COMPATIBILITYTEST*OFTrichodermavirens*AND*Metarhiziumanisopliae*ANDTHEIR ABILITY TO CONTROL *Oryctes rhinoceros* LARVAE IN COMPOST

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Abstract

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Oryctes rhinoceros is a major pest in oil palm plantations. The control often used is *Metarhizium anisopliae,* and also *Trichoderma*sp. whichhavebeeinreportedtobeabletocontrol pests. This study aims to test the compatibility of *Trichoderma virens* and *Metarhiziumanisopliae* 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 andtestingtheeffectivenessof thecombinationof*T.virens* and *M. anisopliae* on the mortality of *O. rhinoceros larvae* with a completelyrandomizeddesign(CRD).Thetreatmentsconsisted 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$g.l^{-1}$+5kgOPEFBcompost.Thedataweretested 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, 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%.

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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 industriessuchasbiodiesel,soap,detergent,surfactants,cosmetics,medicines,andvariousotherhouseholdandindustrial 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 hectaresandcrudepalm oil(CPO)productionof6.3milliontons(CSA,2023).Thishighproductionresults 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 oil palm plants, namely the rhinoceros beetle(*Oryctes rhinoceros*). This pestcanreduce yields by upto 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.lhas 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.

Materialsand 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,cornkernels,70%alcohol,steriledistilledwater,brownsugar,granulatedsugar,aluminumfoil,plastic *wrap* and label paper. Whilethetoolsused ware, LAFC(*Laminar airflowcabinet), autoclave*, petri dish, steamer, stove, plastic tarpaulin, hoe, 1000 ml *beaker g.lass*, analytical balance, bunsen, cork borer, spatula, ose needle, bucket, sprayer, camera and stationery.

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Thestudyusedacompletelyrandomizeddesign(CRD)consistingoffivetreatmentcombinationsnamely *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 $g.l^{-1}$+5kgcompositeOPEFB,*T.virens*50$g.l^{-1}$+*M.anisopliae*75$g.l^{-1}$+5kgcompositeOPEFB,*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. rhinoceroslarvae.*

Theresearchimplementationstartedfromreisolation of*T.virensandM.anisopliae,*compatibilitytest 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 usinganalysis 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.

ResultsandDiscussion Results

1. Compatibilityof*M.anisopliae*and*T. virens*

Table 1

Thecompatibilityof*M.anisopliae*and*T.virens*wastestedinvitroonPDAmedia.

Culturestested	Compatibility
M.anisopliae+T.virens	+

(-)=notcompatible,(+)=compatible

Figure1

Compatibilitytestof(a) *T.virensfungus*,(b)*M.anisopliaefungus*onPDAmedia

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2. Initialtimeofdeathof*O.rhinoceroslarvae*

Table 2

Timeofonsetoflarvalmortalityof *O.rhinoceros*L.followingadministrationofseveralcompatibility treatments of *M. anisopliae* and *T. virens* on compost.

Compatibilitytreatment*ofT.virens*and*M.anisopliae*oncompost	Timeofonsetofdeath(Hours)
*T.virens*0g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	98.4a
*T.virens*25g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	64.8a
*T.virens*50g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	64.8a
*T.virens*75g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	60.0a
*T.virens*100g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	31.2b

Numbersfollowed bydifferentlowercase letters aresignificantlydifferent according totheDNMRTtestat 5%levelafterbeingtransformedwith√y.

3. *Lethaltime*50(LT50)of*O.rhinoceroslarvae*

Table 3

***LethalTimeof*50*O.rhinocerosLarvae*AfterSeveralCompatibilityTreatments *ofM.anisopliae*and *T.virens*inCompost**

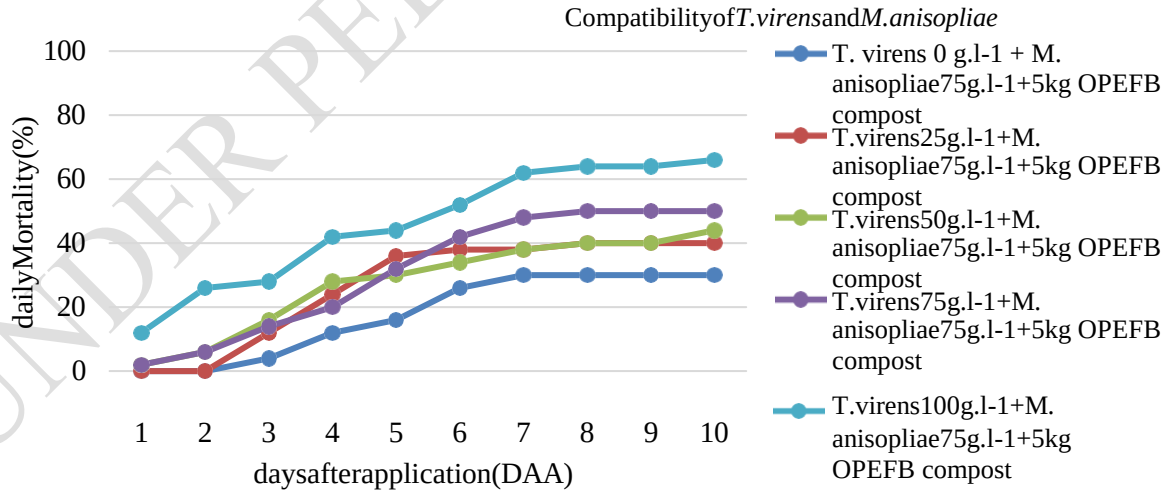
Compatibilitytreatment*ofT.virens*and*M.anisopliae*oncompost	*Lethaltime*50(Hours)
*T.virens*0g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	240.0a
*T.virens*25g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	168.0ab
*T.virens*50g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	192.0ab
*T.virens*75g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	144.0b
T. virens 100 g.l^{-1} + *M. anisopliae* 75 g.l^{-1} + 5 kg OPEFB Compost	120.0b

