

COMPATIBILITY TEST OF Trichoderma virens AND Metarhizium anisopliae AND THEIR ABILITY TO CONTROL Oryctes rhinoceros LARVAE IN COMPOST

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Abstract

Oryctes rhinoceros is a major pest in oil palm plantations. The control often used is *Metarhizium anisopliae*, and also *Trichoderma* sp. which have been reported to be able to control pests. This study aims to test the compatibility of *Trichoderma virens* and *Metarhizium anisopliae* entomopathogenic fungi and the effectiveness of their combination in controlling *Oryctes rhinoceros* larvae in oil palm empty fruit bunches (OPEFB) compost media. This study consists of two stages, namely in vitro compatibility test on potato dextrose agar (PDA) media and testing the effectiveness of the combination of *T. virens* and *M. anisopliae* on the mortality of *O. rhinoceros* larvae with a completely randomized design (CRD). The treatments consisted of *T. virens* 0 g.l⁻¹, 25 g.l⁻¹, 50 g.l⁻¹, 75 g.l⁻¹, and 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ + 5 kg OPEFB compost. The data were tested using the DNMRT advanced test at 5% level. The in vitro test results showed that *Trichoderma virens* and *Metarhizium anisopliae* fungi were compatible, characterized by normal colony growth without inhibition zones. In the semi-field test, the best treatment was obtained from the combination of *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ + 5 kg of compost, which resulted in an initial larval death time of 31.20 hours, LT₅₀ of 120 hours, and total mortality of 66%. This mortality rate is considered virulent but does not meet the ideal bioinsecticide standard of ≥72%.

Introduction:

Oil palm (*Elaeis guineensis* Jacq.) is a major plantation commodity in Indonesia and is experiencing rapid growth. Not only palm oil useful as a food ingredient but it is also useful as a raw material for industries such as biodiesel, soap, detergent, surfactants, cosmetics, medicines, and various other household and industrial needs. The market potential for palm oil is very promising as the demand continues to increase annually, both domestically and internationally (Ardana & Kariyasa, 2016).

Riau Province is the largest palm oil production center in Indonesia, having plantations covering 2,868,300 hectares and crude palm oil (CPO) production of 6.3 million tons (CSA, 2023). This high production results in large quantities of empty fruit bunches (EFB), accounting for approximately 23% of total fresh fruit bunches (Saputra & Stevanus, 2019). EFB is commonly used as organic mulch to improve soil fertility, but it can also serve as a breeding ground for the main pest of oil palms, the rhinoceros beetle (*Oryctes rhinoceros* L.).

The obstacle faced in oil palm cultivation is the main pest attack on palm plants, namely the rhinoceros beetle (*Oryctes rhinoceros*). This pest can reduce yields by up to 60% at the first harvest and cause death of up to 25% in immature plants (Arida *et al.*, 2019). This pest attacks plants characterized by the presence of burrows on then sucking fluids and making holes in the midrib, the presence of distinctive "V" shaped young leaf cuts and attacks on young leaves (Directorate General of Plantations, 2008). In Riau Province, the area of *Oryctes rhinoceros* attack reached 12,384.85 hectares, with the worst damage occurring in Indragiri Hilir Regency at 2,727 ha, Siak at 340 ha, Kampar at 579 ha, Kuansing at 459 ha, and the rest spread across smallholders of oil palm plantations (Riau Province Plantation Service, 2014).

Pest control at the farm level generally still relies on synthetic chemical pesticides. However, excessive pesticide use can cause various negative impacts, such as resistance, pest resurgence, residues that pollute the environment, human health problems, and side effects on non-target organisms (Directorate of Horticultural Plant Protection, 2008). Therefore, more environmentally friendly control alternatives are needed, including the use of natural enemies, entomopathogenic fungi, predatory insects, and parasitoids (Healthy Agriculture Institute, 2008). Entomopathogenic fungi has been reported to be able to control *O. rhinoceros* are *Trichoderma* sp. and *Metarhizium anisopliae*.

