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Pathogenıcıty Beauveria Bassiana (Deuteromycetes), Whıch Provıded By Addıtıonal Chıtın From Sources Tenebrio Molitor And Shrımp Skın On The Reproductıon Medıa Hypothenemus Hampei Ferrari. (Coleoptera: Scolytıdae)

Abdul Rizal AZ[1], Muhamad Rizki Miharja[2], Siwi Hardiastuti EK[3]

[1,2,3] Department of Agrotechnology, Faculty of Agriculture Universitas Pembangunan Veteran Yogyakarta. Jalan Padjadjaran lingkar Utara Yogyakarta. Postcode 55281

Corresponding author: abdul.rizal@upnyk.ac.id

Abstract

Beauveria bassiana is one of the biological agents to control insect pests, including the coffee berry borer. Adding chitin nutrition to the propagation media is essential to improve the quality of the spores and their pathogenicity. The research objective was to determine the effect of adding chitin from sources of Tenebrio molitor and shrimp shells with different concentrations in the propagation media on the spore quality and pathogenicity of beauveria bassiana. The research was conducted at the Protection Laboratory at the National Veterans Development University, Yogyakarta, from November 2022 to February 2023. The study used a completely randomized design as a treatment using multiplication media. beauveria bassiana without adding chitin (C0), media with chitin T. molitor 1 g/200 g (C1); Get it T. molitor 2 g/200 g (C2); Kitten T. molitor 3g/200g (C3); Shrimp shell chitin 3 g/200 g (C4); Shrimp shell chitin four g/200 g(C5); Shrimp shell chitin 5 g/200 g(C6). The treatment was repeated four times. The results showed that spore quality test, T. molitor chitin treatment 2 g/200 g (spore density 7.53x108 spores/mL and viability 84.48%) and shrimp shell chitin 4 g/200 g (spore density 7.34x108 spores) /mL and viability 83.09%) resulted in high-quality mushrooms. While the pathogenicity test, the treatment of T. Molitor chitin 2 g/200 g and shrimp shell 4 g/200 g produced good pathogenicity (the highest percentage of mortality; the fastest death rates were 5.1 and 4.7 days; the most secured 50% Lethal Time was 4.264 and 3.883 days and the lowest eating power of 0.55 and 0.51 grams.

Keywords: spore quality, pathogenicity, chitin, beauveria bassiana, Hypothenemus hampei

1. INTRODUCTION

Coffee berry borer (Hypothenemus hampei Ferrari) is the primary pest harmful to coffee cherries. Globally, it causes an annual loss of 25% in coffee production or more than US $ 500 million worldwide (Wang, S.L.; Liang, 2017). In Indonesia, coffee production was 774.60 thousand tons in 2021, and H. hampei attacks can reach 30% -60%. Symptoms of a seizure due to infestation of H. hampei in young coffee cherries are fruit fall, while investment in fruit relative aging causes the fruit to become hollow and changes the taste of the coffee. H. hampei causes coffee bean quality and decreases productivity if control is not carried out significantly (Laila et al., 2011).

One Integrated Pest Management (IPM) strategy to control insect pests is biological control using entomopathogenic fungi (Tran et al., 2019). Entomopathogenic fungi are an alternative to conventional insecticides, safe for plants, humans, and animals. About 1000 species of entomopathogenic fungi are known to kill insects (Shang et al., 2015). Pathogenic Fungus, beauveria bassiana (Balsamo Crivelli) one of the most promising biological control agents (Rai et al., 2014). Fungus beauveria bassiana is used as a bio-insecticide to control various target pests. (Y.-P. Gao et al., 2022) Several researchers have tested the effectiveness of this fungus against several types of problems, including Spodoptera litura (Lepidoptera: Noctuidae) (Erawati et al., 2018), insect pests on green beans , Frankliniella Occidentalis (Thysanoptera: Thripidae) (Y. Gao et al., 2012), Diaphorina citri (Hemiptera: Liviidae) (Permadi, 2017), Nezara viridula (Hemiptera: Pentatomidae) (Permadi et al., 2018)