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

4. Dailymortalityof*O.rhinoceroslarvae*

Figure2

Dailymortalityof*O.rhinoceros*L.larvaeafterapplicationof*T.virens*and*M.anisopliaecompatibility treatment* in compost.



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5. Totalmortalityof*O.rhinoceroslarvae*

Table 4

Totalmortalityof*O.rhinoceroslarvae*afterseveralcompatibilitytreatmentsof *T.virens*and*M. anisopliae* in compost

Compatibilitytreatment*ofT.virens*and*M.anisopliae*oncompost	Totalmortality(%)
*T.virens*0g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	30.0b
*T.virens*25g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	40.0b
*T.virens*50g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kgOPEFBCompost	44.0b
*T.virens*75g.l^{-1}+*M.anisopliae*75 g.l^{-1}+5kgOPEFBCompost	50.0ab
*T.virens*100g.l^{-1}+*M.anisopliae*75 g.l^{-1}+5kgOPEFBCompost	66.0a

Numbersfollowed bydifferentlowercase letters aresignificantlydifferent according totheDNMRTtestat the5%levelaftertransformingwith√y

Discussion

Compatibilityof*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, 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.

Initialtimeofdeathof*O.rhinoceroslarvae*

The compatibility treatment *of T. virens* and *M. anisopliae* to control *O. rhinoceros in* compost showed significantlydifferent results on the initialtimeof deathof*O. rhinoceros*L. larvaewith 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 ofOPEFB 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 fromthecompatibility treatment of *T.virens*100g.l^{-1}+*M.anisopliae*75g.l^{-1}+5kg 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.

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Thecompatibilitytreatment *of T.virens*100g.l^{-1}+*M.anisopliae*75 g.l^{-1}+5 kg ofOPEFBcompost 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.

Lethaltime*50(LT50)*O.rhinoceros

The results showed that the combination of *T. virens* and *M. anisopliae* resulted in a significant differenceintheLT$_{50}$valueof*O.rhinoceroslarvae*,whichrangedfrom120to240hoursafterapplication.Thesingle 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 of*T.virens*75 g.l^{-1}+ *M.anisopliae*75 g.l^{-1}, *T.virens*50 g.l^{-1}+*M.anisopliae*75g.l^{-1},and*T.virens*25g.l^{-1}+*M.anisopliae*75g.l^{-1}.Thisindicatesthatthepresenceof*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 slowinfectionprocess is alsoinfluenced by the interaction betweentheinoculum andthehost. 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.

Dailymortalityof*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. 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 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. 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 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.

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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.

Mortalitytotal*O.rhinoceros larvae*

The results showed that the combination treatment of *T. virens* and *M. anisopliae* in compost had a significanteffectonthetotalmortalityof *O.rhinoceros larvae*, witharangeof30–66%.Thebesttreatment tended to be obtained from the combination of *T. virens* 100 g.l^{-1} + *M. anisopliae* 75 g.l^{-1} which resulted ina totalmortality of 66%,although it was not significantly different from the treatment of *T.virens* 75g.l^{-1} +*M. anisopliae* 75 g.l^{-1} which resulted in 50%. Furthermore, these two treatments were significantlydifferent 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%,and30%,respectively.Thisdifferenceindicatesthatincreasingthedoseofentomopathogenicfungiis directly correlated with increased larval mortality. Lin *et al*. (2017) stated that the higher the dose ofconidia 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 Noviant*iet 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.

Conclusion

Fromtheresultsofthestudyitcanbeconcludedthatthecompatibilityof*Trichodermavirens*and *Metarhiziumanisopliae*incomposttocontrol*Oryctesrhinoceroslarvae*wasachievedbythefollowing:

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 *Oryctesrhinoceros larvae* is *T. virens* 100g.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%.

Thank-younote

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