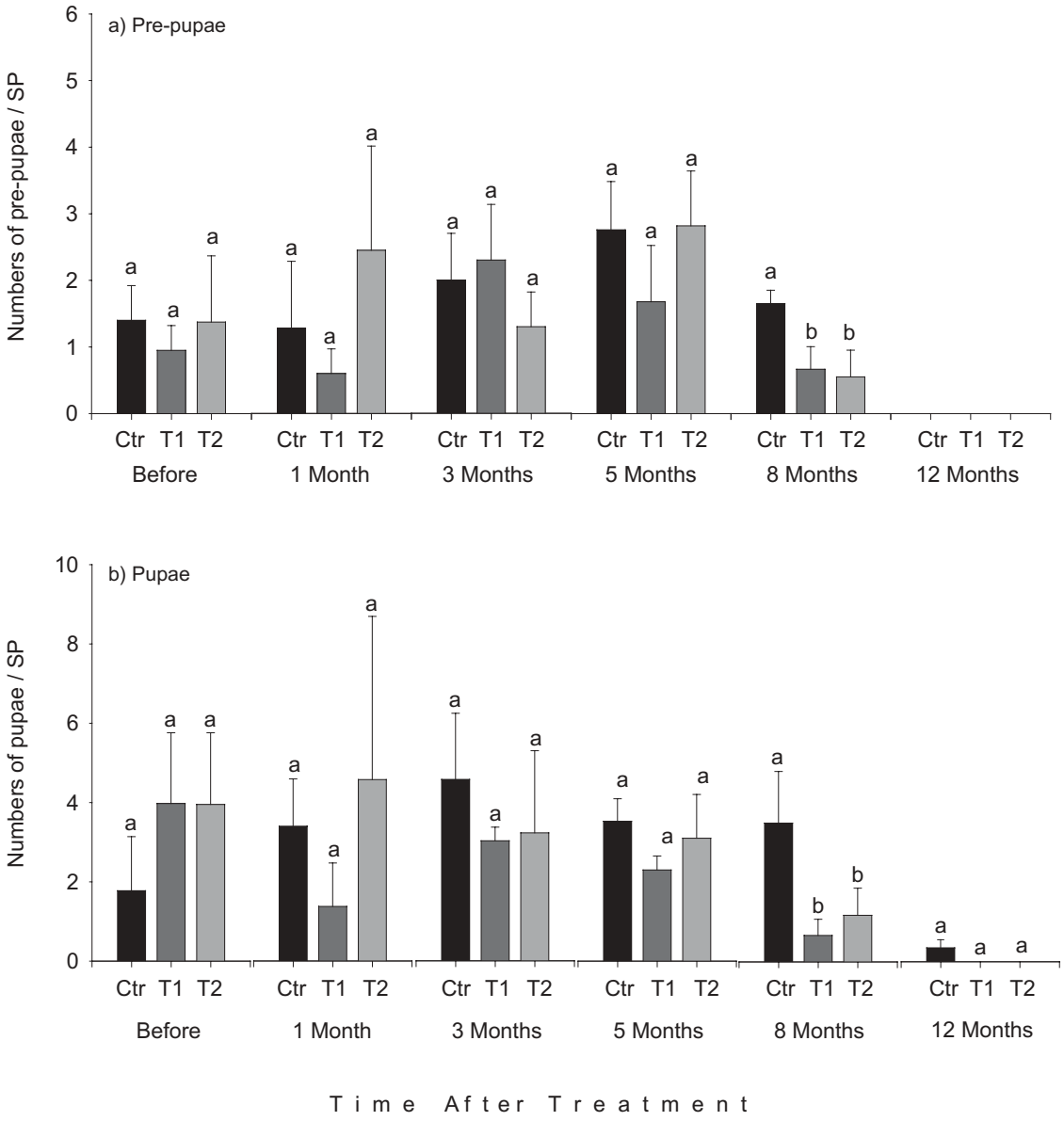


*Figure 4. The effects of* M. anisopliae *applied as powder formulation on population of (a) pre-pupae and (b) pupae of* O. rhinoceros *beetles. Ctr: untreated control, T1: 0.2 g m$^{-2}$ rotting heap, T2: 0.4 g m$^{-2}$ rotting heap.*