Metarhizium anisopliae produces the destruxin compound, which damages insect cell organelles, causing paralysis, tissue damage, and even death (Archana *et al.*, 2022). Application of *M. anisopliae* at a concentration of 75 g/l has been reported to cause total pest mortality of up to 72.5%, making it potentially useful as a biopesticide (Fauzana & Fadilla, 2022). Meanwhile, *Trichoderma* sp. is an antagonistic fungus commonly found in organic soils and is often used for biological control of soil-borne, rhizosphere- and phyllosphere-borne pathogens (Pattikawa *et al.*, 2020). *Trichoderma* sp. has also been reported to suppress the development of *Myzus persicae* by 43.92–74.84% (Trizelia *et al.*, 2021). The results of research by Sidabutra *et al.* (2022), stated that *T. viride* was effective in controlling *O. rhinoceros* larvae, with a mortality rate of 91.67% at a *T. viride* concentration of 60 g per 10 l⁻¹.

The use of *Trichoderma* sp. and *M. anisopliae* alone to control *O. rhinoceros* has been widely used, but compatibility testing is still limited. However, the combination of these two fungi has the potential to increase the effectiveness of sustainable pest control. Therefore, this study was conducted to determine the compatibility of *T. virens* and *M. anisopliae* and their ability to control *O. rhinoceros* both in vitro and in semi-field settings.

The aim of this study was to determine the compatibility of *T. virens* and *M. anisopliae* on an in vitro scale and the ability of *T. virens* and *M. anisopliae* to control *O. rhinoceros* in compost.

Materials and Methods:

This research was conducted at the Plant Pest Laboratory and Experimental Land UPT, Faculty of Agriculture, University of Riau, Bina Widya Campus, Km 12.5, Pekanbaru, Riau, from May to July 2025.

The materials used were *Oryctes rhinoceros* larvae, *M. anisopliae* isolate, *T. virens* isolate, PDA (Potato Dextrose Agar), agar-agar powder, potatoes, OPEFB, chicken manure, urea, TSP, dolomite, sawdust, plastic, corn kernels, 70% alcohol, sterile distilled water, brown sugar, granulated sugar, aluminum foil, plastic wrap and label paper. While the tools used were, LAFS (Laminar airflow cabinet), autoclave, petri dish, steamer, stove, plastic tarpaulin, hoe, 1000 ml beaker glass, analytical balance, bunsen, cork borer, spatula, ose needle, bucket, sprayer, camera and stationery.

The study used a completely randomized design (CRD) consisting of five treatment combinations namely *T. virens* 0 g.l⁻¹+ *M. anisopliae* 75 g.l⁻¹+ 5 kg OPEFB compost, *T. virens* 25 g.l⁻¹+*M. anisopliae* 75 g.l⁻¹+5kg composite OPEFB, *T. virens* 50 g.l⁻¹+*M. anisopliae* 75 g.l⁻¹+5kg composite OPEFB, *T. virens* 75 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹. *T. virens* 100 g.l⁻¹+*M. anisopliae* 75 g.l⁻¹+ 5 kg of OPEFB compost, each was repeated five times, resulting in 25 experimental units. Each experimental unit used 10 *O. rhinoceros* larvae.

The research implementation started from reisolation of *T. virens* and *M. anisopliae*, compatibility test of *T. virens* and *M. anisopliae*, propagation of *T. virens* and *M. anisopliae*, compost making, compost sterilization, making liquid media for *T. virens* and *M. anisopliae*, treatment application, procurement and infestation of *O. rhinoceros* larvae.

Data were collected through series of experiments consisting of compatibility tests of *T. virens* and *M. anisopliae* in paired cultures, initial time of larval death, lethal time 50, daily mortality, and total mortality of *O. rhinoceros* larvae. Compatibility and daily mortality data were analyzed descriptively in the form of tables and graphs, while data on initial time of death, LT₅₀ and total mortality were analyzed using analysis of variance (ANOVA). If the analysis results showed significant differences, further testing would be carried out using Duncan's New Multiple Range Test (DNMRT) at 5% level. Data analysis was carried out using SAS software.

Results and Discussion Results
1. Compatibility of *M. anisopliae* and *T. virens*

Table 1
The compatibility of *M. anisopliae* and *T. virens* was tested in vitro on PDA media.

