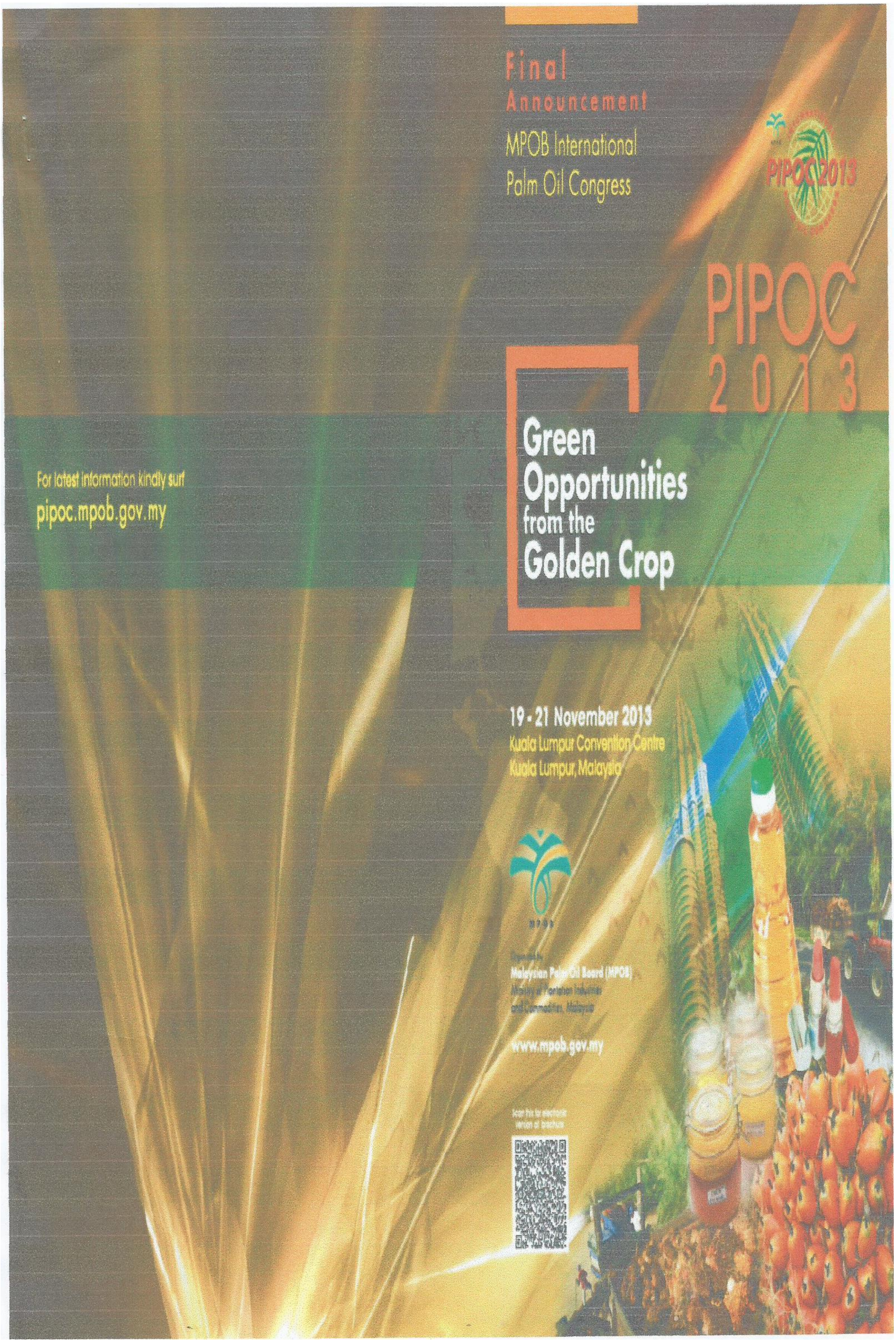
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TTHE EFFICACY OF SPRAYING THE SPORES OF *Metarhizium anisopliae* AS A DELIVERY TEHNIQUE TO CONTROL *Oryctes rhinoceros* L. IN EFB COMPOST

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ABSTRACT

Biological control of *Oructes rhinoceros* using entomopathogen is a promising alternative to the commonly used chemical control, as a part of the integrated pest management in oil palm plantations. The beetle breeds in rotting palm, wood stumps and empty fruit bunches in the field. It is not only a serious pest for young oil palms but for mature palms, especially in peat soil plantations. In some cases the entry hole of the pest on the crown can cause secondary fungy attacks, which cause spear-rot disease.