The use of ground mushrooms beauveria bassiana to handle H. hampei has been reported in Columbia (Grigolli et al., 2015) and India (Kreutz et al., 2004). The results showed a significant decrease in the population of H. hampei due to infection by a mixture of strains beauveria bassiana. H. hampei Infected species fail to reproduce in coffee cherries. Other biocontrol agents, namely, Cephalonomia stephanotis by the way (Hymenoptera: Bethylidae) andPhymastichus coffee LaSalle (Hymenoptera: Eulophidae), were also reported as two types of ants as parasitoids against H. hampei (Nguyen et al., 2017)

The nutrients influence the virulence of entomopathogenic fungi in the mushroom growing medium in the propagation medium. Adding nutrients is very important to improve the quality of mass-produced spores and mushrooms. Chitin is a straight-chain polymer of N-acetyl-d-glucosamine (GlcNAc) units with β-1,4 bonds, a common polysaccharide globally, second only to cellulose. Until now, chitin-containing materials from fisheries by-products (shells from crabs, shrimp, or squid ink) were essential for chitin production. However, the chitinous material also contains high amounts of protein and minerals (Kreutz et al., 2004).

Chitin compounds are one of the carbon sources that can be derived from the integument or outer skin of the body of insects and crustacean shells, such as crabs, shrimp, crayfish, and others [4,5,6].

Whoever is added to the propagation media helps the fungus improve the quality of its spores and pathogenicity, thereby accelerating the efficacy and infection of entomopathogenic fungi against their hosts (Danti et al., 2019). Khitin, on the other hand, showed no antioxidant activity in our assay, which agrees with a possible scavenging mechanism. It was reported that antioxidant activity is related to the presence of hydroxyl groups (C6) and amino groups (C2) so that active hydroxyl and amino groups can react with free radicals.

The research objective was to determine the effect of adding chitin from sources T. molitor and shrimp shells with different concentrations on the propagation media to improve the quality and pathogenicity of the fungus beauveria bassiana to handle H. hampei.

2. RESEARCH METHODS

The research was conducted at the Yogyakarta Veterans National Development University Protection Laboratory from November 2022 to February 2023.

The research consists of 2 stages, and the first stage is quality testing of beauveria bassiana, which was propagated on ground corn media supplemented with chitin nutrition. The second stage was the pathogenicity test of beauveria bassiana reproduced in the media with the addition of source chitin T. molitor and shrimp shells on the coffee berry borer.

The study used a completely randomized design (CRD) with one factor, namely the concentration of chitin added to the propagation medium beauveria bassiana are: propagation media without the addition of chitin (C0) media with the addition of chitin from T. molitor 1 g/200 g (C1); Get it T.molitor 2 g/200 g (C2); Kitin T. molitor 3 g/200 g (C3); Shrimp shell chitin 3 g/200 g (C4); Shrimp shell chitin 4 g/200 g (C5); and Shrimp shell chitin 5 g/200 g (C6). The research was repeated four times. Data were analyzed by analysis of variance (ANOVA) 5%, different treatment tests with Least Significance Different Test 0,05 and Lethal Time 50% is calculated using probit regression analysis with a linear model.

2.1 Spore Density

Spore density is the number of spores per gram of media, calculated using a hemocytometer, 400 times magnification microscope. The spore density formula is Information S = spore density/mL, x = Average number of spores in the box (a, b, c, d and, e),

L = Area of counting box (0.04 mm2), t = Depth of counting area (0.1 mm), d = Dilution factor, 103= Calculated suspension volume (1 mL = 103 mm3) (BBPPTP, 2019).