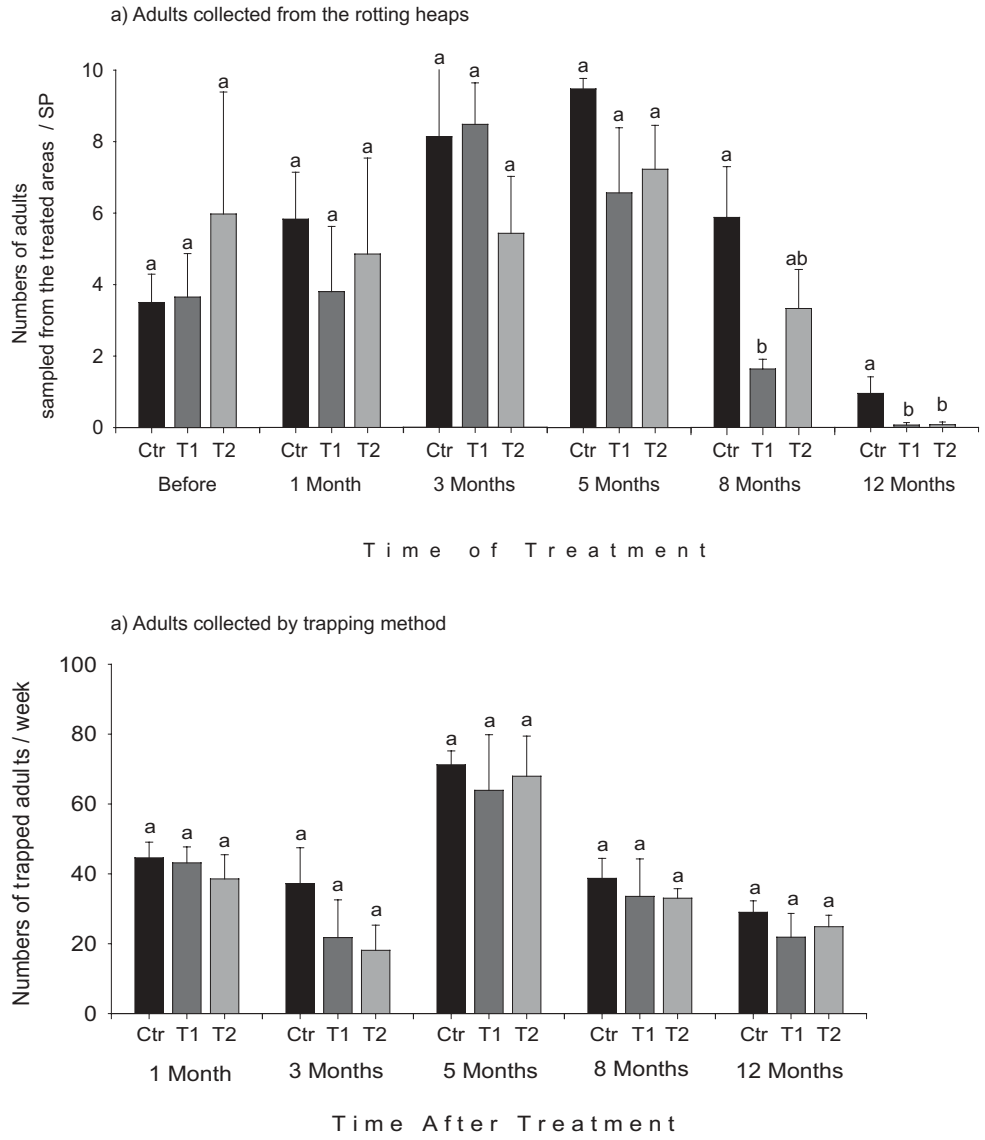


*Figure 5. The effects of* M. anisopliae *applied as powder formulation on population of adults of* O. rhinoceros *census from (a) the breeding site and (b) by trapping method. Ctr: untreated control, T1: 0.2 g m$^{-2}$ rotting heap, T2: 0.4 g m$^{-2}$ rotting heap.*

