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Test concentrations of Riau local rice field isolate entomophatogenic fungus *Metarhizium anisopliae* toward mortality brown planthopper (*Nilaparvata lugens* stal.) in the laboratory

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Abstract. The brown planthopper (BPH) (*Nilaparvata lugens* Stal.) is main pest of rice plants and cause hopperburn and viruses vector. The BPH control uses an entomopathogenic fungus is Riau local rice field isolate *Metarhizium anisopliae*. This isolate was obtained from rice plants in Kampar, Riau. The research was aimed to get the best concentration and the right concentration in killing the BPH. Research was conducted at Laboratory of Plant Pests, Faculty of Agriculture, Riau University from June to April 2023. The research was conducted experimentally using a completely randomized design (CRD) with 6 treatments and 4 replications. The treatment was concentration of 0, 40, 50, 60, 70 and 80 $g.l^{-1}$ aquades to obtain 24 experimental units. The parameters for observation were initial time of death, lethal time 50%, lethal concentrations of 50 and 95, daily mortality and total mortality. The results research shows concentration of 40 $g.l^{-1}$ aquades (0.90 x 10^6 $kon.ml^{-1}$) was the best concentration cause a total mortality of 72.50 ± 9.57%, the initial time of death 163.50 ± 6.45 hours after application and lethal time of 50 206.25 ± 9.77 hours after application. The right concentration in killing 50% and 95% of BPHs was 2.11% and 10.45%..

1 Introduction

The rice (*Oryza sativa* L.) is a food commodity that produces staple food for energy sources for more than 95% of the population in Indonesia [1]. Based on the Riau Province Central Bureau of Statistics (2023), rice production in 2018 reached 266,375 tons, in 2019 the production decreased to 230,874 tons and in 2020 rice production increased to 243,685 tons, while in 2021 and 2022 it decreased again to 223,399 tons and 213,560 tons [2]. Fluctuations in rice production is inseparable from various cultivation factors. One of the factors affecting rice plant production is pest attack. An important pest that attacks rice plants is the brown rice planthopper [3].

Brown planthopper (BPH) (*Nilaparvata lugens* Stal.) is a major pest in rice plants because the damage caused by this pest is quite extensive namely the sucking of the cell fluid of rice

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plants. Typical symptoms of attack are shown by plants which become withered and dry like burning (hopperburn) [4]. BPH is considered dangerous because it easily adapts to new environments and acts as a vector for viral diseases in rice plants [5], namely Rice grassy stunt virus and Rice ragged stunt virus, especially type 2 [3].

Brown planthopper attacks rice plants from nursery to harvest. [6] Reportedly, the attack of 15 BPHs per clump of rice in one-month-old plants within 10 days can cause crop failure. [7] Also reportedly, 4 BPH attacking per clump of rice per 30-day tillering period could reduce yields by 77% and in the pregnant period by about 28%. Efforts to control BPH pests need to be made to keep rice productivity stable.

Biological control agents are the control of plant pest organisms (pest) by natural enemies. Utilization of biological agents is a control technique that is more compatible in application than other biological control components [8]. One of the biological agents is the Riau local entomopathogenic fungus *Metarhizium anisopliae*. The use of local isolates is better because local entomopathogenicfungi are considered adaptable to local ecosystems therefore they are more able to suppress pests and do not disturb the ecological balance [9]. In this study, *M. anisopliae* was isolated from paddy field soil in Kampar. The results of research [10] on local entomopathogenic fungus *M. anisopliae* showed that the concentration of 60 $g.l^{-1}$ aquades was the best concentration that caused mortality of *Leptocorisa oratorius* (F.) with an initial time of death of 90 hours while LT50 at 174,25 hours after application has total mortality of 90%. The purpose of this study was to obtain the best concentration of entomopathogen *Riau local rice field isolate Metarhizium anisopliae* against BPH mortality in the laboratory.

