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The effect of chitin on the effectiveness of *Beauveria bassiana* formulation product to control cocoa pod borer *in vitro*

Pengaruh kitin terhadap efektivitas produk formulasi Beauveria bassiana *untuk mengendalikan penggerek buah kakao secara* in vitro

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Abstrak

Jamur entomopatogen telah disarankan untuk dimanfaatkan sebagai bioinsektisida karena ketahanan biologisnya dan ramah terhadap lingkungan. Beauveria bassiana *merupakan salah satu jamur entomopatogen yang efektif mengendalikan hama serangga, termasuk penggerek buah kakao (CPB). Penelitian ini bertujuan mengetahui tingkat efektivitas formula* B. bassiana *(Bb) asal Palu dalam menekan pupa penggerek buah kakao secara* in vitro*. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL), terdiri atas dua faktor yaitu konsentrasi sel dan perlakuan kitin yang diulang tiga kali. Konsentrasi sel terdiri atas dua taraf yaitu 10^6 dan 10^7 conidia/mL. Perlakuan kitin terdiri atas tujuh perlakuan yaitu isolat asal Kediri tanpa formulasi kitin (Bb-K), isolat asal Palu tanpa formulasi kitin (Bb-P0), empat formulasi isolat asal Palu dengan penambahan kitin pada berbagai media tumbuh (Bb-P1, Bb-P2, Bb-P3, dan Bb-P4), dan kontrol (tanpa suspensi Bb). Perlakuan Bb-P0 dan Bb-P2 pada konsentrasi 10^7 conidia/mL mulai mengkolonisasi CPB pada hari ketiga setelah perlakuan diberikan sedangkan formulasi lainnya mulai pada hari kelima. Akan tetapi, Bb-P0 menunjukkan tingkat infeksi terendah pada akhir pengamatan. Sebaliknya, data rasio pupa terinfeksi memperlihatkan bahwa perlakuan Bb-P2 paling tinggi dibandingkan perlakuan lainnya. Hal ini sesuai dengan rasio kemunculan serangga dewasa dimana Bb-P2 menunjukkan yang terendah, yang berarti Bb-P2 memiliki virulensi paling tinggi dibandingkan formula lainnya. Hasil penelitian menunjukkan bahwa media PDA dan PDB bersuplemen kitin merupakan media yang paling efektif untuk menumbuhkan* B. bassiana *asal Palu sebelum diproduksi secara massal.*

[Kata kunci: bioinsektisida, entomopatogen, jamur, kitin, virulensi]

Abstract

Fungal entomopathogens are suggested to be used as a bioinsecticide due to their biological persistence and ecological friendliness. *Beauveria bassiana* is an entomopathogenic fungus that can effectively control insect pests, including cocoa pod borer. This study aims to determine the level of effectivity of the *B. bassiana* (Bb) formulation product from Palu against cocoa pod borer pupae *in vitro*. This research used a completely randomized design (CRD) consisting of two factors: conidia concentrations and chitin treatments, which were repeated thrice. The conidia concentrations consisted of two levels, that were 10^6 and 10^7 conidia/mL. The chitin treatment included seven treatments: isolate from Kediri without formulation with chitin (Bb-K), isolate from Palu without formulation with chitin (Bb-P0), four formulations of isolates from Palu with the addition of chitin to various growth media (Bb-P1, Bb-P2, Bb-P3, and Bb-P4), and control (without Bb suspension). Bb-P0 and Bb-P2 treatments at a concentration of 10^7 conidia/mL began to colonize CPB on the third day after the treatment application, while the other formulations started on the fifth day. However, Bb-P0 showed the lowest infection rate at the end of the observation. Contrarily, the data on the pupa-infected ratio showed that the Bb-P2 treatment was the highest compared to other treatments. It conformed to the adult emergence ratio that Bb-P2 exhibited the lowest, which means Bb-P2 has the most virulence of other formula. The result showed that PDA and PDB media-supplemented chitin was the most effective for culturing *B. bassiana* origin Palu before mass production.

[Keywords: bioinsecticide, chitin, entomopathogen, fungus, virulence]

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Introduction

Overusing chemical fertilizers has resulted in several ecological and environmental issues, such as contamination and degradation of soil or alleviation of advantageous soil organisms. Recently, there has been a growing awareness of the need for eco-friendly pest management techniques in agricultural production and biological control agents to reduce the utilization of chemically synthesized pesticides (Doolotkelvieva & Ismailova, 2022; Kidanu, 2020). Because of their ecological friendliness and biological persistence, entomopathogens are well-investigated and suggested for killing insects at various life cycle phases (Doolotkelvieva & Ismailova, 2022; Gul et al., 2014).