2.2 Spore Viability

Spore viability is calculated from the number of germinated spores divided by all germinated spores multiplied by 100 percent. As follows:

2.3 Percentage of Mortality

The mortality percentage was calculated from the number H hampei dead divided by the total H. hampei.

2.4 Speed of Death

The formula for determining the speed of death is as follows:

$$V = \frac{T_1 N_1 + T_1 N_2 + T_1 N_3 + \cdots T_n N_n}{n}$$

Description:
V = Death rate,
Tn = 1st, 2nd, 3rd observation time,...,n,
Nn = Number of insects that died 1st, 2nd, 3rd,...,n,
n = Number of insects tested.

2.5 Lethal Time 50% (LT50)

Determination of the time of death (LT50) is the time required beauveria bassiana to cause death in pests H. hampei as much as 50%. Calculations use the S program AS-STAT in SAS 6.12 (SAS Institute, USA) to determine with probit analysis.

2.6 Feeding Power

Feeding capacity is calculated by the formula:
Feeding power = Initial Coffee Bean Feed Weight – Final Coffee Bean Feed Weight

3. RESEARCH PREPARATİON

3.1 Media preparation of beauveria bassiana

Wash and boil 200 g of potato extract with 1 liter of distilled water, then add sugar after boiling dextrose 25g, bacto agar 30 g, and one chloramphenicol capsule. Stir well and

cook again. Then pour 1/3 of the solution into a test tube, then sterilize it with an autoclave for 30 minutes. Air dry the media in a slanted state.

3.2 Stock Media Italic Beauveria bassiana

Isolate beauveria bassiana originating from the protection laboratory collection, inoculated onto slanted media and incubated for 7 days.

3.3 Making Chitin Flour

Integumentary chitin from Hong Kong caterpillar sources (T. molitor) and shrimp shells. Dried in the sun for 3 days, then in the oven at 400 t Celsius for 2 hours. Grind the ingredients until smooth and filtered with 30 mesh. Then ready to use.

3.4 Insect Test H. hampei

Insect exploration H. hampei performed with m take the symptomatic coffee cherries, then the coffee beans are placed in a 3500 mL box jar and given healthy coffee as natural food and moist cotton and covered with tile cloth. Insect H. hampei used in this study were young brown adults, the second generation.\

3.5 Preparation of propagation media beauveria bassiana

The propagation medium used was ground corn. Corn is cooked until half cooked. After cooking, the ground corn is dried, mixed with chloramphenicol until evenly distributed, and placed in Erlenmeyer as much as 200 g. The addition of chitin powder to the media according to the treatment. The propagation medium was covered with cotton and aluminum foil, then sterilized for 30 minutes. The next stage was incubated for 10 days.

3.6 Pathogenicity Test beauveria bassiana to H. hampei.

Make a test solution by stirring, taking as much as 3 grams, crushing and dissolve it in 100 mL of distilled water. To liquefy by vortex for 3 minutes. Pathogenicity test using a small jar with a box-shaped perforated lid. Coffee beans as feed and cotton to regulate humidity in a jar dipped in a mushroom solution beauveria bassiana prepared for 1 minute. Then put it in the test jar and squeeze the cotton until it feels moist. Furthermore, in each jar, 20 insects were added to H. hampei. The test jar was covered with a tile cloth.

4. RESULTS AND DISCUSSION

4.1 Spore Density and Spore Viability

The results of calculating the density and viability of spores can be seen in Table 1. Adding chitin to the propagation media increases the spore density and spore viability. The addition of chitin T. molitor 2 g/200 g and 4 g/200 g shrimp shell chitin showed the highest spore density, while the highest spore viability was in chitin medium T. Molitor at a concentration of 2 g/200 g, while in shrimp shell chitin at a concentration of 4 g/200 g. Mushroom quality is influenced by the availability of nutrients in the propagation media. Adding nutrients to the media in the form of T. Molitor integuments and shrimp shells increased the amount of protein and chitin content. Materials containing chitin can be used as a nutrient source for the bioconversion of microorganisms to produce many bioactive compounds, for example, proteases, chitinases/chitosanase, glucosidase inhibitors, exopolysaccharides (Danti et al., 2019), (Doan et al., 2019), tyrosinase inhibitors (Liang et al., 2015),