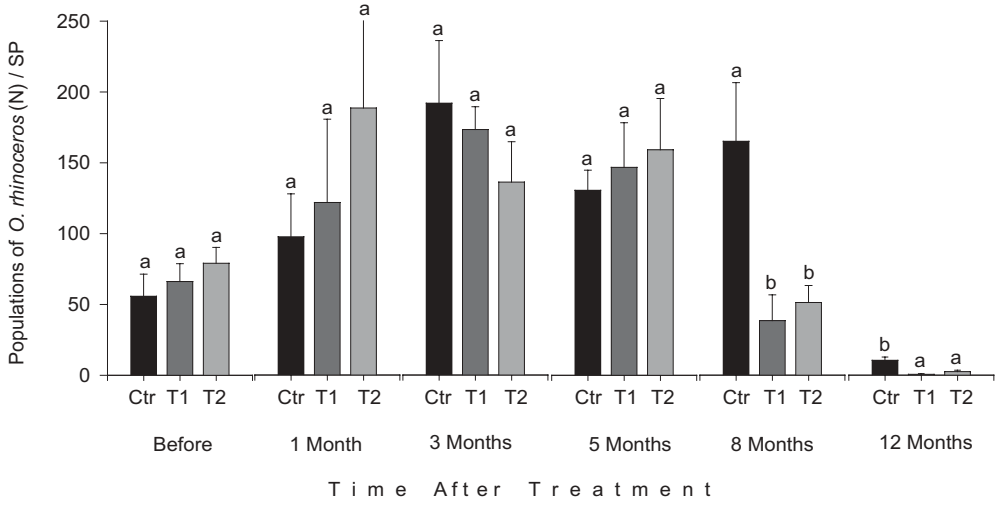


*Figure 6. The effects of* M. anisopliae *applied as powder formulation on overall populations of* O. rhinoceros. *Ctr: untreated control, T1: 0.2 g m$^{-2}$ rotting heap, T2: 0.4 g m$^{-2}$ rotting.*

**Infection of *M. anisopliae* on *O. rhinoceros***

*M. anisopliae* infected all stages of *O. rhinoceros*. On larvae, pre-pupae and pupae, infection was confirmed when there were brownish or black lesions appeared scattered on the insect cuticle. Advanced infection occurred when the dead insect become hardened or slightly shrunken, finally covered by whitish hyphae, which later turned to green as the spores matured. Multiple infections of *M. anisopliae* were commonly observed.

The infection of the fungus in each stage of *O. rhinoceros* was determined (*Table 1*). As expected, the percentage of infection had increased over time. The infected *O. rhinoceros* in the control plot was commonly found, in which the levels were also increased over time as in the treatment plots. At one, three and five MAT, the infection levels in the control and treatment plots, except for T2 at three MAT, were not different (P>0.05). But then at eight MAT, the infection in plots T1 and T2 was increased to 33.3% and 30.4% respectively, significantly higher (P<0.05) as compared to control (12.6%). At 12 MAT, the infection levels increased more than double (T1, 67.8% and T2, 72.9%), although the differences as compared to control (52.0%) was not significant (P>0.05).

Infection of *M. anisopliae* on each developmental stage of *O. rhinoceros* was also monitored (*Table 1*). The infected L1 larvae (0.13%) were only observed in the plot T2 at eight MAT. On L2 larvae, the infection of *M. anisopliae* was only observed in the plot T1 as soon as one MAT. Later, it was observed in both treated and in the control plots. On L3 larvae, infection of *M. anisopliae* was always found at every recording, at the highest level than the other stages. The L3 infection peaked at eight MAT to 8.09% in the control and 20.0% in the plot T1 and 17.7% in the T2. At 12 MAT, infection in treatment plots reduced to 6.9% (T1) and 8.6% (T2).