Entomopathogenic fungal genera used as biopesticides include *Metarhizium*, *Beauveria*, *Isaria*, *Lecanicillium*, *Hirsutella*, and *Paecilomyces*. Since these fungi have a wide range of applications, their characteristic can be applied to kill a wide range of insect pests (Bamisile et al., 2021; Bihal et al., 2023; Litwin et al., 2020). *Beauveria bassiana* was among the initial entomopathogenic fungi to be effectively employed for insect pest myco-biocontrol (Kidanu, 2020), commonly found in soil (Litwin et al., 2020) and has been isolated from infected insects in temperate and tropical locations worldwide (Bamisile et al., 2021). This entomopathogenic fungi can control the population of arthropods naturally and are classified as endophytes of plant leaves, stems, and roots (Jaber & Enkerli, 2017).

Cocoa, a highly competitive commodity in Indonesia, has the main problem in its cultivation: the attack of plant-pest insects. Cocoa pod borer (CPB), *Conopomorpha cramerella*, is the primary agent that damages the cocoa industry and causes yield loss in cocoa production (Fiana et al., 2015; Rahmawati et al., 2017). Attacks by CPB have the potential to harm beans by up to 82% and limit production by up to 80%, so cocoa farmers and business owners are quite concerned about dealing with this issue. It will significantly affect either the weight and quality of the products or raise harvesting expenses due to the lengthy process required to separate the healthy seeds from the damaged seeds (Nurmayulis et al., 2021). It needs the effort to obtain an effective bioinsecticide that contains entomopathogenic fungus in controlling the pest.

The chitin-based formulation in bioinsecticide production is well-known for increasing *B. bassiana* virulence to inhibit pest growth (Saputro et al., 2019; Senthilraja et al., 2010). Adding chitin to growth media can increase the growth rate of *B. bassiana* colony diameter to enhance its function as a bioinsecticide (Rohman et al., 2017). Supporting this, the study by Bagariang et al. (2023) explained that the application of *B. bassiana* in rice solid media significantly inhibited pupa formation and the emergence of *Spodoptera litura* imago. Therefore, our research aims to analyse *in vitro* the ability of our *B. bassiana* formulation products, with chitin-based compound addition in growth media, to suppress the pupal growth of CPB.

Material and Methods

Material

The materials used in this experiment were pupal phase of cocoa pod borer insects, healthy cocoa leaves, plastic boxes, cotton gauze, sterilized water, potato dextrose broth (PDB) medium, potato dextrose agar (PDA) medium, *B. bassiana* (Bb) isolates origin Kediri, East Java (Bb-K) and Palu, Central Sulawesi (Bb-P0), and four products of Bb-P0 formulation. Pupae are obtained by collecting and stacking cocoa pods infected with CPB. Furthermore, the pile of cocoa pods was covered with healthy cocoa leaves (Figure 1). After 2-4 days, these cocoa pods and leaves were observed, and the leaf-attached pupae were separated for the research. The broth medium was commercial PDB (HiMedia®) with chloramphenicol (1 g L^{-1}) added.

The four bioinsecticides were produced by formulation process with 0.5% chitin-based powder addition into a variation of subculture media and rice grains as a carrier substrate for mass production. The Bb-P0 isolate was used to formulate these four bioinsecticides: Bb-P1, Bb-P2, Bb-P3, and Bb-P4. The process sequence is shown in Table 1, where each formulation used solid and liquid media with or without chitin supplementation. The isolate was grown on a solid medium, PDA, or glucose yeast agar (GYA); subsequently, it was transferred into a liquid medium, PDB, or glucose yeast broth (GYB). Then, the mass production was done by inoculating the culture in a liquid medium into rice grains with chitin addition with a ratio of 1:4 (v/w), except for the Bb-K and Bb-P0 that used their cultures on PDB for application. The incubation time was applied depending on the fungal isolate's character and the subculture media type.

Figure 1. Pupae collected from the infected cocoa pods by CPB

Gambar 1. Pupa-pupa dikumpulkan dari buah kakao yang terinfeksi CPB

Table 1. Application of growth media in the *B. bassiana* formulation used in research
Tabel 1. Penggunaan media pertumbuhan pada formulasi B. bassiana *yang digunakan pada penelitian*

Treatments *Perlakuan*	Solid media *Media padat*		Liquid media *Media cair*		Rice grains *Beras*
	Potato dextrose agar (PDA)	Glucose yeast agar (GYA)	Potato dextrose broth (PDB)	Glucose yeast broth (GYB)	
Bb-K	√*)	-	√	-	-
Bb-P0	√	-	√	-	-
Bb-P1	√	-	√	-	√ +
Bb-P2	√ +	-	√ +	-	√ +
Bb-P3	-	√ +	√ +	-	√ +
Bb-P4	-	√ +	-	√ +	√ +

