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RESEARCH ARTICLE

COMPATIBILITYTESTOFTRICHODERMA VIRENS ANDMETARHIZIUM ANISOPLIAE ANDTHEIR 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 beein 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 Oryctesrhinoceros 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 werecompatible, 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, LT_{50} 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 industriessuch 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).

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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 oil 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 Myzuspersicae by 43.92–74.84% (Trizelia et al., 2021).

The results of research by Sidabutar 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 ware Oryctesrhinoceros 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 ware, LAFC (Laminar air flow 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 $g.l^{-1}$ + 5 kg composite OPEFB, T. virens 50 $g.l^{-1}$ + M. anisopliae 75 $g.l^{-1}$ + 5 kg 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 a 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, LT_{50}, 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:-

Compatibility of M. anisopliae and T. virens:-

Table 1

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

Culturestested	Compatibility
M. anisopliae+T. virens	+

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

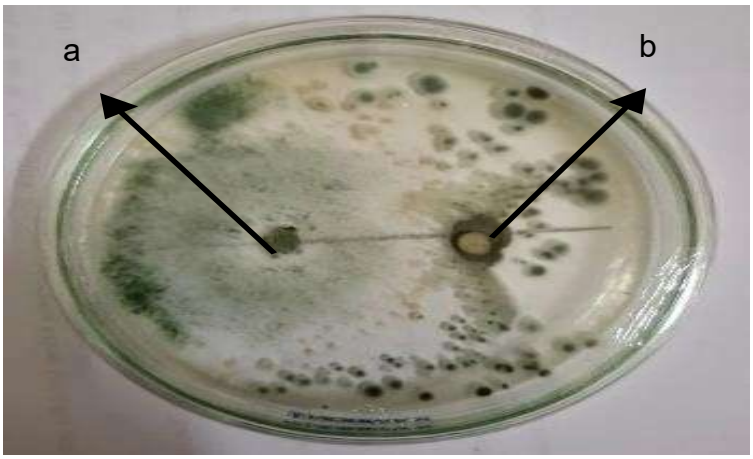


Figure1

Compatibility test of(a) T. virens fungus,(b) M. anisopliae funguson PD Amedia

Initialtime of death of O. rhinoceroslarvae:-

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.

CompatibilitytreatmentofT. virensandM. anisopliaeoncompost	Timeofonsetofdeath(Hours)
T. virens0g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	98.4a
T. virens25g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	68.8a
T. virens50g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	64.8a
T. virens75g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	60.0a
T. virens100g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	31.2b

Numbers followed by different lowercase letters are significantly different according to the DNMRT test at 5% level after being transformed with √y.Lethal time 50 (LT_{50}) of O. rhinoceros larvae:-

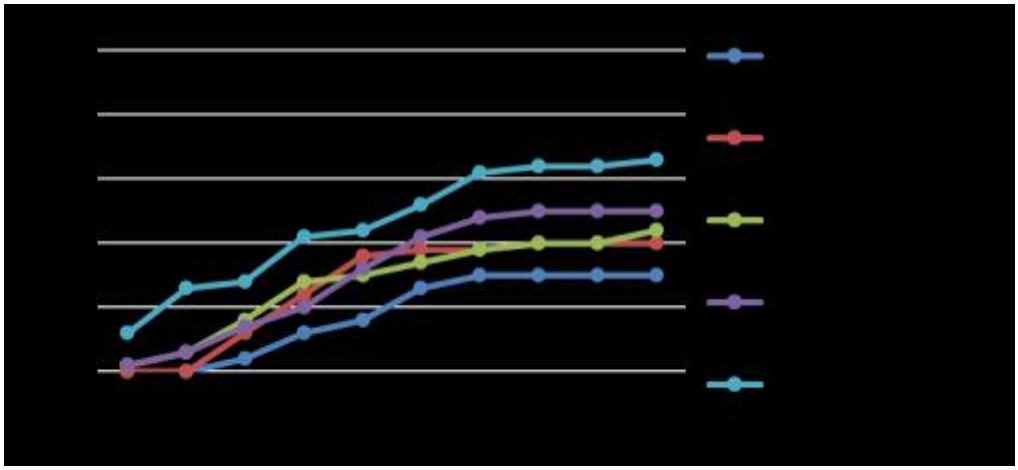
Table 3

Lethal Time of 50 O. rhinoceros Larvae After Several Compatibility Treatments of M. anisopliae and