2 Methodology

This research was conducted at the Laboratory of Plant Pests, Faculty of Agriculture,University of Riau from June to April 2023. The research was conducted experimentally using a completely randomized design (CRD) with 6 treatments and 4 replications. The treatment was the Riau local *M. anisopliae* concentrations of 0 $g.l^{-1}$ aquades, 40 $g.l^{-1}$ aquades, 50 $g.l^{-1}$ aquades, 60 $g.l^{-1}$ aquades, 70 $g.l^{-1}$ aquades and 80 $g.l^{-1}$ aquades, in order to obtain 24 experimental units. Each experimental unit consisted of 10 fourth instar BPH nymphs.

2.1 Rice seedbed (*Oryza sativa* L.)

The rice seeds used were the Inpari 42 variety obtained from the Agricultural Technology Assessment Center Riau province. The rice seeds were washed with water, then the washed seeds were put into 14 cm x 14 cm jars with a stocking system and given water in low water conditions. Seeding was carried out for 14 days until 4 leaves grew and were ready to be used for BPH feeding.

2.2 Provision of BPH (*Nilaparvata lugens* Stal.)

Provision of BPH was taken from rice plants at the Rice Seedling Center Kampar, Riau Province. The BPH taken were macroptera and brachyptera imago using an aspirator.

2.3 Propagation of BPH (*Nilaparvata lugens* Stal.)

Propagation of BPH was carried out in 14 cm x 14 cm jars containing rice seedlings aged 14 days after transplanting. New seeding was done every 3 days in order for the food was always be available for the BPH. When the seedlings in the jar turned yellow during maintenance, the BPH were transferred to new 14-day-old rice seedlings. The method of transferring to new seedlings included placing the yellowed seedlings upside down on top of the new seedlings and supported with a 20 cm wire so that the BPH could move to the new seedlings. Yellowed seedlings were left in the jar because BPH imago lid eggs on the leaves. Imago propagation was carried out up to the F2 generation in order to obtain the 4th instar BPH nymphs.

2.4 Privision of Riau local rice field isolate *Metarhizium anisopliae*

Provision of Riau local rice fields isolate *M. anisopliae* came from rice fields in the Rice Seedling Center of Pulau Rambai village, Kampa District, Kampar Regency, Riau Province. The soil was taken at a depth of 10cm-20 cm and put into black plastic and brought to the Plant Pest Laboratory. Procurement of Riau local rice fields isolate *M. anisopliae* used insect bait method. *Tenebrio molitor* larvae were used as traps for the entomopathogenic fungus Riau local rice fields isolate *M. anisopliae*. The soil taken was put into 10 plastic boxes then 10 newly molted 3rd instar *T. molitor* larvae were put into the plastic container containing the soil and covered with a piece of black cloth. After that, the soil was sprayed with aquades to maintain moisture.

2.5 Isolation of Riau local rice field isolate *Metarhizium anisopliae*

Tenebrio molitor larvae suspected of being infected with entomopathogenic fungi were taken from the plastic box media and placed in petri dishes sterilized with 70% alcohol and rinsed with aquades. Next, the larvae were placed in a petri dish containing filter paper (moist chamber method) and incubated for 3 days before being transferred to PDA media at 24°C-28°C. Infected larvae were taken using tweezers in the isolation room of the laminar air flow cabinet (LAFC) and put into solid PDA media, which previously had been sterilized aseptically.

2.6 Identification of Riau local rice field isolate *Metarhizium anisopliae*

Fungus of Riau local rice fields isolate *M. anisopliae* were identified macroscopically by observing colony shape and colony color and microscopically conidia and conidiophores based on their morphological form referring to Barnett and Hunter [11].

2.7 Propagation of Riau local rice field isolate *Metarhizium anisopliae*

Propagation of the fungus Riau local rice fields isolate *M. anisopliae* was carried out using 0.5 kg of broken corn washed thoroughly then steamed in a steamer until one-third cooked. After cooling, the corn was put into 0.25 kg plastic containing two scoops each and sterilized in an autoclave. Riau local rice fields isolate *M. anisopliae* that grew on PDA media were isolated on broken corn media in an isolation room with the end of the plastic inserted into a PVC pipe with a diameter of 1.5 cm and a length of 3 cm. Cotton was inserted into the pipe, covered with aluminum foil and plastic wrap then incubated for 7 days.