*) Column containing characters: (-) indicates not using the medium, (√) indicates using the medium without chitin addition, and (√+) indicates using the medium with chitin

**) Kolom yang berisi karakter: (-) menunjukkan tidak menggunakan media, (√) menunjukkan menggunakan media tanpa penambahan kitin, dan (√+) menujukkan menggunakan media dengan penambahan kitin*

Methods

For the Bb-K and Bb-P0 preparations, the fungal stock cultures were subcultured on the PDA plate and incubated at 25 °C for 3-5 days. Then, every inoculum was subcultured (cut with a cork-borer) in two 250 mL Erlenmeyer flasks containing PDB 100 mL (Roswanjaya et al., 2021) with shake incubating at 25 °C for 2-3 days. The B. bassiana conidial viability of the Bb-K and Bb-P0 cultures in this liquid medium and the formulation products (Bb-P1, Bb-P2, Bb-P3, and Bb-P4) on the carrier medium were estimated by the total plate count method, referred to Oliveira et al. (2015). The ten-fold serial dilution in this enumeration technique was performed, and 10^6 and 10^7 conidia/mL were used as the final concentration for the research.

The experiment was conducted in the field laboratory of the Maros District's Estate Crops Office. The completely randomized design (CRD) was applied for two-factor experiments, which are concentrations and chitin-based formulation treatments, with three replications. The conidia concentrations were divided into two levels, 10^6 and 10^7 conidia/mL. The chitin treatments consisted of seven treatments, which are the control (without Bb suspension added) and the six Bb formulations (Table 1). The *in vitro* assay of the Bb formulation products was arranged based on Rahayu and Umrah (2012). For every single plastic box (as a replication), healthy leaves were put in the bottom; then, five leaf-attached pupae were transferred to the leaves (Figure 2) and covered with other healthy leaves. The Bb suspension of each treatment was sprayed ±10 mL evenly onto an inner plastic box and then covered with cotton gauze. The observation was conducted daily until the mycelium appeared on the surface of the pupae bodies to count the number of infected pupae. Then it continued until the infected died or the uninfected pupae turned to adults to calculate its number.

For assessing the effects of bioinsecticide formulations, the pupal mortality rate and the pupal survival were quantified using Herlinda et al. (2006) and Li et al. (2019) formulas, respectively. The procedures were used: "Infected pupa ratio" = (Infected pupae number/Total number of testes pupae) x 100% and "Adult emergence ratio" = (Adults emerged number/Total number of testes pupae) x 100%. The number of infected pupae and emerged CPB adults was determined on the sixth day after treatment application, according to Rahayu and Umrah (2012).

The data from the experiment was processed using Microsoft Excel and then subjected to Minitab software to conduct analyses of variance and evaluate the effect of concentrations and bioinsecticide formulations of *B. bassiana* against cocoa pod borer. Since the results differed significantly, the Tukey test was performed at a significance level of 5%. The standard error (SE) was calculated for nine biological replications.

Results and Discussion

Infected ratio of cocoa pod borer pupa

There were variations among the treatments in the bioassay of *Beauveria bassiana* (Bb) formulation products, contrary to the cocoa pod borer (CPB) pupa. After treatment application for three days, the treatment of Bb-P0 and Bb-P2 with 10^7 conidia/mL concentration showed infection of the CPB. However, the other formulations started

the infection activity five days after application (DAA). Both *B. bassiana* from Palu products lead to attack the CPB faster than either its other formulations (Bb-P1, Bb-P3, and Bb-P4) or Bb-origin Kediri (Bb-K).

The data showed that Bb-P0 by 11.11% in two concentrations had the lowest ratio than the Bb treatments, despite its earlier colonization of mycelium appearing on the CPB bodies. On the other hand, Bb-P2, with 10^7 conidia/mL by 77.78%, exhibited a significantly higher rate than its 10^6 conidia/mL density and other treatments in all concentrations (Table 2). The other treatment variations revealed no significant difference compared to the control. It was similar to the two concentrations in every treatment (Figure 3).

Based on the data, the chitin powder addition on the growth media, PDA and PDB, and rice grains as a carrier substrate with 10^7 conidia/mL improved the infectious performance of *B. bassiana* from Palu. At the same time, the other formulations indicate similarities to the *B. bassiana* product without chitin addition (Bb-K and Bb-P0). Nevertheless, the mean of infected ratio among four formulations (Bb-P1 to Bb-P4) showed a higher trend than their origin (Bb-P0) in both concentrations.