Fungi use protein in the media to increase the production and formation of fungal spores. Chitin in the propagation medium also stimulates beauveria bassiana to induce the chitinase enzyme, which plays a role in decomposing the provided chitin material into carbon and nitrogen elements. These two elements form complex molecules in the hyphae cells and the mycelium of the beauveria bassiana fungus. Like cellulose in plant cell walls, the polysaccharide chitin is combined with other compounds to produce a strong network. Both polysaccharides form micro fibrils that differ in length and construction depending on the species and cellular location [5]. In fungi, this involves cross-linking with glucan polymers to create hyphal fused walls [6, 7]. Due to the involvement of other polymers, such as glucans, the fungal cell wall chitin content ranges from 22%–40% (Y. Gao et al., 2012).

Treatment with higher concentrations of chitin (chitin T.molitor 3 g/200 g and shrimp shell 5 g/200 g) decreased spore quality. This is because the multiplication media is saturated with nutrients, so only some nutrients are absorbed by the fungus beauveria bassiana. In addition to the anti-bacterial and anti-fungal properties of chitin in pathogens, adding it at high concentrations will inhibit the growth of beauveria bassiana.

Table 1. Spore density and viability beauveria bassiana

Treatment	Density (Spore/mL)	Spore viability
C0 : Control (Without chitin)	3,38 x 10^8 e	64,87 % c
C1 : T. molitor chitin 1 g/200 g	3,75 x 10^8 de	66,06 % c

C2 : T. molitor chitin 2 g/200 g	7,53 x 10^8 a	84,48 % a
C3 : T. molitor chitin 3 g/200 g	5,56 x 10^8 bc	76,65 % b
C4 : Shrimp shell chitin 3 g/200 g	5,31 x 10^8 cd	73,69 % b
C5 : Shrimp shell chitin 4 g/200 g	7,34 x 10^8 ab	83,09 % a
C6 : Shrimp shell chitin 5 g/200 g	4,31 x 10^8 cde	67,27 % c

Note: values followed by the same letter show no difference significantly based on the LSD follow-up test at α level of 5%.