The infected pre-pupae and pupae were observed as early as one MAT, only in the plot T2, before it was always found in all plots. The infection level was always higher on pre-pupae than pupae.

Infected adults were constantly found at every data recording and the highest level was at 12 MAT.

**The Effect of *M. anisopliae* to Non-Target Organisms**

The application powder formulation of *M. anisopliae* did not affect the stag beetle, *Aegus chelifer*. The population of this beetle in both treated plots T1 and T2 was always not significantly different (P>0.05) than control (*Figure 7a*). Although, infected larvae or adults could be found, the level was very low ranged between 0.1% and 0.6% at five MAT and 1.7 and 2.5% at eight MAT (*Figure 7b*).

The population of pollinating weevil in the treatment and control plots collected from the spikelets of anthesising male inflorescences at eight MAT was maintained higher (*Figure 8*). This suggested that the use of *M. anisopliae* did not affect the development of the pollinating weevil.

**TABLE 1. THE INFECTION LEVEL OF EACH DEVELOPMENTAL STAGE OF *O. rhinoceros* AFTER TREATED WITH POWDER FORMULATION OF *M. anisopliae***

| Month after treatment (MAT) | Treatment | Infection of *M. anisopliae* (%) on each stage of *O. rhinoceros* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | L1 | L2 | L3 | Pre-pupae | Pupae | Adult | Total* |
| 1 | Ctr | 0 | 0 | 0.71 | 0 | 0 | 0.32 | 1.03 a |
| | T1 | 0 | 0.03 | 2.14 | 0 | 0 | 0.50 | 2.67 a |
| | T2 | 0 | 0 | 1.70 | 0.05 | 0.02 | 0.31 | 2.08 a |
| 3 | Ctr | 0 | 0 | 1.45 | 0.02 | 0.02 | 0.66 | 2.15 a |
| | T1 | 0 | 0.06 | 2.93 | 0.10 | 0.02 | 0.64 | 3.75 ab |
| | T2 | 0 | 0.07 | 5.25 | 0.24 | 0.14 | 1.16 | 6.86 b |
| 5 | Ctr | 0 | 0.03 | 2.76 | 0.05 | 0.03 | 1.77 | 4.64 a |
| | T1 | 0 | 0.99 | 11.1 | 0.11 | 0.03 | 2.66 | 14.90 a |
| | T2 | 0 | 1.17 | 8.70 | 0.17 | 0.13 | 2.20 | 12.40 a |
| 8 | Ctr | 0 | 0.69 | 8.09 | 0.15 | 0.06 | 3.63 | 12.60 a |
| | T1 | 0 | 3.39 | 20.00 | 0.28 | 0.28 | 9.38 | 33.30 b |
| | T2 | 0.13 | 3.63 | 17.70 | 0.50 | 0.39 | 8.05 | 30.40 b |
| 12 | Ctr | 0 | 5.83 | 14.60 | 0.40 | 0.40 | 30.80 | 52.00 a |
| | T1 | 0 | 3.47 | 6.94 | 0.69 | 0 | 56.70 | 67.80 a |
| | T2 | 0 | 2.15 | 8.60 | 0.27 | 0 | 61.90 | 72.90 a |

Notes: Ctr: untreated control, T1: 0.2 g $m^{-2}$ rotting heap, T2: 0.4 g $m^{-2}$ rotting heap.
*Number at each MAT with the same letters are not significantly different (P>0.05) after least significant difference.