This finding is in accord with previous research declaring that chitin is an essential source of carbon and nitrogen, where the main components of carbohydrates, nucleic acids, proteins, and lipids (Gerding et al., 2007), for mycelium development and the generation of fungal conidia (Afandhi et al., 2023; Barreto et al., 2004). For chitinolytic organisms, this carbon source can improve their efficiency of growth and multiplication (Gerding et al., 2007; Saputro et al., 2019). In addition, Afandhi et al. (2022) deduced that supplementing the medium with a chitin source might enhance conidia persistence and infection capability, where the density and viability of conidia are associated with entomopathogenic fungus virulence.

Figure 2. A plastic box containing five leaf-attached pupae each
Gambar 2. Wadah plastik berisi lima pupa yang menempel di daun

Table 2. Percentage of infected pupa and adult emergence ratio at 6 day after application
Tabel 2. Persentase pupa terinfeksi dan rasio kemunculan serangga dewasa pada 6 hari setelah aplikasi

Treatments *Perlakuan*	Infected pupa ratio (%) *Rasio pupa terinfeksi (%)*		Adult emergence ratio (%) *Rasio kemunculan serangga dewasa (%)*	
	10^6 conidia/mL	10^7 conidia/mL	10^6 conidia/mL	10^7 conidia/mL
Control *Kontrol*	0 ± 0*)	0 ± 0	100 ± 0	100 ± 0
Bb-K	22.22 ± 11.11	33.33 ± 0	88.89 ± 11.11	66.67 ± 0
Bb-P0	11.11 ± 11.11	11.11 ± 11.11	88.89 ± 11.11	88.89 ± 11.11
Bb-P1	66.67 ± 0	55.56 ± 11.11	33.33 ± 0	44.44 ± 11.11
Bb-P2	44.44 ± 22.22	77.78 ± 11.11	55.56 ± 22.22	22.22 ± 11.11
Bb-P3	44.44 ± 22.22	44.44 ± 29.4	55.56 ± 22.22	55.56 ± 29.4
Bb-P4	44.44 ± 22.22	44.44 ± 11.11	55.56 ± 22.22	55.56 ± 11.11

*) Numbers before and after the ± character represent mean and SE values of nine biological replicates, respectively
**) Angka sebelum dan sesudah karakter ± mewakili nilai rata-rata dan SE dari sembilan ulangan biologis*

Adult emergence ratio of cocoa pod borer

The effectiveness of bioinsecticide efficacy on the CPB pupa is exhibited by the lower percentage of adult emergence measured after the *B. bassiana* infection. The lowest adult emergence was 22.22% in CPB on day six after treatments, which was seen in Bb-P2 with 10^7 conidia/mL concentration (Table 2). In contrast, other treatments were statistically no different from the control, which showed 100% at day five (Figure 4). The finding showed that Bb-P2 had the highest efficacy compared to other bioinsecticides, killing the most CPB pupae. It was in line with the infected ratio data that Bb-P2 was the fastest and the most infecting pupae. Nonetheless, no difference in significance was seen across treatment concentrations (Figure 4).

The result revealed the different efficacy among the formulations of *B. bassiana* origin Palu due to the type of subculture media used. This bioassay resulted in Bb-P2, applied chitin addition into PDA and PDB as the subculture media and the rice for mass production, is the most suitable method for formulation of the *B. bassiana* to kill the CPB pupa. The carbon and nitrogen (CN) ratio of each medium affects germination (Mustafa & Kaur, 2009), morphological growth (Deb et al., 2017), and conidia production (Afandhi et al., 2023; Mustafa & Kaur, 2009) of *B. bassiana* isolates. Afandhi et al. (2023) explained that the rise of conidia production is related to the content of the CN ratio. It may imply that the difference in nutrient content between carbon and nitrogen as a nutrient source became an essential factor in producing the fungal bioinsecticide.

Based on the subculture media combination used before supplemented with chitin powder, the CN ratio of PDA might be higher than GYA. The CN ratio of the PDA medium is known as 10:1, as a low category (Mustafa & Kaur, 2009; Safavi et al., 2007), while the GYA mainly contains 0.2% glucose and 0.5% yeast extract predicted less than it. The glucose content of the PDA medium is higher than the GYA, which may explain it. The PDA also contains the simple sugar dextrose and vitamins that support the conidial production of fungus (Indriyanti et al., 2021). However, Safavi et al. (2007) explained that the *B. bassiana* isolate yielded its maximum spore number in the medium with a low CN ratio. It was also explained by Vega et al. (2003) that a liquid medium with a 10:1 CN ratio generated the maximum production of spore in *B. bassiana*, *Metarhizium anisopliae,* and *Paecilomyces fumosoroseus*. Carbon and nitrogen levels in the media influence mycelial growth and conidial germination (Safavi et al., 2007).