2.8 Making of suspension Riau local rice field isolate *Metarhizium anisopliae*

Fungus of Riau local rice fields isolate *M. anisopliae* aged 7 days contained in broken corn media weighed according to the treatment, namely 40 g, 50 g, 60 g, 70 g and 80 g each mixed with 1000 ml of aquades then stirred and filtered. The fungus suspension of Riau local rice fields isolate *M. anisopliae* was added with sugar as much as 1% of the concentration level to accelerate cell division.

2.9 Counting of conidia density Riau local rice field isolate *Metarhizium anisopliae*

The suspension of Riau local rice fields isolate *M. anisopliae* was calculated by serial dilution method to obtain spore density of up to 10^{-2}. A suspension of 1 ml of solution was diluted with 9 ml of aquades then stirred for 5 minutes on a vortex at 100 rpm and repeated until a dilution of 10^{-2} was obtained. Conidia density was calculated using a haemocytometer under a light microscope at 400x magnification. Conidia density was calculated using the formula [12] as follows:

$$J = \frac{t \times d}{0{,}25 \times n} \times 10^{6} \quad (1)$$

Description :
J = Conidia density in 1 g media
t= Sum of densities in all calculated squares n = The number of squares counted
d = The dilution factor 10^{-8} 0,25 = The constant

2.10 Preparation of rice plant, BPH infestation and calibration for treatment

The rice plants used were 14 days old from seeding in jars. BPH instar IV nymphs were taken from the rearing container using an aspirator and infested with 10 tails each into plastic cups. Calibration was carried out using a 250 ml volume hand sprayer filled with aquades to the brim and then sprayed on rice plants in plastic cups evenly until wet. Application was carried out at 07.00 AM.

2.11 Observation parameter

The observation parameters in this research were identification of Riau local rice field isolate *M. anisopliae,* changes in behavior and morphology, the initial time of death (hours), *lethal time* 50 (LT50) (hours), *lethal concentration* (LC50 dan LC95) (%), daily mortality (%), total mortality (%), temperature and humidity.

3 Results and discussion

The concentration test of the entomopathogenic fungus Riau local rice fields isolate M. anisopliae on the mortality of BPH in rice plants was carried out at the Plant Pest Laboratory, Faculty of Agriculture, Riau University at an average temperature of 26.7°C and an average humidity of 85.92% with the following results:

3.1 Identification of isolate Riau local rice fields Metarhizium anisopliae

The identification of Riau local rice fields isolate *M. anisopliae* was carried out macroscopically and microscopically. Macroscopic observations were made by directly observing the shape and color of fungal colonies that grew in petri dishes. The fungus was initially white then turned green in 2-3 days. This agrees with Susandi et al. [13] that the *M. anisopliae* fungus is initially characterized by the appearance of white colonies and turns into a dark green with increasing fungal age.

The fungus Riau local rice fields isolate *M. anisopliae* microscopically has concentrated hyphae with oval-shaped spores and conidiophores that swell and secrete sprouting tubes. According to Susandi et al. [13] the hyphae of *M. anisopliae* have insulation with conidiophores arranged upright with different sizes, layered and *branched* and filled with spores that are cylindrical, hyaline and single-celled. Microscopic observations were made based on morphological forms using the Illustrated Genera of Imperfect Fungi book written by Barnett and Hunter [11], namely a) infected insects, b) conidiophores, c) conidiophores and d) conidia. Macroscopic and microscopic fungus Riau local rice fields isolate *M. anisopliae* can be seen in Figure 1.