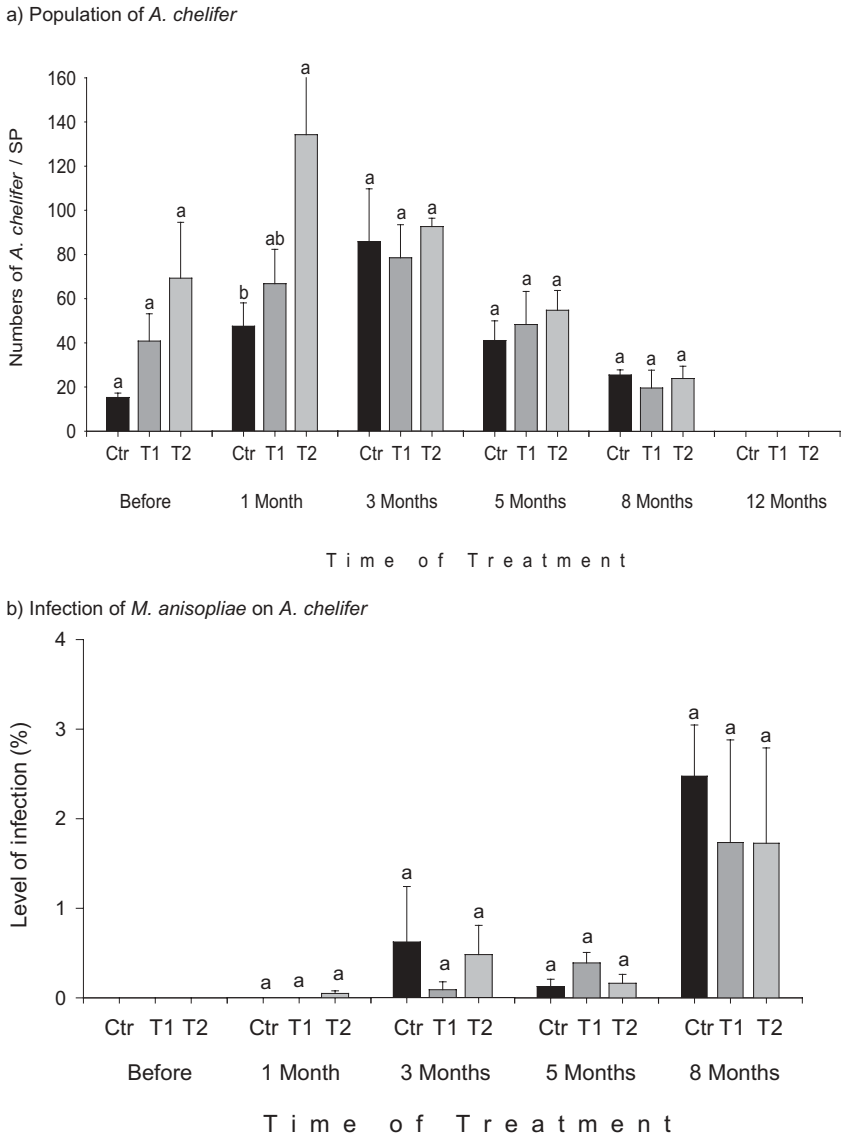


*Figure 7. The effects of* M. anisopliae *on (a) the population of A. chelifer and (b) the level of infection of* M. anisopliae. *Ctr: untreated control, T1: 0.2 g m$^{-2}$ rotting heap, T2: 0.4 g m$^{-2}$ rotting heap.*

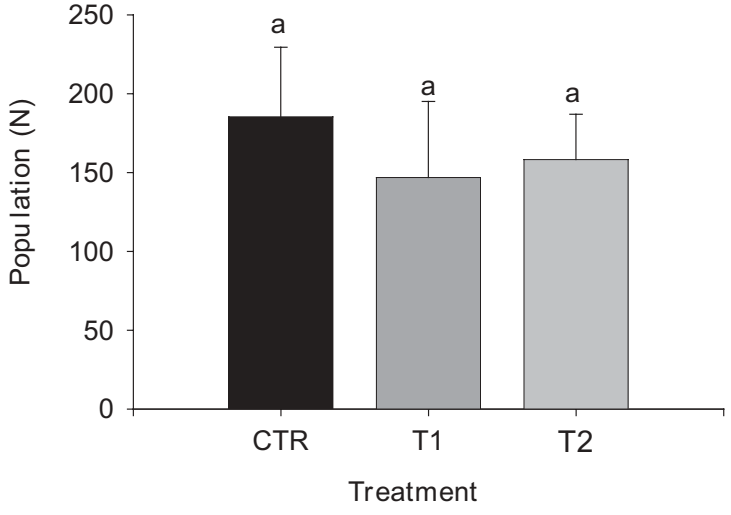


*Figure 8. The effects on the use of* M. anisopliae *as powder formulation on the population of oil palm pollinating weevil,* E. kamerunicus. *Ctr: untreated control, T1: 0.2 g m$^{-2}$ rotting heap, T2: 0.4 g m$^{-2}$ rotting heap.*