Our results showed that the chitin-based flour addition to the solid and liquid of potato dextrose media and the rice improved the effectiveness of *B. bassiana* for biological control of cocoa pod borer. According to Saputro et al. (2019), adding chitin to the growth media can increase the effectivity of *B. bassiana* in controlling *Cyclas formicarius* pest. Furthermore, Afandhi et al. (2023) study informed that for mass production of virulent *B. bassiana*, rice bran combined with cricket flour was the most appropriate waste medium. The insect's mortality caused by using entomopathogenic fungus happens due to the presence of poisonous chemicals and enzymes in their host. This fungus is also able to produce protease, chitinase, lipase, carboxypeptidases, and phosphatase (Harith-Fadzilah et al., 2021; Vidhate et al., 2023; Wang et al., 2021).

The initial phase in fungal entomopathogenic infection is the insect's cuticle layer penetration by the enzymes and hyphae-secreted specialized infection structures (appressoria) that infiltrate the host cells (Wang et al., 2021). Protease degrades the cuticle's outer layer proteins, enabling chitinase to penetrate the pro-cuticular chitin (Vidhate et al., 2023; Włóka et al., 2022) and hyphae to reach the insect hemolymphoid (Wang et al., 2021). Moreover, lipase breaks down lipoproteins, lipids, and wax layers, which are then utilized as a source of nutrients by the fungus (Włóka et al., 2022).

Chitin-based powder addition to the culture media of *B. bassiana* can induce chitinase enzymes used to control insect pests (Afandhi et al., 2023). This condition may result in maintaining entomopathogenic fungal infection (Herlinda et al., 2006). Chitinase enzyme activity is recognized to be one of the factors of the ability of entomopathogenic fungus infection since it plays a role in the penetration of fungi into the insect body and occurs from the commencement of fungal development (Afandhi et al., 2023; Pelizza et al., 2012).

However, various factors relating to the host, the pathogen, and the environment influence the pathogenesis of insects by fungi. Several processes, including the mechanical movement of the fungal hyphae and enzyme activity, improve fungal invasion into the host's body (Pelizza et al., 2012). This study shows that our Bb isolate formulated in the Bb-P2 treatment potentially enhances its effectiveness, in particular through increasing activities of hyphal growth and extracellular enzyme releases, which directly hydrolyze the host's cuticular components.

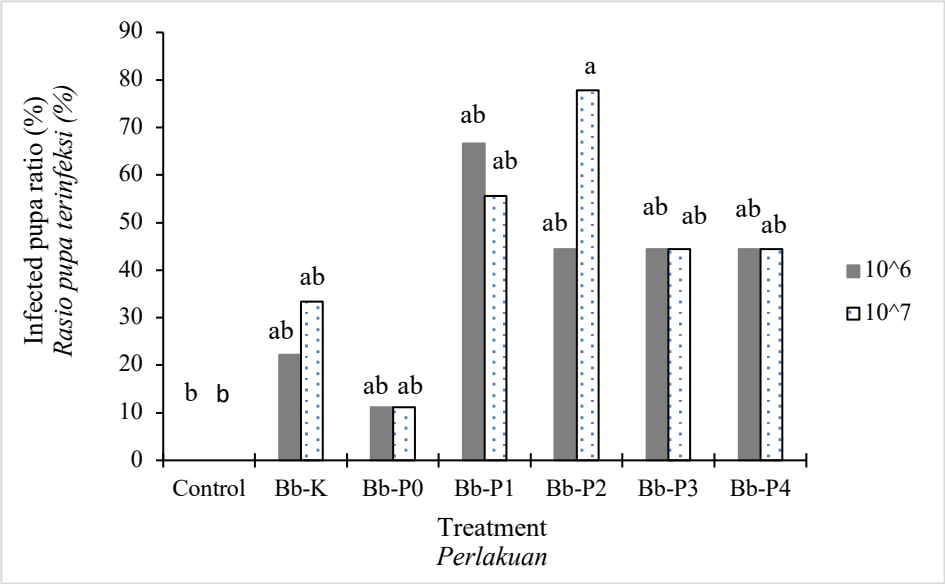

Figure 3. Percentage of infected pupa. Different letters represent significant different values according to the Tukey test at p<0.05. Bb-K = *Bacillus bassiana isolates* Kediri origin, Bb-P0-P4 = *Bacillus bassiana isolares* Palu origin