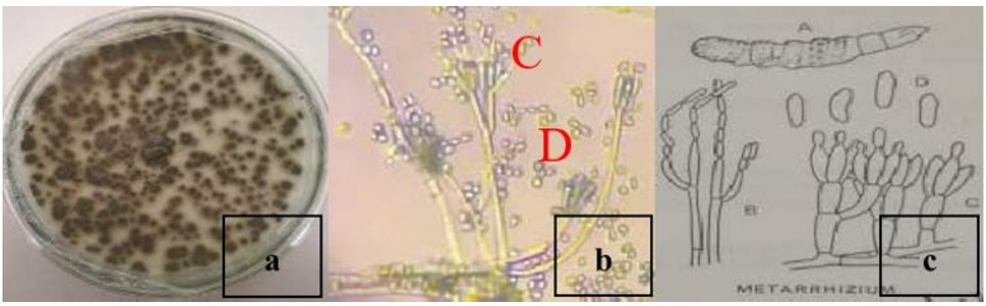

Fig. 1. Identification of Riau local rice fields isolate *M. anisopliae* a) 7-day-old macroscopy, b) 400x magnification microscopy and c) reference microscopy [8]; C (conidiophores) dan D (conidia).

3.2 The changing behavior and morphology

Application of Riau local rice fields isolate *M. anisopliae* fungus concentration to BPH caused changes in BPH behavior and morphology. It takes time for the entomopathogenic fungus Riau local rice fields isolate *M. anisopliae* to infect BPH. Changes in BPH behavior are decreased activity. This is in accordance with the results of research Rustama et al. [14] that the symptoms of *M. anisopliae* infection are the decreased feeding activity, uncoordinated movements and finally the insect dies.

Changes in BPH morphology, namely the dark brown body is then overgrown by white fungal hyphae and turns green. The dark brown discoloration of BPH is due to melanization which is a form of insect body defense against pathogens [15]. The black discoloration or melanization is due to the activity of the enzyme phenoloxidase. This enzyme is known to play a role in the wound healing process, sclerotization of the cuticle and plays a role in the melanization process against foreign objects that enter the hemocoel [16].

The surface of the BPH body is covered by the mycelium of the fungus Riau local rice fields isolate *M. anisopliae.* According to Pracaya [17] pests infected with *M. anisopliae* fungus are initially characterized by the appearance of white flour which then turns into dark green and the pest's body will be stiff and hardened (mummification). The stages of Riau local rice fields isolate *M. anisopliae* infecting BPH can be seen in Figure 2.

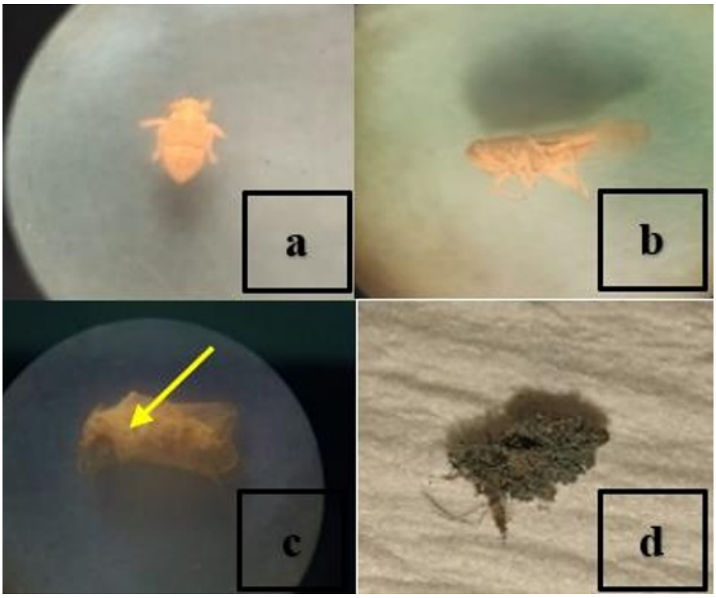

Fig. 2. The changing morphology BPH after application concentration of Riau local rice fields isolate *M. anisopliae.*

3.3 The initial time of death

The results of variance showed that the treatment of several concentrations of Riau local rice fields isolate *M. anisopliae* on rice plants had a significant effect on the initial time of BPH death. The results of the initial time of BPH death after the DNMRT test at the 5% level can be seen in Table 1.