## DISCUSSION

The use of powder formulation of *M. anisopliae* onto the rotting heaps of oil palm materials which was fully covered by the leguminous cover crops affected the L2 and L3 larvae. The population of L1 larvae was not affected as their population density in both T1 and T2 was always not significant (P>0.05) than the control, except in the T1 at eight MAT. This might suggest that the L1 larvae developed deep in the layer between top soils and rotting tissues, giving them less chance to make contact with the spores. This was supported as only one dead L1 larva or 0.13% was found confirmed infected by the fungus at eight MAT (*Table 1*). Short developmental period of the L1 larvae (about two weeks) might also be a reason to the low infection of *M. anisopliae* (Bedford, 1980).

The impact of *M. anisopliae* on the L2 and L3 larvae was about the same (*Figures 3b* and *c*). From one to five MAT, the population of both stages in the treatments was not significant (P>0.05) than control. But at eight MAT, the impact of *M. anisopliae* was obviously seen as the populations in the treatments were significantly reduced (P<0.05) than the control. The numbers of L2 larvae in the T1 and T2 were five ISP and 10 ISP, significantly lower (P<0.05) as compared to the control (31 ISP). For the L3 larvae, the number in T1 and T2 was about the same (20 ISP), 75% lower as compared to control (80 ISP). It was noticed that the application of the product at rate T1, 0.2 g $m^{-2}$ reduced both L2 and L3 larval populations as good as the treatment at rate T2, 0.4 g $m^{-2}$.

Prior work by Latch and Falloon (1976) and Ramle *et al.* (1999b) reported that the use of the fungus had greatly reduced the L3 population, and not the L2 population as shown in this study. The sizes, movement and longer developmental period of the L2 (16.5 days) and the L3 larvae (112.3 days) might play a role in increasing the infection of *M. anisopliae.* The bigger size, the active movement and the longer life span, will give higher probability for them to be contacted with the spores and caused infection to occur. This fact is supported as higher infection level was observed on the individual stage (*Table 1*). The infected L2 was found as early as one MAT only in the plot T1, at three MAT in the both treatment plots and later in all plots. The infected L3 larvae were observed in all plots as early as one MAT and peaked at the highest level of 8.09% in the control plot, 20.0% in the T1 plot and 17.7% in the T2 plot at five MAT. Finding on the infection of *M. anisopliae* in each developmental stages of *O. rhinoceros*, however, cannot be compared to other studies as it was not determined by previous workers (Latch and Falloon, 1976; Tey and Ho, 1995; Ramle *et al.*, 1999b).

The reduction on L3 larvae had affected the populations of pre-pupae and pupae of *O. rhinoceros* (*Figure 4*). At eight MAT, the populations of the pre-pupae in the respective T1 and T2 were 0.7 and 0.6 ISP, significantly lower (P<0.05) as compared to control (1.7 ISP). For pupae, the same reduction was also observed, in which the number in the plots T1 (0.7 ISP) and T2 (1.2 ISP) was significantly lower (P<0.05) as compared to the control (3.5 ISP).

As early as one MAT, the infected pre-pupae (0.05%) and pupae (0.02%) was found only in the plot T2 (*Table 1*). Then, it was constantly found in all plots and the levels were gradually increased. However, at 12 MAT, no infected pupae were found in the plots T1 and T2.

