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Pathogenicity of Indigenous Entomopathogen Liquid Formulation to Rice Bug Nymphs (*Leptocorisa acuta* Thunberg)

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ABSTRACT

The purpose of this study was to determine the effectiveness of bio insecticide liquid formulations made from indigenous entomopathogens against paddy bugs nymphs. The design of this experiment was a non-factorial Complete Randomized Design with 9 treatments and 1 control with five replications. The treatments studied consisted of E0: Control, E1: *Beauveria* sp Pky isolate + coconut water (CW), E2: *Metarhizium* sp isolate Jjt + CW, E3: *Beauveria* sp isolate Jts + CW, E4: *Metarhizium* sp isolate Lcc + CW, E5: *Beauveria* sp Pky isolate + shrimp shell extract (SSE), E6: Metarhizium sp isolate Jjt + SSE, E7: *Beauveria* sp isolate Jts + SSE, E8: *Metarhizium* sp isolate Lcc + SSE, E9: Synthetic insecticide Carbamate 2 ml L^{-1}). The result showed that liquid bioinsecticide formulations were effective against the pest, infected nymphs and shortening nymph lethal time. All entomopathogenic fungi isolates were able to cause infections in rice bugs nymphs 68% - 84%. The isolates of *Beauveria* sp Pky and *Metarhizium* sp Jjt caused nymph mortality of 72% - 84%, which were not different from insecticide Carbamate. Conidia viability of all entomopathogenic isolates in liquid media within 24 hours reached more than 80%. The fastest time to lethal of paddy bugs nymph occurred at 5.44 days (*Metarhizium* sp. isolate) and 5.92 days (*Beauveria* sp. isolate) in coconut water media. *Metarhizium* sp. and *Beauveria* sp. in the medium of coconut water or shrimp shell extract has a very high potential to be developed as a bio insecticide.

INTRODUCTION

In 2015, The Ministry of Agriculture issued a policy on special efforts for three food crop commodities, respectively rice, corn and soybeans in order to achieve food self-sufficiency. The pests that often attack lowland rice are brown plant hopper, rice bugs, and rice stem borers. The cumulative *Leptocorisa acuta* attack in Central Kalimantan in 2017 reached 388.90 ha or 15.18% of all attacks. The peak of the attack occurred in January and February with affected area of 102.5 ha and 83.5 ha (Central Kalimantan BPTPH, 2017), while in 2018 the cumulative attack area was 273.70 ha (BPTPH, Central Kalimantan 2018). This pest damages by absorbing the grains of rice to the ripe phase of milk so that the grain becomes empty. Severe attacks can reduce production to crop failure.

The use of insecticides continuously will cause damage to the ecosystem, kill beneficial

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organisms, the occurrence of pest explosion and require expensive costs. One alternative for pest control is the use of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* Herlinda *et al.* (2008a) suggested *B. bassiana* and *Metarhizium anisopliae* effective in turning off the green leafhopper and brown plant hopper nymphs in the fastest time of about 4 days. In addition, Effendy et al. (2010) also reported that the two entomopathogenic fungi isolates were able to kill 50% of rice bugs for 5-8 days after infection.

Development of liquid bio insecticide formulations are made from indigenous entomopathogenic fungi in controlling pests and plant diseases. The variations of development of liquid bio insecticide formulations, it is necessary to study the factors that influence the virulence of fungal isolates during the process of making bio insecticide formulations, so that effective products can be obtained in terms of killing pest insects. Coconut water can be used as growth medium for mold spores because coconut water contains a variety of nutrients that are adequate for fungus growth. Juliana et al., (2017 reported that coconut water could be used as a growing medium for *Trichoderma* sp. Herlinda et al., (2006) enrichment of the media by the addition of cricket flour containing chitin was able to increase the density of *B. bassiana* spores. Based on this statement it can be analogous to shrimp shell broth containing chitin which is also useful as a medium for the entomopathogenic fungus propagation. Entomopathogenic fungal spores in liquid formulations tend to have higher viability, virulence and produce mycotoxins compared to solid media. The better the media for fungal propagation, the higher the mycotoxins produced by entomopathogenic fungi (Hasyim et al. 2011). Entomopathogenic fungi have a high infection ability and the presence of a carrier that contains a lot of nutrients in the form of protein, carbohydrates, chitin can maintain the effectiveness of liquid bioinsecticides with active ingredients B. *bassiana* and M. *anisopliae* (Herlinda et al., 2008a).