Table 1. The initial time of BPH death after application concentration of Riau local rice field isolate *M. anisopliae*.

Concentration of isolate Riau local rice field *M. anisopliae*	Initial Time of Death ± SD (hours)
0 $g.l^{-1}$ aquades (0.00 x 106 $kon.ml^{-1}$)	238.00 ± 0 a
40 $g.l^{-1}$ aquades (0.90 x 106 $kon.ml^{-1}$)	163.50 ± 6.45 b
50 $g.l^{-1}$ aquades (1.05 x 106 $kon.ml^{-1}$)	158.00 ± 7.93 bc
60 $g.l^{-1}$ aquades (1.40 x 106 $kon.ml^{-1}$)	156.50 ± 10.98 bc
70 $g.l^{-1}$ aquades (1.75 x 106 $kon.ml^{-1}$)	152.50 ± 8.34 bc
80 $g.l^{-1}$ aquades (1.90 x 106 $kon.ml^{-1}$)	139.25 ± 12.17 c

The numbers in the column followed by small letters that are not the same are significantly different according to the DNMRT test at the 5% level, after being transformed by the formula $\sqrt{y}$

Table 1 shows that the application of several concentrations of Riau local rice fields isolate *M. anisopliae* caused significant differences in the initial time of BPH death with a range of 139.25 hours-163.50 hours. The treatment of 40 $g.l^{-1}$ aquades concentration showed the longest initial time of death, 163.50 hours, which was not significantly different from that of the the concentrations of 50 $g.l^{-1}$ aquades, 60 $g.l^{-1}$ aquades and 70 $g.l^{-1}$ aquades with the initial time of BPH death, namely 158 hours, 156.50 hours and 152.50 hours but significantly different from 80 g.l^{-1} aquades, namely 139.25 hours.

The treatment of 50 g.l^{-1} aquades concentration is the best concentration because it is not significantly different from the treatments of 40 g.l^{-1} aquades, 60 g.l^{-1} aquades, 70 g.l^{-1} aquades and 80 g.l^{-1}, namely 158 hours, 156.50 hours, 152.50 hours and 139.25 hours. This is because the fungus is still making adjustments to the environment. In addition, according to Ferron [18] that the successful use of entomopathogenic fungi in pest control is partly determined by the density of conidia and spore germination, the higher the density and germination of spores, the chance of fungi infecting insects is faster and vice versa the lower the density and germination power, the chance of fungi in killing is also slower.

3.4 Lethal time 50 (LT 50)

The results of variance analysis showed that the application of several concentrations of Riau local rice fields isolate *M. anisopliae* on rice plants gave a real effect on the lethal time of 50 (LT50) BPH. The average results of LT50 BPH after DNMRT test at 5% level can be seen in Table 2.

Table 2. Lethal Time of 50 BPH after application concentration of Riau local rice field isolate *M. anisopliae*.

Concentration of isolate Riau local rice field M. anisopliae	Lethal Time 50 ± SD (hours)
0 g.l^{-1} aquades (0.00 x 106 kon.ml^{-1})	238.00 ± 0 a
40 g.l^{-1} aquades (0.90 x 106 kon.ml^{-1})	206.25 ± 9.77 ab
50 g.l^{-1} aquades (1.05 x 106 kon.ml^{-1})	204.25 ± 10.04 bc
60 g.l^{-1} aquades (1.40 x 106 kon.ml^{-1})	197.50 ± 3.10 bc
70 g.l^{-1} aquades (1.75 x 106 kon.ml^{-1})	193.75 ± 5.12 bc
80 g.l^{-1} aquades (1.90 x 106 kon.ml^{-1})	189.25 ± 9.17 c

The figures on the lane followed by lowercase letters were not significantly different according to the DNMRT test at the 5% level after being transformed with formula √y

