

# The virulence improvement of *Beauveria bassiana* in infecting *Cylas formicarius* modulated by various chitin based compounds

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**Abstract.** Saputro TB, Prayogo Y, Rohman FL, Alami NH. 2019. The virulence improvement of *Beauveria bassiana* in infecting *Cylas formicarius* modulated by various chitin based compounds. *Biodiversitas* 20: 2486-2493. Sweet potato (*Ipomoea batatas* L.) is one of alternative carbohydrate source with an important role in food production, animal feed industries, and as raw materials for other derivatives. However, severe losses are constantly occurred in its production due to a notorious pest known as sweet potato weevil (*Cylas formicarius*). The chemical insecticides commonly used to treat the pest, but have not been efficient in eliminating *C.formicarius*. *Beauveria bassiana* is well known as a biological control agent, has been identified to effectively eliminate the pest. The aim of this research is to observe the optimum concentration of several chitin-based compounds in improving the virulence of *B. bassiana*. The research was conducted by the addition of chitin from various sources - insects, crustaceans and mollusk shells in a growth medium of *B. Bassiana*. Moreover, conidia produced by *B.bassiana* was exposed to *C.formicarius* to investigate the mortality rate of this insect. The results showed four different characteristics of colonies - velvety, wooly, pellicular, and farinaceous. The 1% chitin from *Tellina* sp, (P9), was found to be the best treatment which increased the growth of the colony diameter attaining 6.7cm in 20 days, increased the conidia viability that reaches 93.5%. In addition, the highest mortality percentage of *C. formicarius* was 91.67% in P9 at 6 days after infection. Overall, this research gave new potential sources of chitin that can be applied in improving the virulence of *B. Bassiana* in eliminating *C. formicarius*.

**Keywords:** Entomopathogenic, *Beauveria bassiana*, *Cylas formicarius*, *Ipomea batatas*, chitin

## INTRODUCTION

Sweet potato is one of primary commodity that has high content of starch. Many efforts were conducted to increase the production rate of sweet potatoes in Indonesia. Unfortunately, those efforts still face various challenges such as no superior varieties, suitable cultivation, pests and diseases. *Cylas formicarius* is one of the main pests of sweet potato, also known as a tube-borer pest (Reddy et al. 2012). Smith and Hammond (2006) reveal that the utilization of chemical insecticide to control *C.formicarius* has not shown positive results because of the application of chemical insecticide unable to access the tube regions that penetrates by the larvae of *C.formicarius*.

A series of studies confirm the other negative effects of chemicals usage including the reduction of biodiversity, pest resistance, pest resurgence as well as having residues of harmful compounds. Ondiaka et al. (2008) develop entomopathogenic fungi *B. bassiana* and *Metarhizium anisopliae* to eliminate the prevalence of *Cylas puncticollis*. *Beauveria bassiana* is well known as one of the entomopathogenic fungi which infect almost all the order and various stadia of insects, and has a very high potential to be used as an alternative substitute for chemical insecticides (Meyling and Eilenberg in 2007; Reddy et al.

2014). Khosvari et al. (2015) clearly stated that the advantages of *B. bassiana* as biological agents for pest control, including (i) easy to propagate or cultivated in either a natural or an artificial media, (ii) does not cause resistance to the target pests, (iii) safe for the environment, water sources, and livestock, and (iv) the increment of product quality since it is free of residues from harmful compounds.

continuous mass production in media is considered as one of limiting factor in the development of biopesticides that causing decrement in virulence. The different level of virulence is highly determined by the sources of isolate and its culture medium. The virulence rate is directly related to the medium compositions. Herlinda et al. (2006) conclude that the addition of chitin to the growth medium has a positive impact to increase the density of *B. bassiana* conidia. Rohman et al. (2017) found that the addition of chitin has the ability to improve the growth of *B. bassiana*. Therefore, in this research, we analyze the potential status of different chitins obtained from Insects, Crustaceans, and Mollusca shells in improving the virulence status of *B. bassiana*. The importance of this research is to provide information on the best source of several chitins from arthropods that highly abundant in the environment to induce a vigorous *B. bassiana*.