This study aimed to determine the pathogenicity of the bio insecticide liquid formulation of entomopathogenic fungi, from mortality infected nymphs, the time of death of paddy bugs nymphs (*Leptocorisa acuta* Thunberg) and the viability of conidia.

MATERIALS AND METHODS

Research location was in Tumbang Liting Village, Subdistrict of Katingan Hilir, Katingan Regency and Laboratory of Agriculture Cultivation, Faculty of Agriculture, University of Palangka Raya from January to July 2019. The treatments studied consisted of E0: Control, E1: *Beauveria* sp isolate Pky + coconut water (CW), E2: *Metarhizium* sp isolate Jjt + CW, E3: *Beauveria* sp isolate Jts + CW, E4: *Metarhizium* sp isolate Lcc + CW, E5: *Beauveria* sp isolate Pky + shrimp shell extract (SSE), E6: Metarhizium sp Jjt + SSE isolates, E7: Beauveria sp Jts + SSE isolates, E8: *Metarhizium* sp Lcc + SSE isolates, E9: Synthetic insecticides Carbamate 2 ml L^{-1})

Exploration of entomopathogenic fungi used the baiting method in which *Tenebrio molitor L*. larvae was incubated for 7 days in rice rhizosphere soil samples, mustard greens and legume cover crops (LCC). Larvae that were infected by fungal colonies were isolated to obtain entomopathogenic isolates. Pure isolates *Metarhizium* sp. and *Beauveria* sp. were grown on PDA media. Test insects used were noticeable 3rd time instar (offspring) which previously had been reproduced.

Mass-culture of entomopathogenic conidia refered to the method of Herlinda et al.(2006), the composition of 1 L coconut water and 40 g dextrose. Shrimp broth media were made by boiling 250 g of shrimp shells in 1 L of water for 15-20 minutes, then adding 40 g dextrose. Each liquid media was added with 5 g of cricket flour to enrich the medium nutrition. Then, the media was sterilized with an autoclave of 121^0 C for 30 minutes. As the media was getting colder, each according to treatment, was in pure inoculation of *Beauveria* sp. and *Metarhizium* sp. 10 pieces,it was done aseptically . Each culture was incubated for 7 days before being applied to the test insects. An entomopathogenic pathogenicity test was carried out by spraying 10 mL conidia suspension with 10^8 conidia / mL densities on each treatment

unit containing 5 nymphs (Koswanudin, 2014), then the nymphs were kept in plastic jars containing a sprig of ready harvested rice and then covered with gauze (Christina & Jantje, 2015).

Observations were made on: 1) Infected nymphs, marked by decreased appetite, mobility, hardened body and discoloration of the body into whiteness and greenness covered by fungus mycelium. Calculation of percentage of infected nymphs refers to Prayogo et al. (2011), 2) Nymphs mortality, by counting the number of dead nymphs and the number of nymphs remaining to form an imago was recorded. Percentage of death of paddy bugs nymphs was calculated with the formula,

$$P = \frac{n}{N} \times 100\%$$

where P = Percentage of nymph mortality, n = Number of dead nymphs N = Initial number of nymphs tested, 3) Lethal time of nymphs, length of time of death of nymphs is calculated by referring to Karmila (2006). Observations were carried out every day for 14 days after application, 4) Conidia Viability, by counting conidia germination in each bio insecticide 6 hours and 24 hours after incubation. Conidia germination was observed with a light microscope with a magnification of 100x-400x, and the percentage calculated was based on Prayogo et al. (2011).

RESULTS AND DISCUSSION

The highest percentage of infected nymphs was obtained at treatment E1 (84%), significantly different from treatments E3, E7 and E8. The ability to kill and the rate of infecting test insects for each isolate with liquid propagation media were varied, but all isolates were generally able to infect test insects (Table 1).