Table 2 shows that the application of several concentrations of Riau local rice filed isolate *M. anisopliae* gave a significantly different effect on lethal time of 50 with a range 189.25 hours-206.25 hours after application. The entomopathogenic fungus Riau local rice field isolate *M. anisopliae* in the treatment of 40 g.l^{-1} aquades caused LT_{50} of 206.25 hours and was not significantly different from 50 g.l^{-1} aquades, 60 g.l^{-1} aquades and 70 g.l^{-1} aquades with LT_{50} of 204.25 hours, 197.50 hours and 193.75 hours. This is due to the resistance factor of BPH as a host of entomopathogenic fungi therefore it shows the same LT_{50} results. The process of fungal infection of the host involves several physiological responses or host immunity to xenobiotic and pathogenic types [19]. In addition, the conidia factor also affects BPH. According to Prayogo [20], not all conidia of entomopathogenic fungi that are applied successfully reach the target because of the high mobility of insects and the existence of molting events, consequently the poison cannot work optimally.

The length of time for LT_{50} death against BPH is 189.25 hours to 238 hours. This is because the fungus needs time to go through the infection mechanism from contact to destruction. The growth of *M. anisopliae* conidia attached to BPH takes time to germinate into hyphae before penetrating the insect's integument. According to Herlinda et al. [21] the length of time required for entomopathogenic fungus isolates from insect infection to death ranges from 2 to10 days under appropriate environmental conditions.

3.5 Lethal concentration 50 and 95 (LC_{50} and LC_{95})

Based on the results of probit analysis using the POLO-PC program, the concentration of Riau local rice field isolate *M. anisopliae* showed LC50 and LC95 which were 2.11% and 10.45%, respectively. The results of probit analysis can be seen in Table 3.

Table 3. Lethal concentrations of 50 and 95 BPH after application concentration of Riau local rice field isolate *M. anisopliae*.

Lethal concentration (LC)	Concentration	Range CI (%)
LC 50	2.11	0.57 – 3.10
LC 95	10.45	7.81 – 26.32

CI = Confidence interval

Table 3 shows that the concentration of Riau local rice field isolate *M. anisopliae* capable of killing 50% of the BPH population is 2.11% or equivalent to 21.1 $ml.l^{-1}$ aquades with a confidence interval in the range of 0.57%-3.10%. The concentration capable of killing 95% of the BPH population was 10.45% or equivalent to 104.5 $ml.l^{-1}$ aquades with a confidence interval in the range of 7.81%-26.32%.

3.6 Daily mortality

The observation of the percentage of daily mortality of BPH on rice plants with the application of different concentrations of Riau local rice fueld isolate *M. anisopliae* showed fluctuations in BPH mortality. Daily mortality of BPH can be seen in Figure 3.

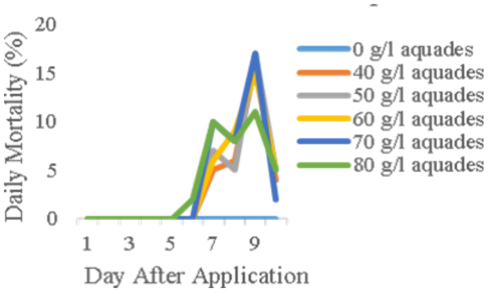

Fig. 3. Daily mortality of BPH after application concentration of Riau local rice field isolate *M. anisopliae*

Figure 3 shows that the daily mortality of BPH started on day 6 after the application of Riau local rice field isolate *M. anisopliae* in the treatment of 80 $g.l^{-1}$ aquades, on day 7 after the treatment concentrations of 40 $g.l^{-1}$ aquades, 50 $g.l^{-1}$ aquades, 60 $g.l^{-1}$ aquades and 70 $g.l^{-1}$ aquades then decreased on day 8. The peak mortality of all then treatments occurred on day 9 and decreased on day 10. This is because the *M. anisopliae* fungus needs time to infect BPH.

The peak of BPH mortality occurred on day 9, since the process of infection of entomopathogenic fungi causes death to BPH on day 9. *M. anisopliae* can infect by making contact between the propagule and the insect's body then the attachment and germination of fungal propagules on the insect's integument occurs. The fungus uses compounds found in the insect's integument. Next, penetration occurs and this penetration reaches the integument to form a sprouting tube. The last stage is destruction at the point of penetration and the formation of blastospores that invade other tissues [22].