Culture tested	Compatibility
<i>M. anisopliae</i> + <i>T. virens</i>	+

(-) = not compatible, (+) = compatible

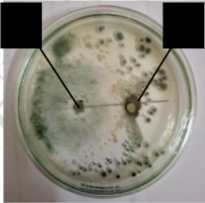


Figure 1
Compatibility test of (a) *T. virens* fungus, (b) *M. anisopliae* fungus on PDA media

2. Initial time of death of *O. rhinoceros* larvae

Table 2

Time of onset of larval mortality of *O. rhinoceros* L. following administration of several compatibility treatments of *M. anisopliae* and *T. virens* on compost.

Compatibility treatment of <i>T. virens</i> and <i>M. anisopliae</i> on compost	Time of onset of death (Hours)
<i>T. virens</i> 0 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	98.4a
<i>T. virens</i> 25 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	64.8a
<i>T. virens</i> 50 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	64.8a
<i>T. virens</i> 75 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	60.0a
<i>T. virens</i> 100 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	31.2b

Numbers followed by different lowercase letters are significantly different according to the DNMR test at 5% level after being transformed with $\sqrt{y}$.

3. Lethal time 50 (LT50) of *O. rhinoceros* larvae

Table 3

Lethal Time of 50 *O. rhinoceros* Larvae After Several Compatibility Treatments of *M. anisopliae* and *T. virens* in Compost

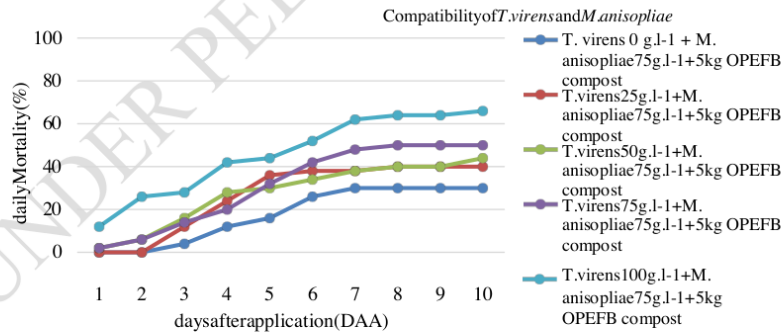
Compatibility treatment of <i>T. virens</i> and <i>M. anisopliae</i> on compost	Lethal time 50 (Hours)
<i>T. virens</i> 0 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	240.0a
<i>T. virens</i> 25 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	168.0ab
<i>T. virens</i> 50 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	192.0ab
<i>T. virens</i> 75 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	144.0b
<i>T. virens</i> 100 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFB Compost	120.0b

Numbers followed by different lowercase letters are significantly different according to the DNMR test at 5% level after being transformed with $\sqrt{y}$.

4. Daily mortality of *O. rhinoceros* larvae

Figure 2

Daily mortality of *O. rhinoceros* L. larvae after application of *T. virens* and *M. anisopliae* compatibility treatment in compost.



5. Total mortality of *O. rhinoceros* larvae

Table 4

Total mortality of *O. rhinoceros* larvae after several compatibility treatments of *T. virens* and *M. anisopliae* in compost

Compatibility treatment of <i>T. virens</i> and <i>M. anisopliae</i> on compost	Total mortality (%)
<i>T. virens</i> 0 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFBCompost	30.0b
<i>T. virens</i> 25 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFBCompost	40.0b
<i>T. virens</i> 50 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFBCompost	44.0b
<i>T. virens</i> 75 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFBCompost	50.0ab
<i>T. virens</i> 100 g.l ⁻¹ + <i>M. anisopliae</i> 75 g.l ⁻¹ + 5 kg OPEFBCompost	66.0a

Numbers followed by different lowercase letters are significantly different according to the DNMRT test at the 5% level after transforming with $\sqrt{y}$

Discussion

Compatibility of *T. virens* and *M. anisopliae*

T. virens and *M. anisopliae* isolates showed a highly beneficial and compatible interaction. This was indicated by the formation of clear colony boundaries, green spore color, normal colony growth, the absence of mutual inhibition, the absence of growth dominance (overgrowth), and the presence of hyphal fusion at the colony junction area (Figure 1). Tabacchioni *et al.* (2021) stated that compatible interactions between isolates were characterized by the absence of inhibition zones, while incompatible interactions were characterized by the formation of inhibition zones between the two isolates. This is supported by the results of research by El-Refai *et al.* (2013) that incompatible fungi were characterized by the formation of inhibition zones, overgrowth, and the accumulation of conidia on one side.