Gambar 3. Persentase pupa terinfeksi. Huruf berbeda menunjukkan perbedaan nilai yang signifikan berdasarkan uji Tukey pada p<0,05. Bb-K = Isolat Bacillus bassiana *asal Kediri*, *Bb-P0-P4 = Isolat* Bacillus bassiana asal Palu

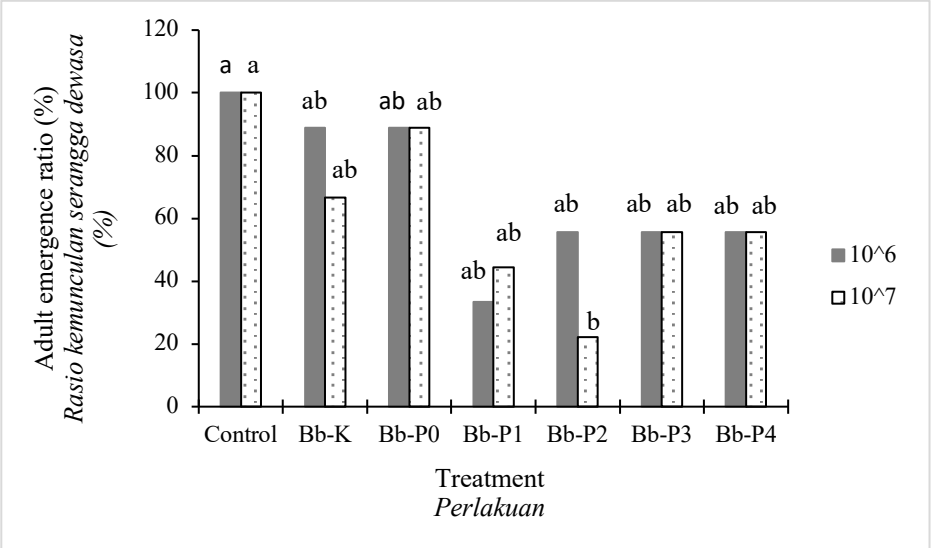

Figure 4. Percenntage of adult emergence. Different letters represent significant differences according to the Tukey test at p<0.05. Bb-K = *Bacillus bassiana isolates* Kediri origin, Bb-P0-P4 = *Bacillus bassiana isolates* Palu origin

Gambar 4. Persentase kemunculan serangga dewasa. Perbedaan huruf menunjukkan perbedaan yang signifikan menurut uji Tukey pada p<0,05 . Bb-K = Isolat Bacillus bassiana *asal Kediri*, *Bb-P0-P4 = Isolat* Bacillus bassiana asal Palu

Conclusion

In conclusion, the formulation through enrichment of subculture media and rice grains as a carrier substrate by chitin flour increased the effectivity of *B. bassiana* origin Palu compared to the without formulating and the control treatment. Applying PDA and PDB media-supplemented chitin in pre-mass bioinsecticide production was the most effective in suppressing the growth of CPB pupa in an *in vitro* assay. Our findings open an approach to developing a formulation of fungal insecticide through media optimization for fungal growth and development before mass production. However, a suitable proportion of chitin supplementation for suppressing CPB pests required further examination.

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References

Afandhi, A., Choliq, F. A., Fernando, I., Marpaung, Y. M. A. N., & Setiawan, Y. (2022). Occurrence of soil-inhabiting entomopathogenic fungi within a conventional and organic farm and their virulence against *Spodoptera litura*. *Biodiversitas Journal of Biological Diversity*, *23*(2), Article 2. https://doi.org/10.13057/biodiv/d230263

Afandhi, A., Rachmawati, R., Syib'li, M. A., & Zain, H. A. U. (2023). Performance and virulence of the entomopathogenic fungi *Beauveria bassiana* grown in media derived from biodegradable agricultural wastes enriched with cricket powder. *AGRIVITA, Journal of Agricultural Science*, *45*(2), Article 2. https://doi.org/10.17503/agrivita.v45i2.4113

Bagariang, W., Kurniati, A., Lestrari, T. M. P., Mahmudah, D., Suyanto, H., & Cahyana, N. A. (2023). Uji media padat *Beauveria Bassiana* terhadap mortalitas, pembentukan pupa dan kemunculan imago *Spodoptera litura* Fabr. *Agro Wiralodra*, *6*(1), 1–8. https://doi.org/10.31943/agrowiralodra.v6i1.86

Bamisile, B. S., Akutse, K. S., Siddiqui, J. A., & Xu, Y. (2021). Model application of entomopathogenic fungi as alternatives to chemical pesticides: Prospects, challenges, and insights for next-generation sustainable agriculture. *Frontiers in Plant Science*, *12*. https://www.frontiersin.org/articles/10.3389/fpls.2021.741804