On day 10, there wa a decrease in daily mortality of BPHs, this was caused by many BPH that died on days 7-9. The percentage of BPHs tha decreased indicated that presumably the *M. anisopliae* infention mechanism had reached an advanced stage.

3.7 Total Mortality

The results of variance showed that the application of several concentrations of Riau local rice field isolate *M. anisopliae* had a significant effect on total BPH mortality. The average results of total BPH mortality after the DNMRT test at the 5% level can be seen in Table 4.

Table 4. Total mortality of BPH after application concentration of Riau local rice field isolate *M. anisopliae*.

Concentration of isolate Riau local rice field *M. anisopliae*	Total Mortality ± SD (hours)
0 $g.l^{-1}$ aquades (0.00 x 10^6 $kon.ml^{-1}$)	00.00 ± 0 c
40 $g.l^{-1}$ aquades (0.90 x 10^6 $kon.ml^{-1}$)	72.50 ± 9.57 b
50 $g.l^{-1}$ aquades (1.05 x 10^6 $kon.ml^{-1}$)	85.00 ± 10 ab
60 $g.l^{-1}$ aquades (1.40 x 10^6 $kon.ml^{-1}$)	87.50 ± 5 ab
70 $g.l^{-1}$ aquades (1.75 x 10^6 $kon.ml^{-1}$)	87.50 ± 5 ab
80 $g.l^{-1}$ aquades (1.90 x 10^6 $kon.ml^{-1}$)	90.00 ± 8.16 a

The figures on the lane followed by lowercase letters were not significantly different according to the DNMRT test at the 5% level after being transformed with formula $\sin^{-1} \sqrt{y/100 + 0.5}$

Table 4 shows that the applications of several concentrations of Riau local rice field isolate *M. anisopliae* caused BPH death with a total mortality of 72.50%-90.00%. The concentration of 40 $g.l^{-1}$ aquades was the best concentration that could cause 72.50% total mortality and was not significantly different from the treatment concentrations of 50 $g.l^{-1}$ aquades, 60 $g.l^{-1}$ aquades 70 $g.l^{-1}$ aquades which caused total mortality of 87.50% and 90,00% and was significantly different 80 $g.l^{-1}$ aquades is 90.00%. This shows that Riau local rice field isolate *M. anisopliae* is well used to BPH where at a concentration of 50 $g.l^{-1}$ aquades has caused a total mortality of 85.00%. This agrees with [23] which states that fungi that can be categorized as biopesticides are fungi that are successful in controlling insects with mortality of 72%-95%.

The results showed that the lowest concentration of 40 $g.l^{-1}$ aquades was classified as a biopesticide which caused a total mortality of 72.50%. It is suspected that the local Riau rice field isolate has a high ability to infect BPH. The fungus Riau local rice fields isolate *M. anisopliae* infects BPH in contact with the insect cuticle and germinates. The penetration of Riau local rice fields isolate *M. anisopliae* with the help of peptidase and chitinase enzymes, then with the help of mechanical pressure, the enzymes destroy the skin by lysis. The *M. anisopliae* fungus produces six enzymes, namely lipase, chitinase, amylase, proteinase, pospatase and esterase [24]. After the fungus Riau local rice field isolate *M. anisopliae* enters the body of the pest, the conidia quickly multiply themselves therefore the blastospores immediately spread in the host body Ferron [18].

4 Conclusion

According to the study's findings concentration of 40 g.l^{-1} aquades (0.90 x 10^{6} kon.ml^{-1}) is the best concentration because it can cause a total mortality BPH of 72.50 ± 9.57%, the initial time of death BPH of 163.00 ± 6.45 hours after application and the lethal time of 50 206.25 ± 9.77 hours after application. The right concentration in killing 50% of BPHs is 2.11% and the concentration of 95% in killing BPHs is 10.45%.

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