T. virens and *M. anisopliae* fungi are able to grow on PDA media and form a combination that is not detrimental to each other and does not inhibit the growth of each biological agent. Figure 1 shows that the hyphal growth of both fungi does not overlap due to the competition for space. The performance of both biological agents does not show an antagonistic mechanism, as seen from the absence of an inhibition zone between the two fungi. This indicates that *T. virens* and *M. anisopliae* fungi can coexist without disrupting each other's growth.

T. virens and *M. anisopliae* fungi produce various types of different antimicrobial compounds, so the combination of these two fungi is more effective in controlling various types of plant pests. This is supported by the opinion of Siregar & Prayitno (2016) who stated that the combination of compatible microorganisms could produce better performance compared to the use of a single isolate, because the enzymes produced by each microorganism could work synergistically and utilize the same nutrient sources without inhibiting each other.

Initial time of death of *O. rhinoceros* larvae

The compatibility treatment of *T. virens* and *M. anisopliae* to control *O. rhinoceros* in compost showed significantly different results on the initial time of death of *O. rhinoceros* larvae with a range of 31.20 to 98.40 hours after application. The compatibility treatment of *T. virens* 0 g.l⁻¹, 25 g.l⁻¹, 50 g.l⁻¹, 75 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ + 5 kg of OPEFBCompost was not significantly different among treatments on the initial time of death, namely 96.40 hours, 64.80 hours, 64.80 hours and 60.00 hours, but significantly different from the compatibility treatment of *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ + 5 kg OPEFBCompost which caused the initial death of *O. rhinoceros* larvae at 31.20 hours after application. This is presumably due to the low dose given and the small amount of toxin produced and the fungus that was not working optimally hence the initial time of death is not significantly different. According to Neves & Alves (2004) the ability of entomopathogenic fungi to kill insects is influenced by the level of virulence and density of the entomopathogenic fungus's conidia itself.

The compatibility treatment of *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ + 5 kg of OPEFB compost was the fastest treatment to kill the initial death of larvae, which was 31.20 hours. This is presumably because the compatibility treatment of *T. virens* and *M. anisopliae* with high doses shows synergy that can accelerate the infection process of *O. rhinoceros* larvae. The high dose given causes more conidia to come into contact with the larval body, therefore more enzymes and toxins are released by the fungus into the larval body. Siswanto & Trisawa (2017) stated that the number of conidia entering the larval body could affect the speed at which the fungus kills its host. The more conidia enter the insect's body, the faster the insect's integument is damaged and body fluids are released, hence insects die faster.

Lethal time 50 (LT₅₀) *O. rhinoceros*

The results showed that the combination of *T. virens* and *M. anisopliae* resulted in a significant difference in the LT₅₀ value of *O. rhinoceros* larvae, which ranged from 120 to 240 hours after application. The single treatment of *M. anisopliae* without the addition of *T. virens* had not reached 50% mortality at the end of the observation period (240 hours). This indicates that the treatment of only *M. anisopliae* without *T. virens* had not worked optimally. Nurjayanti (2017) stated that the effectiveness of entomopathogenic fungi in causing mortality of target insects was often less than optimal because the infection process took a long time.

Treatment with the addition of *T. virens* at various doses accelerated the time of larval death. The highest combination of *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ resulted in the fastest LT₅₀ at 120 hours, but was not significantly different from the treatments of *T. virens* 75 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹, *T. virens* 50 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹, and *T. virens* 25 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹. This indicates that the presence of *T. virens* supports *M. anisopliae* colonization and infects larvae. However, the physiological resistance of larvae also affects the mortality rate. Sihombing *et al.* (2014) stated that even though the virulence of entomopathogenic fungi increased, *O. rhinoceros* larvae still maintained their ability to fight infection, so that the difference in mortality between treatments was not always significant.