Barreto, C. C., Staats, C. C., Schrank, A., & Vainstein, M. H. (2004). Distribution of chitinases in the entomopathogen *Metarhizium anisopliae* and effect of N-acetylglucosamine in protein secretion. *Current Microbiology*, *48*(2), 102–107. https://doi.org/10.1007/s00284-003-4063-z

Bihal, R., Al-Khayri, J. M., Banu, A. N., Kudesia, N., Ahmed, F. K., Sarkar, R., Arora, A., & Abd-Elsalam, K. A. (2023). Entomopathogenic fungi: An eco-friendly synthesis of sustainable nanoparticles and their nanopesticide properties. *Microorganisms*, *11*(6), 1617. https://doi.org/10.3390/microorganisms11061617

Deb, L., Rajesh, T., & Hajong, M. (2017). Growth of *Beauveria bassiana* in different solid media. *Trends in Biosciences, 10*(23), 4815–4817

Doolotkelvieva, T., & Ismailova, E. (2022). In vitro and in vivo screening of *Beauveria bassiana* strains for endophytic and insecticide activity. *IV International Agricultural, Biological & Life Science Conference, Turkey.*

Fiana, Y., Nurbani, N., & Danial, D. (2015). Study on the effectiveness of biological agent Beauveria bassiana and pod sleeving for controlling cocoa pod borer pest in east Kalimantan. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*, *1*(5), Article 5. https://doi.org/10.13057/psnmbi/m010545

Gerding, M., France, A., Sepulveda, M., & Campos, J. (2007). Use of chitin to improve a *Beauveria bassiana* alginate-pellet formulation. *Biocontrol Science and Technology*, *17*, 105–110. https://doi.org/10. 1080/09583150600937717

Gul, H., Saeed, S., & Khan, F. Z. A. (2014). Entomopathogenic fungi as effective insect pest management tactic: A review. *Applied Sciences and Business Economics*, *1*, 10–18

Harith-Fadzilah, N., Abd Ghani, I., & Hassan, M. (2021). Omics-based approach in characterising mechanisms of entomopathogenic fungi pathogenicity: A case example of *Beauveria bassiana*. *Journal of King Saud University - Science*, *33*(2), 101332. https://doi.org/10.1016/j.jksus.2020.101332

Herlinda, S., Utama, M. D., Pujiastuti, Y., & Suwandi, D. (2006). Kerapatan dan viabilitas spora *Beauveria bassiana* (Bals.) akibat subkultur dan pengayaan media, serta virulensinya terhadap larva *Plutella xylostella* (Linn.). *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *6*(2), 70–78. https://doi.org/10.23960/j.hptt.2670-78

Indriyanti, D. R., Bintari, S. H., Setiati, N., & Alfiyan, J. M. Z. (2021). The density and viability of *Metarhizium anisopliae* conidia on several growth media. *Biosaintifika: Journal of Biology & Biology Education*, *13*(2), Article 2. https://doi.org/10.15294/biosaintifika.v13i2.31408

Jaber, L. R., & Enkerli, J. (2017). Fungal entomopathogens as endophytes: Can they promote plant growth? *Biocontrol Science and Technology*, *27*(1), 28–41. https://doi.org/10.1080/09583157.2016.1243227

Kidanu, S. (2020). Research and application of entomopathogenic fungi as pest management option: A review. *Journal of Environment and Earth Science*, *10*(3), 31

Li, Z., Chambi, C., Du, T., Huang, C., Wang, F., Zhang, G., Li, C., & Juma Kayeke, M. (2019). Effects of water immersion and soil moisture content on larval and pupal survival of *Bactrocera minax* (Diptera: Tephritidae). *Insects*, *10*(5), 138. https://doi.org/10.3390/insects10050138

Litwin, A., Nowak, M., & Różalska, S. (2020). Entomopathogenic fungi: Unconventional applications. *Reviews in Environmental Science and BioTechnology*, *19*(1), 23–42. https://doi.org/10.1007/s11157-020-09525-1

Mustafa, U., & Kaur, G. (2009). Effects of carbon and nitrogen sources and ratio on the germination, growth and sporulation characteristics of *Metarhizium anisopliae* and *Beauveria bassiana*. *African Journal of Agricultural Research*, *3*, 922–930

Nurmayulis, Hastuti, D., Eris, F. R., & Mujahidah. (2021). The symptomp rate of cocoa pod borer (*Conopomorpha cramerella*) due to the combination of several concentrations of Neem (*Azadiracta indica*) solution given the biosurfactant of diethanolamide olein palm and different fruit sizes. *IOP Conference Series: Earth and Environmental Science*, *715*(1), 012048. https://doi.org/10.1088/17551315/715/1/012048