Laboratory trials by Central Plantations Services (CPS) to investigate the growth rates and sporulation capacities of *Metarhizium anisopliae* showed that 12 days after inoculations, *M. aniosoliae* had grown fully in petridishes. It then sporulated profusely on corn media in 14 days post inoculation with a population of 2.7 x 10^9 spores/gram. *O. rhinoceros* beetle larvae werw obtained from the field and grown in non-sterile empty fruit bunch (EFB) composts, produced using aerated bunker composting *anisopliae* infected 97% of *O.rhinoceros* larvae within 14 days.

Preliminary field trial was conducted ian a peat soil plantation with a heavy infestation of *O.Rhinoceros* beetless. The result showed that 88% larvae in the compost were infected within 35 days after treatment with *M. anisopliae.* The treatment was especially effective when larvae were in the 3rd instars stage.

Research had shown that *M.anisopliae* was effective in controlling *O.rhinoceros*, our study si the first to show that the fungi can also be inoculated in the EFB compost. Despite their values as organic fertilisers, EFB compost are still not widely used, especially in peat soil plantations, mainly because *O. rhinoceros beetles* tend to breed in EFB compost. Here the study showed that compost can be used both as organic fertilisers and also as agents for pest control.

Ketwords : Metarhizium anisopliae, Oryctes rhinoceros, *oil palm mill by products, compost, sustainable, peat soil.*

INTRODUCTION

Oryctes rhinoceros L. is an important pest in oil palm and has become a major problem in newly planted or replanted oil palm areas. The adult beetle attack palms by boring through young leaf bases and feeding the soft tissue in the crown region. The damages could cause crop losses of up to 72 % in the first year of harvesting.

During replanting, trunk chips from two rows of felled palms are normally stacked to form heaps of biomass in between the rows. The heaps of biomass are potential breeding sites for rhinoceros beetles, if not properly managed and controlled. Currently, the conventional control measure for *O. rhinoceros* is by spraying with contact insecticides. Due to problems associated with beetle resurgences or outbreaks in certain cases, as well as other environmental and regulatory concerns, research to develop alternative control strategies is needed.

Entomopathogenic fungus, *Metarhizium anisopliae* has been used widely as a mycopesticide to control many insect pests, include *O. Rhinoceros*.0ne of the common factors that affect the effectiveness of *M anisopliae* in infecting *Oryctes* beetle is its delivery into the beetles' breeding sites. A field application trial showed that application of *Metarhizium anisopliae* by a mist blower or a power sprayer onto the breeding sites were both effective in controlling *O. rhinoceros*. In both methods, applications of *M. anisopliae* at dosage of 2.0 g/L were sufficient to induce 80% mortality as early as 5 weeks after treatment (WAT). The result strongly suggests that applications *M. anisopliae* to the breeding sites could reduce the larval population of *O. rhinoceros*, and subsequently reduce the emergence of new adults.

MATERIALS AND METHODS

Metarhizium anisopliae isolates were obtained from the culture collection of CPS Pathology laboratory. The growth rate of M anisopliae was assessed on potato dextrose agar (PDA), with five replicates in a completely randomized design. *M. anisopliae* growth rates were measured using an estimation of slope and an assessment of spomlation was also made by measuring spore density on corn media with five replicates in a completely randomized design. The corns were sterilized at 121 ^{0}C for 30 minutes and then inoculated with *Metarhizium anisopliae* from cultures growing on PDA. After 12 days incubation in the dark, *M. anisopliae* had completely colonized and sporulated profusely on corn media.

Laboratory trials were conducted to investigate the effectiveness of M anisopliae as a biological control of larvae (*Oryctes rhinoceros*). *M. anisopliae* was grown in the laboratory as explained above. The larvae were caught from the compost piles in the field. The compost were obtained from Bangun Tenera Riau (BTR) mill, produces with aerated bunker composting (ABC) system (silalahi *et al.* 2010). The number of larvae was counted before treatment and put in the non-sterilised compost piles, which were then sprayed with *M. anisopliae* in two different spores density per gram ($10^0$and 10^9). There were ten combined treatments with five replicates in a completely randomized design. The effectiveness of *Metarhizium anisopliae* was assessed by recording the infestation of larvae after treatment. Dead larvae (*O. rhinoceros*) were then isolated and planted in vitro on PDA media (Pelzcar, 1986) to investigate whether the infection of M. *anisopliae* was indeed the cause of death. The field trial is conducted in an oil palm plantation planted on peat soil. The area and its vicinity were all first generation of oil palms. Heavy infestations by *O. Rhinoceros* were found on many individual palms, and these have been attributed to the availability of trunks and compost applications, both of which formed the breeding sites for the beetles. Block B8, with size of about 23 hectares, was selected for the study. 120 kg composts were applied per palm in this block. Each compost pile was sprayed with either 1 liter solution of M. anisopliae contains 2.7 x 10^9 spores per gram or water (control treatment) with 15 replications in a randomised block design. The effectiveness of *M. anisopliae* to infest larvae of *Oryctes* beetle was assessed by recording the infected larvae after treatment.