## MATERIALS AND METHODS

### Rearing of *Cylas formicarius* insects

The imagines of *C. formicarius* were reared and maintained in clean and sterile plastic jars, in order to keep it away from pathogens. This is vital because the pathogens generally increase the mortality rate of pest at this rearing stage. Subsequently, the plastic jars were covered with gauze to prevent the insects from escaping. The rearing jars were filled with fresh sweet potato daily as feed. This breeding of insects was a continuous process to meet up with the population needed to complete the study.

### Medium preparation, inoculation, and growth of *Beauveria bassiana*

The chitins used in this study were from different sources i.e, insects (*Gryllus asimilis* and *Oecophylla smaragdina*), crustacean (*Scylla olivacea*), and mollusca shells (*Tellina* sp.). All of chitins were air-dried under the sunlight for 3 days and oven-dried at 65°C. These dried shells were grounded into a fine powder and filtered using 0.600 mm sieve. Afterward, each type of chitin was added to a growth medium of Potato Dextrose Agar (PDA) in two concentrations - 0.5% and 1% in 200 mL of PDA medium. And the media was sterilized for 30 minutes in the autoclave maintained at 121°C. But the control treatment was only PDA without adding any chitin.

Immediately after the sterilization process, these growth media were poured into a petri dish with a diameter of 9 cm, and 10 mL each was added 1 mL of 0.5% lactic acid. All these treatments were replicated 3 times. The *B. bassiana* isolate was obtained from the collection of Biopesticide Laboratory Indonesian Research Center of Tubes and Beans (BALITKABI), which was purified on PDA medium later on. Shortly thereafter, the isolates were incubated for 21 days at room temperature 27°C. Subsequently, after 21 days, the isolates which had been purified and grown on PDA media were perforated using sterile reaction tube with a diameter of 1cm. These isolates were then inoculated on each media type containing chitin and incubated at the same room temperature for 20 days, while the colony diameter was measured every 5 days. In general, the treatments are: P1 (control without any addition of chitin); P2 (Chitin of *G. asimilis* 0,5%); P3 (Chitin of *G. asimilis* 1%); P4 (Chitin of *O. smaragdina* 0,5%); P5 (Chitin of *O. smaragdina* 1%); P6 (Chitin of *S. olivacea* 0,5%); P7 (Chitin of *S. olivacea* 1%); P8 (Chitin of *Tellina* sp. 0,5%); and P9 (Chitin of *Tellina* sp. 1%).

### The character of the colony and conidial production

The characterization of the fungal colonies was conducted after 20 days inoculation (DAI) by comparing their growth patterns based on the shape or pattern, color, and growth rate of these colonies. And the conidia produced by the fungi were collected by adding 10 mL of sterile distilled water into each petri dish, and the colony was scraped with a fine brush. The conidia suspension of *B. bassiana* from each treatment was homogenized using vortex. The calculation of conidia density was measured by taking 1 to 2 drops and placed on the hemocytometer and

then observed with a compound microscope at 100 or 400 times magnification. Subsequently, the conidial densities were measured to obtain 10<sup>7</sup>/mL. While, the viability of conidia was determined based on method of Herlinda et al. (2006) by incubating a conidia suspension for 10, 24, and 48 hours. the number of conidia forming the germ tube was calculated using this formula:

$$V = \frac{G}{G+U} \times 100\%$$

Where:

V = The viability of conidia after 10, 24, 48 hours incubation (%)

G = Number of germinated conidia

U = Number of non-germinated conidia

### Bioassays of *Beauveria bassiana* against *Cylas formicarius*

Total of 5 insects from the rearing jars was transferred into each reaction tube with a diameter of 2 cm and filled with 5 pieces of fresh sweet potato, 0.5cm<sup>2</sup> in size. The suspension of *B. bassiana* conidia with a density of 10<sup>7</sup>/mL is sprayed onto the body of the insects and then observed by counting the number of *C. formicarius* that died due to the infection of *B. bassiana*. This observation was conducted every 3 days until mycelium was formed on the surface of insect body. In this research, we use 3 and 6 days after treatment. This mycelium was observed under a stereo-microscope. The mortality rate of the insect was calculated based on Herlinda et al. (2006) using the formula:

$$\text{Percentage of dead insect} = \frac{\sum \text{dead insect}}{\sum \text{Total insect}} \times 100\%$$