Oliveira, D. G. P., Pauli, G., Mascarin, G. M., & Delalibera, I. (2015). A protocol for determination of conidial viability of the fungal entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. *Journal of Microbiological Methods*, *119*, 44–52. https://doi.org/10.1016/j.mimet.2015.09.021

Pelizza, S. A., Elíades, L. A., Saparrat, M. C. N., Cabello, M. N., Scorsetti, A. C., & Lange, C. E. (2012). Screening of Argentine native fungal strains for biocontrol of the grasshopper *Tropidacris collaris*: Relationship between fungal pathogenicity and chitinolytic enzyme activity. *World Journal of Microbiology and Biotechnology*, *28*(4), 1359–1366. https://doi.org/10.1007/s11274-011-0935-8

Rahayu, R., & Umrah, U. (2012). Uji kemampuan formula *Beauveria bassiana* Balsamo. bentuk sediaan tablet untuk mengendalikan penggerek buah kakao *Conopomorpha cramerella* Snellen. *Biocelebes*, *6*(1), Article 1. https://bestjournal.untad.ac.id/index.php/Biocelebes/article/view/3888

Rahmawati, D., Wagiman, F. X., Harjaka, T., & Putra, N. S. (2017). Detection of cocoa pod borer infestation using sex pheromone trap and its control by pod wrapping. *Jurnal Perlindungan Tanaman Indonesia*, *21*(1), Article 1. https://doi.org/10.22146/jpti.22659

Rohman, F. L., Saputro, T. B., & Prayogo, Y. (2017). Pengaruh penambahan senyawa berbasis kitin terhadap pertumbuhan cendawan entomopatogen *Beauveria bassiana*. *Jurnal Sains dan Seni ITS*, *6*(2), Article 2. https://doi.org/10.12962/j23373520.v6i2.23827

Roswanjaya, Y. P., Saryanah, N. A., Nawfetrias, W., Rosdayanti, H., & Putri, A. L. (2021). Selection of culture medium and incubation time for growth and production of beauvericin by local *Beauveria bassiana*. *Proceedings of the International Seminar on Promoting Local Resources for Sustainable Agricultural and Development,* 59–65. https://doi.org/10.2991/absr.k.210609.011

Safavi, S. A., Shah, F. A., Pakdel, A. K., Reza Rasoulian, G., Bandani, A. R., & Butt, T. M. (2007). Effect of nutrition on growth and virulence of the entomopathogenic fungus *Beauveria bassiana*. *FEMS Microbiology Letters*, *270*(1), 116–123. https://doi.org/10.1111/j.1574-6968.2007.00666.x

Saputro, T. B., Prayogo, Y., Rohman, F. L., & Alami, N. H. (2019). The virulence improvement of Beauveria bassiana in infecting *Cylas formicarius* modulated by various chitin based compounds. *Biodiversitas Journal of Biological Diversity*, *20*(9), Article 9. https://doi.org/10.13057/biodiv/d200909

Senthilraja, G., Anand, T., Durairaj, C., Raguchander, T., & Samiyappan, R. (2010). Chitin-based bioformulation of *Beauveria bassiana* and *Pseudomonas fluorescens* for improved control of leafminer and collar rot in groundnut. *Crop Protection*, *29*(9), 1003–1010. https://doi.org/10.1016/j.cropro.2010.06.002

Vega, F., Jackson, M., Mercadier, G., & Poprawski, T. (2003). The impact of nutrition on spore yields for various fungal entomopathogens in liquid culture. *World Journal of Microbiology and Biotechnology*, *19*, 363–368. https://doi.org/10.1023/A:1023924304456

Vidhate, R. P., Dawkar, V. V., Punekar, S. A., & Giri, A. P. (2023). Genomic determinants of entomopathogenic fungi and their involvement

in pathogenesis. *Microbial Ecology*, *85*(1), 49–60. https://doi.org/10.1007/s00248-021-01936-z

Wang, H., Peng, H., Li, W., Cheng, P., & Gong, M. (2021). The toxins of *Beauveria bassiana* and the strategies to improve their virulence to insects. *Frontiers in Microbiology*, *12*. https://www.frontiersin.org/articles/10.3389/fmicb.2021.705343

Włóka, E., Boguś, M. I., Wrońska, A. K., Drozdowski, M., Kaczmarek, A., Sobich, J., & Gołębiowski, M. (2022). Insect cuticular compounds affect *Conidiobolus coronatus* (Entomopthorales) sporulation and the activity of enzymes involved in fungal infection. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-17960-z