The slow infection process is also influenced by the interaction between the inoculum and the host. Conidia present in compost media require time to attach to the integument, germinate, and penetrate before causing death. Other influencing factors include the level of pathogenicity of the isolate, the resistance of the larvae to toxic compounds, and the application method. Siswanto and Trisna (2017) emphasized that a single application would often reduce effectiveness because molting in larvae could release attached conidia. Furthermore, Wicaksono *et al.* (2015) reported that direct application to the insect body resulted in faster death than application through compost, due to the shorter infection pathway.

Daily mortality of *O. rhinoceros* larvae

Observations of daily mortality showed that mortality of *O. rhinoceros* larvae began to occur faster in high treatment combination treatments. Treatments of *T. virens* 100 g.l⁻¹, 75 g.l⁻¹, and 50 g.l⁻¹ caused deaths to occur on the 1st day after application, while treatments of *T. virens* 25 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ and *T. virens* 0 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ caused deaths to only occur on the 3rd day after application. This shows that the higher the dose given, the faster the fungal infection process in the larvae.

Entomopathogenic fungal infections occur through four stages: inoculation, penetration, infection, and invasion (Shin *et al.*, 2020). Conidia attach to the larval cuticle, germinate and penetrate the integument, then develops in the hemolymph, producing toxins that weaken the insect's immune system. The fungus then exploits the host's nutrients for growth, causing tissue damage, motor impairment, and ultimately death.

The highest dose of compatibility of *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ showed an increase in mortality that tended to be the highest from day 1 to day 10. This was caused by the administration of a high dose, thus more conidia thereby increased the chance of contact and successful infection. Sterkel *et al.* (2021) stated that the increase in conidia density was directly proportional to the host's infection capacity.

Afandhiet al. (2020) also added that conidia that successfully attached would germinate, produce toxins, and suppress the insect's immune system, thereby accelerating the death process. The relatively limited conditions of larvae in the compost medium also increase the intensity of contact with conidia, thereby increasing the effectiveness of infection.

Mortality to *O. rhinoceros* larvae

The results showed that the combination treatment of *T. virens* and *M. anisopliae* in compost had a significant effect on the total mortality of *O. rhinoceros* larvae, with a range of 30–66%. The best treatment tended to be obtained from the combination of *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ which resulted in a total mortality of 66%, although it was not significantly different from the treatment of *T. virens* 75 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ which resulted in 50%. Furthermore, these two treatments were significantly different compared to lower doses, namely *T. virens* 50 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹, 25 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹, and *T. virens* 0 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹, which only resulted in mortality of 44%, 40%, and 30%, respectively. This difference indicates that increasing the dose of entomopathogenic fungi is directly correlated with increased larval mortality. Lin et al. (2017) stated that the higher the dose of conidia given, the greater the chance of infection and contact with the host was, thus accelerating the death process.

The combination of *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ showed the highest total mortality 66%, but yet cannot be categorized as a bioinsecticide. According to Noviantier et al. (2021), entomopathogenic fungi are classified as bioinsecticides if their mortality rate reaches 72–95%. Thus, although the combination of these two fungi shows promising potential, its effectiveness cannot yet be categorized as an ideal bioinsecticide.

The low total mortality rate is presumably due to the application method used, which involves spraying the compost medium. This indirect application slows the infection process, as the fungus needs time to develop in the medium before infecting the larvae. Wicaksono et al. (2015) explained that application via compost medium generally resulted in lower mortality rates than direct application to the insect's body.

Overall, these results indicate that the combination of *T. virens* and *M. anisopliae* has high potential to control *O. rhinoceros* larvae. However, its effectiveness still needs to be improved by selecting more virulent isolates, increasing the application dose, and modifying the application method to meet bioinsecticide standards.

Conclusion

From the results of the study it can be concluded that the compatibility of *Trichoderma virens* and *Metarhizium anisopliae* in compost to control *Oryctes rhinoceros* larvae was achieved by the following:

1. *Trichoderma virens* and *Metarhizium anisopliae* were shown to be compatible in vitro, as indicated by the non-inhibition of colony growth.
2. The treatment that tends to be the best for the mortality of *Oryctes rhinoceros* larvae is *T. virens* 100 g.l⁻¹ + *M. anisopliae* 75 g.l⁻¹ + 5 kg of OPEFB compost, with an initial death time of 31.20 hours, a lethal time of 120 hours, and a total mortality of 66%.

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