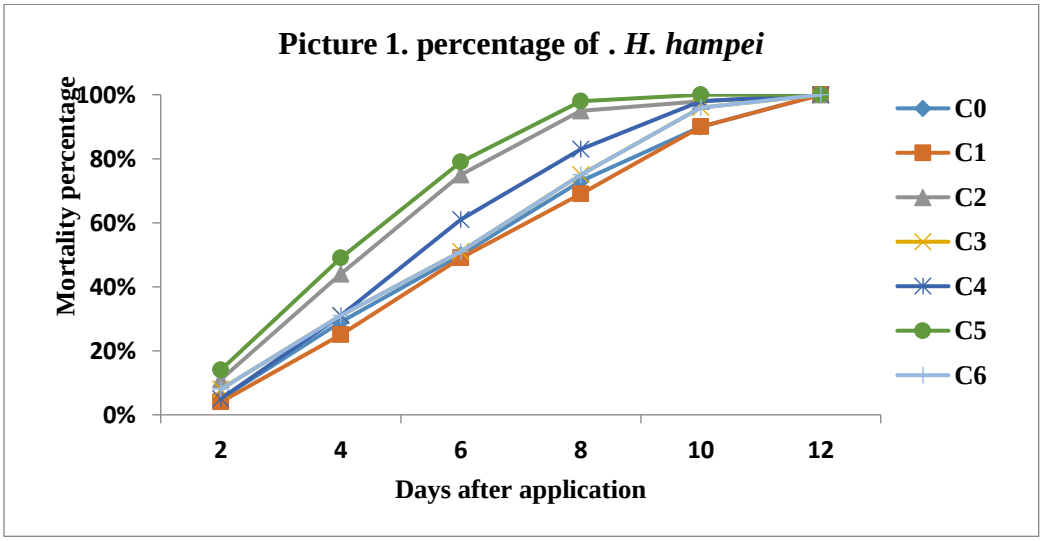
4.2 Mortality Percentage H. hampei

Table 2. Shows the percentage of each mortality H. hampei, observed from the second to the eighth day after application. Treatment of chitin chitin T. molitor 2 g/200 g was not significantly different from shrimp shell chitin 4 g/200 g and higher than the other treatments, the two treatments gave a mortality percentage of 98% and 100% on the tenth day (Pic.1). This parameter relates to the quality and viability of the spore's beauveria bassiana the better the quality applied, the higher the mortality percentage (Table 1). This indicates the addition of chitin plays a role in increasing the percentage of fungal mortality beauveria bassiana to H. hampei. Increasing the concentration of spore density will cause a high percentage of mortality. The presence of chitin in the propagation media induces the chitinase enzyme in fungi beauveria bassiana is more active and can hasten death H. hampei.

Table 2. Mortality percentage of H. hampei,

Treatment	Mortality percentage of H. hampei (DAA)					
	2	4	6	8	10	12
C0 : Control (without chitin)	5 **c**	29 **bc**	50 **c**	73 **d**	90 **a**	100 **a**
C1 : T. molitor chitin 1 g/200 g	4 **c**	25 **c**	49 **c**	69 **d**	90 **a**	100 **a**
C2 : T. molitor chitin 2 g/200 g	11 **ab**	44 **a**	75 **a**	95 **a**	98 **a**	100 **a**
C3 : T. molitor chitin 3 g/200 g	8 **bc**	31 **bc**	51 **c**	75 **cd**	96 **a**	100 **a**
C4 : Shrimp shell chitin 3 g/200 g	5 **c**	31 **bc**	61 **b**	83 **b**	98 **a**	100 **a**
C5 : Shrimp shell chitin 4 g/200 g	14 **a**	49 **a**	79 **a**	98 **a**	100 **a**	100 a
C6 : Shrimp shell chitin 5 g/200 g	8 **bc**	31 **bc**	51 **c**	75 **cd**	96 **a**	100 **a**

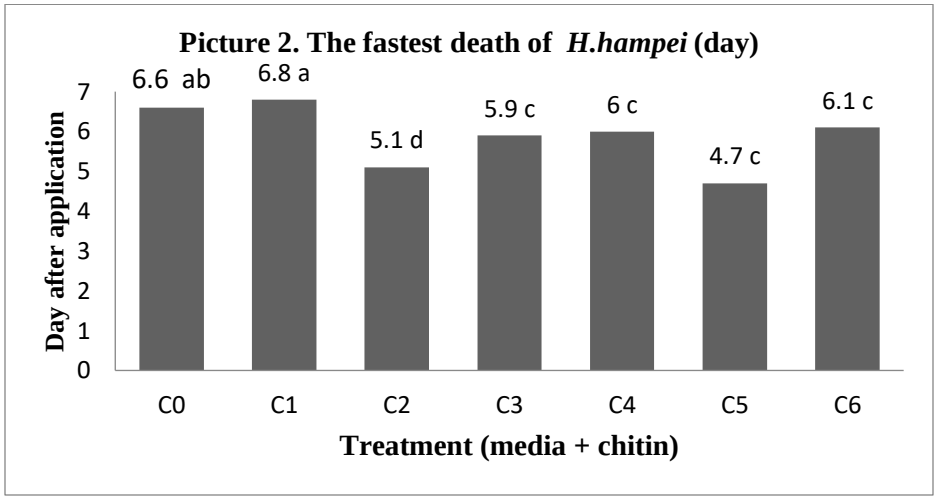
Description: Values followed by the same letter in one column indicate there was no significant difference based on the LSD follow-up test at α level of 5%.