Previous studies showed that the infected pre-pupae and pupae were hardly found as they developed deep in the soil, giving them less possibility to contact with the spores or contaminated materials (Latch, 1976; Ramle *et al.*, 1999b). But in this study, the infected pre-pupae and pupae were not only commonly observed, but it caused a significant reduction to the population. This is possibly because of conducive conditions in the rotting oil palm materials under the cover crops, favours the persistence of spores to initiate infection. The moisture content of the rotting heaps was about 80% (Norman *et al.*, 2003) and the temperature was between 27°C-29°C (Bedford, 1980). These conditions as had been reported could enhance the spores longevity in soil and increase the sporulation rate on the infected cadavers of other entomopathogenic fungi, including *M. anisopliae* (Arthurs and Thomas, 2001; Sun *et al.*, 2003).

The great impact of *M. anisopliae* has reduced the L2 and L3 larvae, pre-pupae and pupae, contributing to the significant reduction (P<0.05) of the overall populations of *O. rhinoceros* (*Table 1*).

The adults of *O. rhinoceros* collected in the SP can be categorized as new emergent (neonates and newly emerged adults) and the migrated adults. The neonates were easily recognized as they stayed in the cocoon while the newly emerged adults had a shiny elytra of blackish to brownish in colour. Although, the adult population was not sorted according to the above category, it was noticed that the newly emerged adults were commonly found. Possibly, about 95% from the total adult samples were the newly emerged adults.

As the *M. anisopliae* affected the pupation, it also affected the emergent rate of adults in the treatment plots. This was obviously seen at five MAT, as the number of adults in the treated plots decreased to between 6.5 and 7.2 ISP, slightly lower than the control at 9.5 ISP (*Figure 5a*). At eight MAT, only the number of adults in the T1 (1.6 ISP) was significantly lower than control (5.8 ISP), but not in the T2 (3.3 ISP). At 12 MAT, the number of adults in both treatments T1 and T2 was significantly lower than control (one ISP). This finding was not observed in the previous study conducted by Ramle *et al.*

(1999b). He found that the population of adults in the treated plots was not significantly different (P>0.05) as compared to control for the whole period of the study.

In this study, the pressure of migrated adults that coming into the experimental area was suspected to be greater. This is because the experimental area (120 ha) was placed within the huge replanting areas of more than 800 ha (Norman and Basri, 2004). This is also the reason why the population of adults captured by the pheromone traps in the treatments was not significantly different (P>0.05) as compared to population in the control plot (*Figure 5b*). The migration of adults into the experimental areas was expected, as they could fly for 2 to 3 hr with the projection of distance about 2-4 km (Hinckley, 1973). In the field, however, the adults could fly much shorter, which only about 150-200 m from the released point.

The significant impact of *M. anisopliae* on overall population of *O. rhinoceros* can only be seen as early as eight MAT, much slower as compared to only three MAT in the study conducted by earlier workers, that applied the fungus on the rotting heaps without or less cover crops growing on the heaps (Tey and Ho, 1995; Ramle *et al.,* 1999b). The cover crops could function as a physical barrier, slowing the down movement of the spore solutions into the rotting debris, thus delayed the time of impact to the pest. However, the presence of the cover crops gave a protection to the spores from the sunlight ultraviolet radiation, high soil temperature and low soil moisture and low relative humidity, which were reported detrimental to them (Moore *et al.,* 1993; Ekesi *et al.,* 2003). This fact explained why higher infection of *M. anisopliae* was found in this study especially on the L2 and L3 larvae, pre-pupae and pupae, but it was hardly seen in the earlier studies (Latch, 1976; Tey and Ho, 1995; Ramle *et al.,* 1999b).

As the spores survived better in the rotting materials, it did not require higher number to cause the significant impact to the *O. rhinoceros* population. In this study, the number of spores in the T1 was 2.2 x $10^7$ spores $m^{-2}$ or 2.1 x $10^{10}$ spores $ha^{-1}$ and T2 was 4.4 x $10^7$ spores $m^{-2}$ or 4.4 x $10^{10}$ spores $ha^{-1}$. Both rates were proven equally effective in reducing the *O. rhinoceros*. These rates were 100 folds lower than the rate used by Ramle *et al.* (1999b) to control *O. rhinoceros* in the rotting materials that less or no growing of cover crops. He found that the L3 larvae was significantly reduced after treated at the highest rate of $5.0^9$ spores $m^{-2}$ or 3.0 x $10^{12}$ spores per hectare.