RESULTS AND DISCUSSIONS

The laboratory trial result showed that the maximum growth of M. anisopliae on PDA occurs at 12 days after inoculation (DAI) with 50.3 cm2 (Figure 1), while the optimum growth occurred at 7 days after inoculation (DA!) with 36.3 cm^2. Microorganisms utilize the nutrients in the media in the form of small molecules are assembled to construct components of cells. Function of a culture media is giving the place and conditions that support the growth and proliferation of microorganisms grown. The quality and quantity of nutrients from the media greatly influence the virulence of entomopathogen.

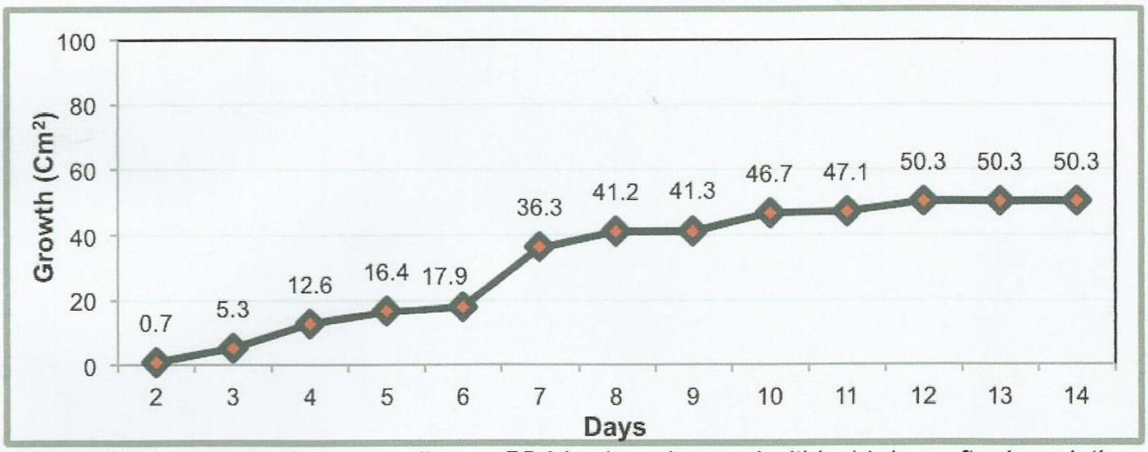

Figure 1. The growth of M. anisopliae on PDA in vitro *observed within 14 days after inoculation*

The effectiveness of *M. anisopliae* as an entomopathogen was tested in the laboratory. The infection symptoms were first observed at 6 days after treatment. By the end of the trial, 97% of the larvae in the compost piles, where fungi spores were sprayed on, had been infected and dead (Figure 2). Comparing this to the 5% death rate of the larvae (no symptom of *Metarhizium* infection) in the control treatment (Figure 2), it was clearly significantly higher (p value 0.01).

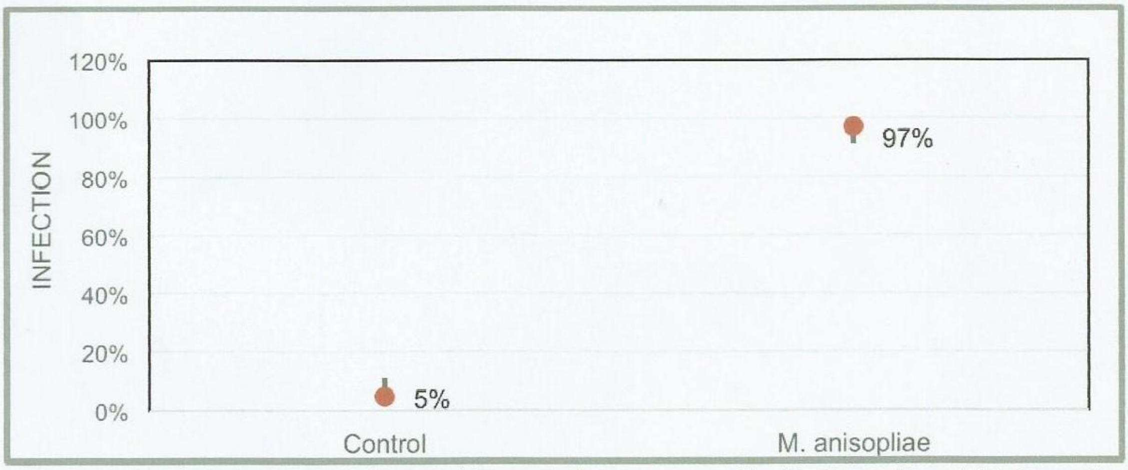

Figure 2. Predicted mean of infected rhinoceros larvae caused by M. Anisopliae at 14 days after treatment.

in the field trial, the symptoms were first observed at 14 days after treatment. The results showed that within 35 days of treatment, 88% larvae had been infected, compared with control treatment where only 6% larvae was dead (Figure 3). Both laboratory and field trials showed that M. anisopliae can be used effectively as a biological control of Oryctes beetles. Interestingly, the fungi can grow well in empty fruit bunch composts. Since the beetles use compost piles as breeding sites, the composts can act both as fertilizers and pest control agents.