Note: C0: control (without chitin), C1: T. molitor chitin 1 g/200 g, C2: T. molitor chitin 2 g/200 g, C3 : T. molitor chitin 3 g/200 g, C4 : Shrimp shell chitin 3 g/200g, C5 : Shrimp shell chitin 4 g/200 g, C6 : Shrimp shell chitin 5 g/200 g

4.3 Speed of Death

Figure 2. Shows the speed of death of chitin treatment T. molitor 2 g/200g and 4 g/200 g shrimp shells were not significantly different and were faster than other treatments. The lower the value of the speed of death, the quicker the treatment in infesting pests H. hampei. This is because this treatment has mushroom quality and a higher mortality percentage value compared to other treatments, in addition to the addition of flour T. molitor and shrimp shells, which contain high chitin and protein so they can stimulate fungi beauveria bassiana produces chitinase and protease enzymes to accelerate the degradation process of pest cuticles H. hampei. The more enzymes, the faster the fungus penetrates its host's body. According to Herlinda (2006), the ability of mushrooms beauveria bassiana to degrade the cuticle of the host insect is influenced by the presence or absence of the chitinase enzyme. At times the fungal hyphae can penetrate the pest's cuticle. H. hampei will issue one of the taxon, i.e., beauvericin, a toxin to disrupt the digestive system and hemolymph of pests H.hampei.

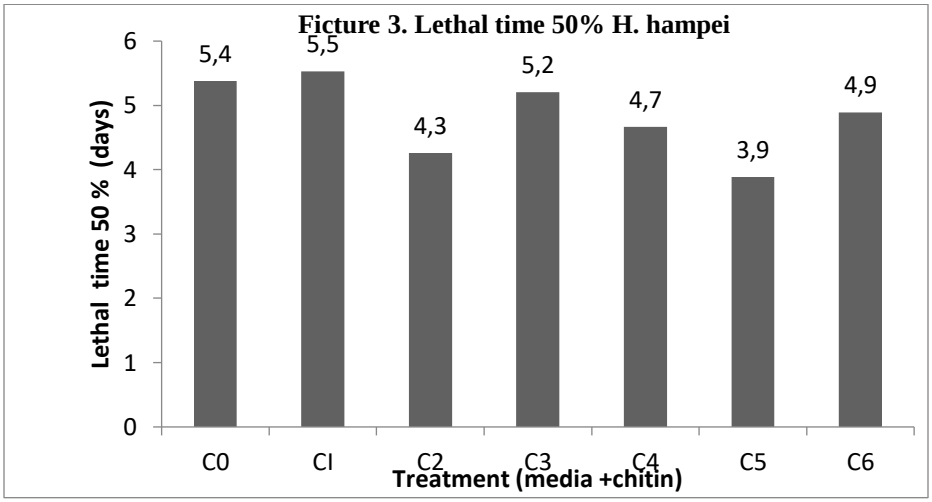


Note: the value followed by the same letter shows no significant difference based on the LSD follow-up test at α level of 5%

C0: control (without chitin), C1: T. molitor chitin 1 g/200 g, C2 : T. molitor chitin 2 g/200 g, C3: T. molitor chitin 3 g/200 g, C4 : Shrimp shell chitin 1g/200g, C5 : Shrimp shell chitin 2 g/200 g, C6 : Shrimp shell chitin 3g/200g