Perlakuan kesesuaian M. anisopliaedan T. virensmengendalikan O. rhinoceros dalam kompos	Waktu mematikan 50 (Jam)
T. virens0 gl $^{-1}$ + M. anisopliae75 gl $^{-1}$ + 5 kg Kompos TKKS	240.00a
T. virens25 gl $^{-1}$ + M. anisopliae75 gl $^{-1}$ + 5 kg Kompos TKKS	168.00ab
T. virens50 gl $^{-1}$ + M. anisopliae75 gl $^{-1}$ + 5 kg Kompos TKKS	192.00ab
T. virens75 gl $^{-1}$ + M. anisopliae75 gl $^{-1}$ + 5 kg Kompos TKKS	144.00b
T. virens100 gl $^{-1}$ + M. anisopliae75 gl $^{-1}$ + 5 kg Kompos TKKS	120.00b

followed by different lowercase letters are significantly different according to the DNMRTtest at 5% level after being transformed with √y

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.



Total mortality of O. rhinocero
Table 4
Total mortality of O. rhinoceros larvae after several compatibility treatments of T. virens and M. anisopliae in compost.

CompatibilitytreatmentofT. virensandM. anisopliaeoncompost	Totalmortality(%)
T. virens0g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	30.0b
T. virens25g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	40.0b
T. virens50g.l^{-1}+M. anisopliae75g.l^{-1}+5kgOPEFBCompost	44.0b
T. virens75g.l^{-1}+M. anisopliae75 g.l^{-1}+5kgOPEFBCompost	50.0ab
T. virens100g.l^{-1}+M. anisopliae75 g.l^{-1}+5kgOPEFBCompost	66.0a

Numbers followed by different lowercase letters are significantly different according to the DNMRT test at the 5% level after transforming with √y.

Discussion:-

Compatibility of T. virens and M. anisopliae:-

T. virensand 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, theabsence 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 produces 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 coul 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 L. 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 OPEFB compost 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 OPEFB compost 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.

Mortality total 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 Novianti 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 resultesd 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.

Thecompatibilitytreatment of T. virens 100g.l-1 +M. anisopliae 75 g.l-1 +5 kg ofOPEFB compost wasthe 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 acceleratethe infection process of O. rhinoceros larvae. The high dose given causes more conidia to come intocontact 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 would 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 LT_{50} 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}resultedin the fastest LT_{50},at 120 hours, but was notsignificantly differentfrom the treatments ofT. virens75 g.l^{-1}+ M. anisopliae 75 g.l^{-1}, T. virens50 g.l^{-1} +M. anisopliae75g.l^{-1},andT. virens25g.l^{-1}+M. anisopliae75g.l^{-1}.Thisindicatesthatthepresence 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 slowinfection 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. rhinoceroslarvae:-

Observations of daily mortality showed that mortality of O. rhinoceros larvae began to occur faster in high treatment combination treatments. Treatments of T. virens100 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. virens25 g.l^{-1}+ M. anisopliae75 g.l^{-1}and T. virens 0 g.l^{-1} + M. anisopliae75 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 attaches to the larval cuticle, germinates and penetrates theintegument, 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. virens100 g.l^{-1} + M. anisopliae75 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 ahigh dose, thus more conidia thereby increased the chance of contact and successful infection. Sterkel et al. (2021) stated that the increase in conidia densitywas directly proportional to the host's infection capacity.

Afandhi et 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.

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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