Rice bugs nymphs infected with entomopathogenic fungi initially showed wrinkled body shape, hardened and there were black spots on the cuticle of the abdomen as the first point of infection that caused insects to become weak, then, the nymphs die and the entire surface of the body was covered with white mycelia as sign that was infected by *Beauveria* sp and green hypha as a sign of being infected with *Metarhizium* sp. Later, hthe bugs harden as mummy. This is in line with the statement of Shinde et al. (2010). Symptoms of infected nymphs are shown in Figure 1.

The effect of bioinsecticide liquid formulations on The highest mortality due to the bioinsectice treatment was found in the treatment of *Metarhizium* sp. The isolates of Jjt isolate and *Beauveria* sp. Pky iso in coconut water media had the same infection ability that were equal to 84% and 80% and both had the same effectiveness with synthetic insecticide Carbamat.

Entomopathogenic fungus isolate *Beauveria*

Table 1. Percentage of infected nymphs, mortality of nymphs and lethal time of nymphs affected by entomopathogenic liquid formulation treatments

Treatment	Variabel		
	Infected Nymphs (%)	Nymphs Mortality (%)	Lethal Time of Nymphs (day)
Control (withoutEP)(E0)	0 a	0 a	0 a
Bv isolat Pky+CW (E1)	84 c	80 de	5.92 cd
Ma isolat Jjt + CW (E2)	72 bc	84 de	5.44 bc
Bv isolat Jts+CW (E3)	48 b	56 bc	10.40 f
Ma isolat Lcc + CW(E4)	52 bc	76 cd	7.44 de
Bv isolat Pky+SSE (E5)	68 bc	72 bcd	8.56 e
Ma isolat Jjt+SSE (E6)	56 bc	72 bcd	7.24 de
Bv isolat Jts+SSE (E7)	40 b	52 b	12.16 g
Ma isolat Lcc+SSE(E8)	44 b	76 cd	8.44 e
Carbamat 500 EC (E9)	0 a	100 e	1 a
LSD(0.05)	33.46	23.98	1.56

Notes: EP = entomopathogen; Bv = *Beauveria* sp; Ma = *Metarhizium* sp.; CW = coconut water; SSE = Shrimp Shell Extract

sp. in-

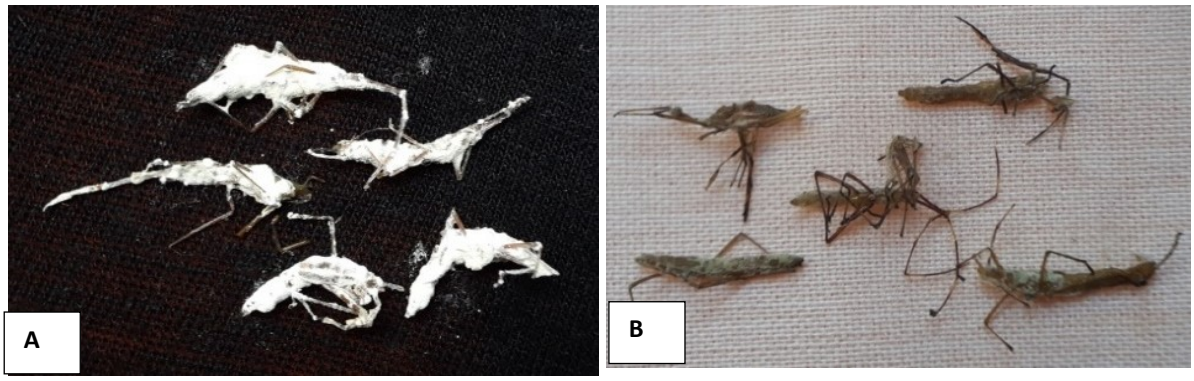

Figure 1. A. Nymphs infected with *Beauveria* sp. 12 dai; B. Nymphs infected with *Metarhizium* sp. 12 dai

digenous (E1) showed a higher pathogenicity than other isolates in infecting paddy bugs nymph up to 84%. Paddy bugs nymphs infected after application of entomopathogenic fungi had behavioral and morphological changes which were slow movements, tended to stay away from food, decreased appetite, and tended to separate from other populations. This is in accordance with the statement of Priyanti (2009) this phenomenon is known as summit diseases, where insects infected with entomopathogenic fungi show behavior rising to the surface of plants, attaching themselves away from other populations.