4.4 Lethal Time 50% (LT50)

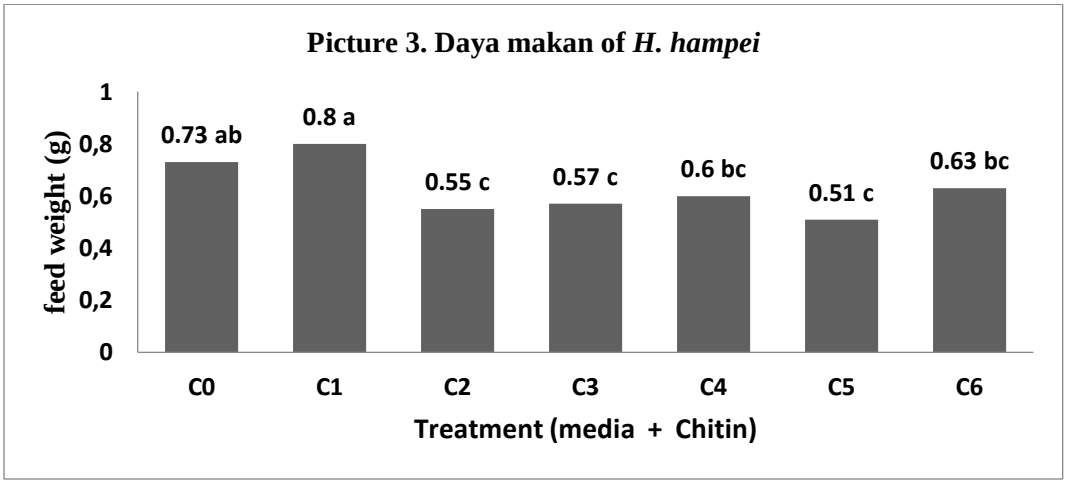
Picture 3 shows the treatment of shrimp shell chitin 4g/200 g has a value lethal time 50% faster than other treatments. The smaller the LT50 value, the more influential the insecticide used. High or low-quality beauveria bassiana influences the LT50 value. This treatment has a high quality, so it will also affect the percentage value of mortality and the speed of death. Propagation media beauveria bassiana has a higher spore density, thus having a faster LT50 value than media with a lower spore density.



Note: C0: untreatment, C1 : Chitin T. molitor 1 g/200 g, C2 : Chitin T. molitor 2 g/200 g, C3: Chitin T. molitor 3 g/200 g, C4: Chitin shrimp shell 1 g/200 g, C5 : Chitin shrimp shell 2 g/200 g, C6: Chitin shrimp shell 3g/200g

4.5 Feeding Power

Table 3 shows the power to eat H. hampei is on chitin treatment T. molitor 2 g/200 g, and shrimp shell chitin 4 g/200 g were not significantly different and significantly different from other treatments. The smaller the power value to eat, the less H hampei eat coffee beans. The two treatments had good spore quality and a high mortality rate. According to Nuryanti et al. (2012), adding chitin has a good effect on the quality and pathogenicity of beauveria bassiana because the chitin source used has characteristics that follow the host insect, namely H. hampei.



Values followed by the same letter show no significant difference based on the LSD test at α level of 5%.

Note: C0: Control (Without chitin), C1: Chitin T. molitor 1 g/200 g, C2: Chitin T. molitor 2 g/200 g, C3: ChitinT. Molitor 3 g/200 g, C4: Chitin shrimp shell 1 g/200 g, C5: Chitin shrimp shell 2 g/200 g, C6: Chitin shrimp shell 3g/200g

5. CONCLUSION

Adding chitin from different sources in the propagation media affects the quality of the mushrooms beauveria bassiana. Spore quality test, chitin treatment T. molitor 2 g/200 g (7.53x108 spores/mL and 84.48%) and 4 g/200 g shrimp shell chitin (7.34x108 spores/mL and 83.09%) produced high-quality mushrooms. While the pathogenicity test, chitin treatment T. molitor 2 g/200 g and 4 g/200 g shrimp shells made good pathogenicity (highest mortality percentage; the fastest death rates were 5.1 and 4.7 days; the most secured 50% Lethal Time were 4.264 and 3.883 days, and the lowest feed ability as low as 0.55 and 0.51 grams.

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