### Data analysis

The Completely Randomized Design (CRD) was applied for this study. Microsoft Excel was used to process the data obtained from experiment and then subjected to SPSS 22.0 software to perform analyses of variance and determine the effect of different types and concentrations of chitin in increasing the virulence of *B.bassiana*. Since the result is significantly different, the Tukey test was conducted at the significant level  $\alpha = 5\%$ . Each datum was expressed as the mean  $\pm$  standard error (SE) driven from 3 statistical replications.

## RESULTS AND DISCUSSION

### Character of colony

The observation of colony morphology was performed at 20 DAI. Based on the observation, there are four main colony characters were identified: velvety (short, straight, and thick hyphae), wooly (hypha is rather long, thickened, woolly-shaped), pellicular (thin colonies interconnected with concentric lines), and farinaceous (flour-like colony). The velvety character showed in five treatments, which are P1, P4, P5, P6, and P7. The wooly character was only showed in P8, farinaceous colonies only in P9, while the pellicular characters showed in P2 and P3. The detailed

information is shown in Table 1, while Figure 1 shows the performance of colonies. This different morphology is an indication that *B. bassiana* has a high genetic variation. Bidochka (2002) hypothesize that *B. bassiana* is distinct in the sense that it has the ability to adapt to a certain habitat and does not co-evolve with certain taxon of insect host. Furthermore, Ownley et al. (2010) reported that the ability of entomopathogenic fungi to adapt to various environmental conditions is a key factor for being able to successfully infect many insects host.

The addition of chitin with different concentrations did not cause any color change of the colony. All of the growing colony on medium containing chitin from *G. asimilis*, *O. smaragdina*, *S. olivacea*, and *Tellina* sp. are white. Increasing the incubation period turns the colony to pale yellow and slightly murky. According to Rehner et al. (2011), *B. bassiana* with high virulent status are usually the farinaceous with cotton-like shape, starchy and with white colonies which turn yellowish after prolonged periods. The treatment P9 has this farinaceous character and faster growth compared with other treatments. Prayogo (2009) conclude that the characters of the colonies are associated with other physiological characters like virulence, conidia production, as well as germination period.

#### The growth rate of *B. bassiana* colony

There were variations among the treatments in the development of *B. bassiana* colonies after 5, 10, 15, and 20 DAI in the chitin-rich medium (Fig. 2). The colony diameter in the control treatment reached 4.2 cm after 20 DAI. While, P9 had the fastest growth compared with other treatments and its diameter reached 6.7cm within 20 days. The growing medium with 1% chitin from *Tellina* sp. was

able to provide nutrients suitable for the fungus growth. Kusumaningsih et al. (2004), the average dry shell of arthropods contains 20-50% chitin in the Mollusca shell. Arias and Ferna'ndez (2002) reveal that the shell of *Tellina* sp. contains about 98% of calcium carbonate ( $\text{CaCO}_3$ ) and 2% organic content, including chitin.

The growth of *B. bassiana* on other treatments was within a range of 3.5 and 5 cm while the lowest was showed in P6 treatment with 3.5 cm. And the comparison between the highest and lowest growth diameter of the colonies is very significant. The detailed results are shown in Table 2. The growth of *B. bassiana* colonies is influenced by substrates or media containing nutrient components required for the growth of fungi (Jaronski, 2014). The chitin substrate is a major source of carbon and nitrogen required for the growth of mycelium and the formation of fungal conidia (Baretto et al. 2004). These carbon and nitrogen present in the medium are the main constituents of carbohydrates, nucleic acids, proteins, and lipids. Chitin is considered as a carbon source for chitinolytic organisms and able to increase their growth rate and multiplication (Gerding-Gonza'lez et al. 2007)

#### Number of conidia

The number of conidia produced from each treatment was related to the growth rate of the fungus. And the number of conidia produced by each treatment during the 20 days of incubation showed significant results. The highest sporulation ability was showed in treatment P5 with the conidia production of  $14.73 \times 10^7$  conidia/petri dish. The lowest number of conidia was showed in P6 (Table 2). The fungi virulence could be changed by the addition of chitin and chitosan into the medium (Nithya et al. 2015).