Types of entomopathogenic isolates have differences in average time in causing death of nymphs. The time taken for entomopathogenic fungi from infection to dead insects showed the pathogenicity of the isolate. This difference in mean death time is related to isolate virulence and host susceptibility. The shorter the average time of death, the more virulent the isolate was. Neves and Alves (2004) suggested that the time of death of an insect is influenced by the application dose and virulence of the isolate. The difference in the time of death caused by each isolate due to fungal conidia requires time to turn off the host, conidia attached to the insect integument must germinate first. The fastest time to die in the treatment of *Metarhizium* sp. isolate Jjt (5.44 days) and was not different from the treatment of *Beauveria* sp. Pky isolate (5.92 days) on coconut water media.

The percentage of conidia germinated at 6 hours after incubation reached more than 70% and at 24 hours of observation the percentage of conidia germinated increased to more than 80% (Figure 2).

According to Kassa (2003), the conidia of entomopathogenic fungal germination that can be used as biological agents is at least 80%. The addition of cricket flour to each entomopathogenic fungus propagation medium also influences the viability of fungal conidia, since cricket flour contains chitin as a source of nutrition that resembles to the nutrition of the host insect. However, the ability of entomopathogens can be categorized as biological agents not only based on their viability, but also some other things such as the ability to produce enzymes such as proteases, chitinase, amylase and lipolytic and secondary metabolites that function as degeneration of insect cuticles and kill the nervous system of insects. The morphological shape of conidia and germ tube of entomopathogenic fungi *Beauveria* sp and *Metarhizium* sp as in Figure 3.

The insect body that has just changed its skin in instar 3 also influenced the effectiveness of

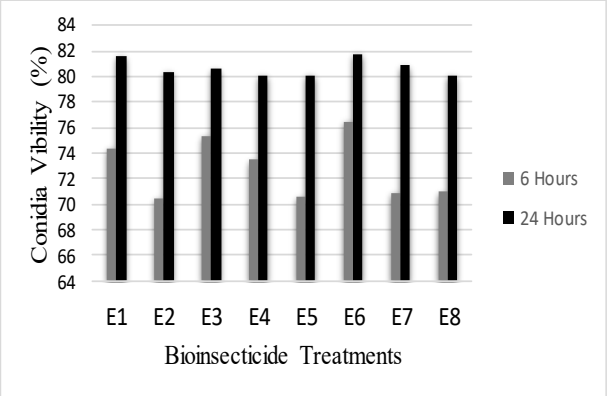

Figure 2. Viability of entomopathogenic conidia in liquid media.

Notes: Bv isolat Pky+AK (E1), Ma isolate Jjt + AK (E2); Bv isolate Jts+CW (E3); Ma isolate Lcc + CW (E4); Bv isolate Pky+SSE (E5); Ma isolate Jjt+SSE (E6); Bv isolate Jts+SSE (E7); Ma isolate Lcc+SSE (E8). Bv = *Beauveria* sp; Ma = *Metarhizium* sp.; CW = coconut water; SSE= shrimp shell extract

the fungus in infecting nymphs because the cuticle layer of the nymph was still very thin, making it easier for entomopathogenic fungi to infect the parasitic nymph. Unfavorable environmental conditions, the development of entomopathogenic fungi only takes place in the