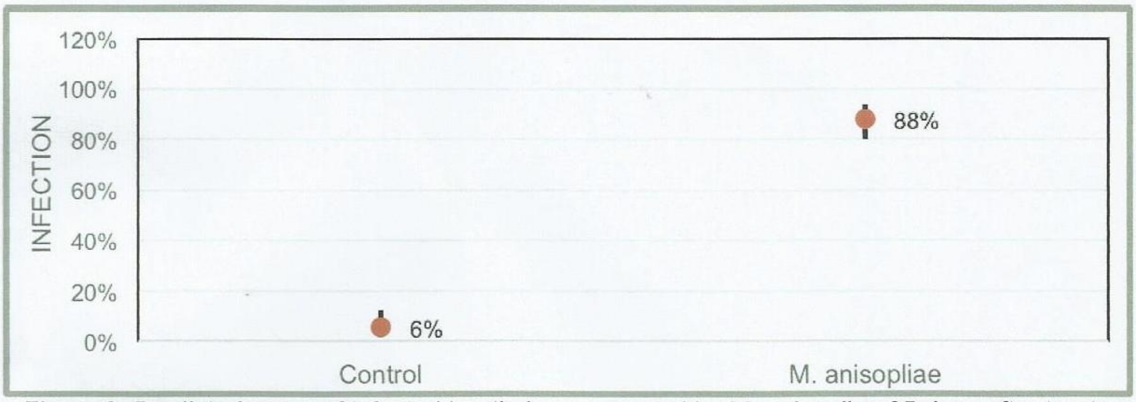

Figure 3. Predicted mean of infected beetle larvae caused by M. anisopliae 35 days after treatment

The early infection is characterized by dark brown spots on the surface of larvae cuticles (Figure 4). Subsequent infection occurs when the infected beetles become harder (mummification), then covered by the hyphae of the fungus, and finally turned green as the fungi mature. The mummification of the body is caused by enzymes secreted by *M. anisopliae*, which then absorb all larvae tissues and body fluids. These enzymes are lipase, khitinase, amylase, proteinase, posphatase, and esterase (Freimoser et al, 2003).

Figure 4. A. Early infection with dark brown spots on the surface of the larvae cuticles but larvae still alive, B. The dead larvae and mummification occur until the entire cuticles of larvae covered by M. anisopliae hyphae and spores

Figure 4. A. Early infection with dark brown spots on the surface of the larvae cuticles but larvae still alive, B. The dead larvae and mummification occur until the entire cuticles of larvae covered by M. anisopliae hyphae and spores

Huge quantities of palm oil mill effluent (162,000 m3) and pressed empty fruit bunches (35,100 tonnes) are produced annually by a 45 t FFB/h capacity mill operating for 20 hours daily. These materials are highly nutritive and can become a valuable nutrient source if composted correctly. Conventional composting methods such as lagoon and windrow composting system have been developed by Schuchardt *et al.* (2002), Siregar *et al.* (2002) and Silalahi and Foster (2006). The latest system developed by Silalahi *et al.* (2010) is an Aerated Bunker Composting (ABC) system in which the nutrients values are stable over the year of production, less nutrient losses, small composting yard and minimum methane released. This study and another (Ginting, *et al.* 2013) showed that in addition to their function as fertilisers, composts can also be used as a biological control agent of oil palm pests.

CONCLUSIONS

1. Laboratory trial showed that the maximum growth of *M. anisopliae* on PDA occurs at 12 days after inoculation (DAI) with 50.3 cmz, while the optimum growth occurred at 7 days after inoculation (DAI) with 36.3 cmz. The spraying of beetle larvae in the non—sterilised compost with *Metarizium* spores gave 97% infected larvae 14 days after treament.

2. The spraying of beetles larvae on the compost piles in the field with formulated *Metarizium* gave 88% larvae were infected within 35 days after treatment in which indicated that *M. anisopliae* can be used as a biological control of *O. rhinoceros*. The application of *M anisopliae* spores by spraying onto compost without affecting its virulence.

3. Compost produced by using aerated bunker composting (ABC) system can be used as replacement of inorganic fertilisers and also as trapping agents. Spores of entomopathogen *M. anisopliae* sprayed to compost piles, can then easily infect the beetles larvae attracted to the compost.

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