**Table 1.** Characteristics of texture and color of *B. bassiana* colony on chitin medium

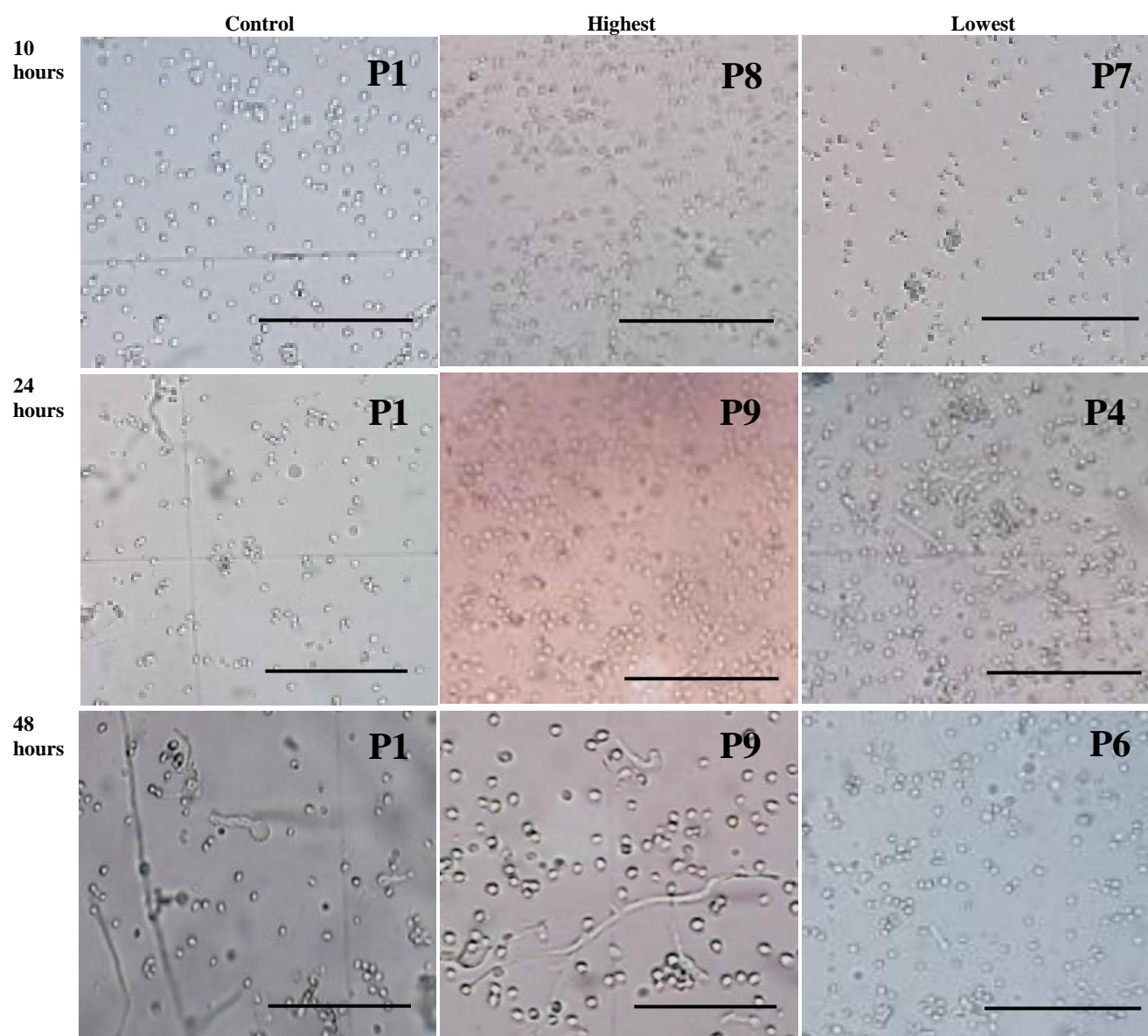
Treatment	Color	Characteristic	Description
P 1	White	Velvety	The hyphae are short, straight, and thick
P 2	White	Pellicular	Thin colonies, hyphae are interconnected with concentric lines
P 3	White	Pellicular	Thin colonies, hyphae are interconnected with concentric lines
P 4	White	Velvety	The hyphae are short, straight, and thick
P 5	White	Velvety	The hyphae are short, straight, and thick
P 6	White	Velvety	The hyphae are short, straight, and thick
P 7	White	Velvety	The hyphae are short, straight, and thick
P 8	White	Wooly	The hyphae or hyphae groups are rather long, thickened
P 9	White	Farinaceous	Colonies like flour