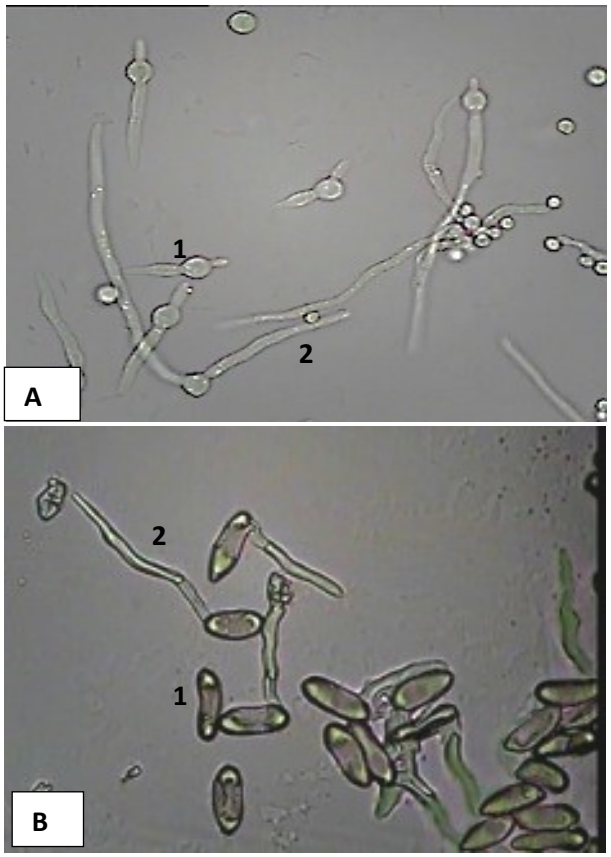

Figure 3. Morphology of Conidia a) *Beauveria* sp; b) *Metarhizium* sp; 1) Conidia; 2) Germ tube

body of insects without going out through the insect integument. The age of the test insect instar also determines the success rate of fungi for penetration and infection. The younger the instar is tested, the skin layers of insects will be more susceptible to entomopathogenic fungi.

The highest mortality rate of nymphs of 84% occurred in the isolate *Metarhizium* sp isolate Jjt (E2) higher than other isolates. The high percentage of nymph mortality related to the origin of the fungus isolates obtained, it was in the rice plant rhizosphere which is a habitat for the host pest. Virulent entomopathogenic fungi can be obtained from the target pest or from the rhizosphere in the plant ecosystem where the pest is located since the soil is a natural habitat for entomopathogenic fungi.

Beauveria sp Pky isolate (E1) was also able to cause a mortality to 80%. This showed the difference in habitat, for example, the origin of the isolate influences the ability of the function to kill insects. Isolates which have a high level of pathogenicity when applied outside the original habitat will have different pathogenicity to the host insect.

Coconut water media used in the process of making entomopathogenic liquid formulations is a better media for fungal pathogenicity than shrimp broth, because coconut water causes higher mortality than shrimp broth media. The high mortality was suspected because coconut water is an organic media that contains nutrients needed for growth, development and fungal activity. The addition of cricket flour containing protein and chitin can engineer artificial media so that it resembles the nutrients in the original host. This is in line with research Herlinda et al. (2006b), that media enrichment with the addition of cricket flour containing chitin can increase the density of *B. bassiana* spores, and cause the death of *Plutella xylostella* larvae to 78.33%.

The fastest death of nymph was 5.44 days, it was assumed that indigenous entomopathogenic isolates were highly adaptable to environmental factors, conidia germinate immediately and penetrate the insect integument. According to Tanada & Rich (2011), the period of the initial process of infection until insect death occurs within a short period of time that is only 3 days and no later than 12 days, but generally occurs within 5-8 days and the period can differ depending on the size host. Virulent isolates are capable of killing insects in a short time and less virulent isolates need a long time to cause chronic infections.

In this study the origins of different fungal isolates and different propagation media did not affect the fungus to germinate. Microscopic conidia seen from all entomopathogenic fungi isolates 6 hours after incubation had formed a sprout tube with viability of more than 70% and after 24 hours of incubation, and there was an increase in viability to more than 80%. High conidia density affected its viability, impacting on the high mortality of nymphs. This showed that the isolate has the potential to be developed as a bio insecticide to control rice pests.

CONCLUSION

All entomopathogenic fungi isolates were able to infect the rice bug nymphs in the range between 68% to 84%. *Beauveria* sp Pky isolate and *Metarhizium* sp Jjt isolate caused nymph mortality 72% - 84%. The effectiveness did not differ from insecticide Carbamate. Conidia viability of all entomopathogenic isolates in liquid media within 24 hours reached more than 80%. The fastest death time of paddy bugs nymph occurred at 5.44 days (isolate *Metarhizium* sp) and 5.92 days (*Beauveria* sp isolate) in coconut water media. In the media of coconut water or shrimp shell extract, *Metarhizium* sp. and *Beauveria* sp. have a very high potential to be developed as a bioinsecticide.

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