For the stag beetle, the population density in both treated and control plots was always not different at all times of data recording (*Figure 7a*). Although, the *M. anisopliae* could infect the larvae and adults, but the percentage was low, ranged between 0%- 0.6% at five MAT and 1.7 and 2.5% at eight MAT (*Figure 7b*). This beetle shared their breeding habitat with the *O. rhinoceros*. Previously, it was reported as a fruit, flower or leaf feeder of oil palm (Wood, 1968), but field damage caused by the beetle was hardly seen. Field observation by Ramle *et al.* (2004) found that those rotting trunks occupied by this beetle were decomposed faster than the other trunks without the beetle. Laboratory test found that the *M. anisopliae* could kill the larvae of the beetle between 33.3% - 83.3% (Ramle *et al.,* 2004), but in the field, the fungus did no affect the beetle population, as shown in this study.

As expected, the use of *M. anisopliae* in powder form did not affect the oil palm pollinating weevil, *E. kamerunicus.* The weevil population was maintained higher and not different as compared to the control (*Figure 8*). Assessment by direct spraying on the adults weevil with the spore solution showed that the weevil was more susceptible to infection of *M. a*. var. *anisopliae*, but not to *M. a.* var. *major* (Ramle *et al.,* 1999a). Furthermore, the fungus did not affect the immature stages of the weevils, as they developed inside the male spikelets. None of the newly emerged adults were infected when the spikelets were directly treated with the *M. a.* var. *major* (Ramle *et al.,* 1999a).

## CONCLUSION

The use of powder formulation of *M. anisopliae* var. *major* to control *O. rhinoceros* breeds in the rotting oil palm heaps that already covered by the leguminous cover crops had proven effective. The application of product by spraying technique at rates T1, 0.2 g (2.1 x $10^{10}$ spores $ha^{-1}$) and T2, 0.4 g product $m^{-2}$ (4.4 x $10^{10}$ spores $ha^{-1}$) effectively reduced the L2 larvae, L3 larvae, pre-pupae and pupae at eight MAT. Although leguminous cover crop affected the movement the spore solution into the rotting heaps, delaying its impact to *O. rhinoceros*, but on the other hand it gave a protection to the spores from the detrimental abiotic factors. It is then making the rotting heaps conducive for the fungus to grow, initiate infection and finally reducing the *O. rhinoceros* population. As the spores survived well under this favourable regimes, therefore, only low number spores, about 2.1- 4.4 x $10^{10}$ spores is required, to give great depression to *O. rhinoceros*. This rate is 100 times lower when the fungus is used to control *O. rhinoceros* in the rotting heaps without or less cover crop.

Higher infection of *M. anisopliae*, especially on the L3 larvae had affected the pupation, later reducing the rate of newly emerged adults in the treated area. However, the reduction was unable to give a significant impact to the overall adult populations, mainly because of migration of adults into the experimental area from the surrounding areas. For the effective management of the beetle in the field, it

is recommended that to apply the product much earlier, say at six to eight months after felling, not at 15 months after felling as done in this study. If possible, it should be applied before the leguminous cover crops had fully covered the rotting heaps. This will give an ample time to the fungus to initiate infection to the L2 and early L3 larvae, as only these stages were mostly found in the rotting heaps (Samsudin *et al.*, 1983). As the fungus developed the disease, which may take about three months, the rotting heaps were already covered by the planted cover crops, enhancing the infection on the L3 larvae and affected the pupation. If the product was applied in the entire area, it possibly would reduce adult population in the field. Further reduction of adults could be achieved by incorporating the *O. rhinoceros* virus (Ramle *et al.*, 2005b), another potential biological agent of the pest.

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