**Figure 1.** Character of *B. bassiana* colonies on media with various types of chitin: A. Velvety, B. Wooly, C. Pellicular, D. Farinaceous

**Table 2.** Effect of chitin in growth parameters of *Beauveria bassiana*

Treat- ments	Diameters of colony (cm) $\pm$ SD				Germination rate (%) $\pm$ SD			Amounts of conidia $10^7/\text{mL} \pm$ SD
	5 days	10 days	15 days	20 days	10 hours	24 hours	48 hours	
P 1	1.73 $\pm$ 0.05e	2.73 $\pm$ 0.047e	3.77 $\pm$ 0.09c	4.17 $\pm$ 0.05c	37.17 $\pm$ 1.65de	53.4 $\pm$ 0.37d	70.20 $\pm$ 1.51de	8.43 $\pm$ 0.05de
P 2	1.83 $\pm$ 0.05cd	2.93 $\pm$ 0.05d	3.37 $\pm$ 0.05e	4.10 $\pm$ 0.08c	37.17 $\pm$ 1.65de	54.63 $\pm$ 0.66cd	75.23 $\pm$ 2.1cd	9.0 $\pm$ 0.08d
P 3	1.87 $\pm$ 0.05c	3.10 $\pm$ 0.00c	3.73 $\pm$ 0.05c	5.03 $\pm$ 0.05b	55.60 $\pm$ 1.99b	67.80 $\pm$ 0.88a	82.10 $\pm$ 1.27b	8.40 $\pm$ 0.22de
P 4	1.67 $\pm$ 0.05e	2.77 $\pm$ 0.05e	3.43 $\pm$ 0.05e	4.17 $\pm$ 0.12c	39.67 $\pm$ 1.89cd	52.83 $\pm$ 0.54d	71.90 $\pm$ 2.68cde	13.30 $\pm$ 0.22b
P 5	2.07 $\pm$ 0.05b	3.27 $\pm$ 0.13b	4.03 $\pm$ 0.05b	4.97 $\pm$ 0.05b	41.97 $\pm$ 1.31c	54.77 $\pm$ 2.6cd	73.67 $\pm$ 1.27cd	14.73 $\pm$ 1.46a
P 6	1.63 $\pm$ 0.05e	2.43 $\pm$ 0.05f	3.17 $\pm$ 0.09f	3.53 $\pm$ 0.05d	51.87 $\pm$ 0.66b	60.13 $\pm$ 1.53b	68.37 $\pm$ 0.66e	7.57 $\pm$ 0.05e
P 7	1.93 $\pm$ 0.05c	2.87 $\pm$ 0.05de	3.53 $\pm$ 0.05d	4.20 $\pm$ 0.08c	35.20 $\pm$ 0.28e	56.73 $\pm$ 1.35c	71.20 $\pm$ 0.8de	10.33 $\pm$ 0.47c
P 8	1.67 $\pm$ 0.05e	2.83 $\pm$ 0.05de	3.70 $\pm$ 0.00c	4.13 $\pm$ 0.17c	57.7 $\pm$ 2.92a	70.70 $\pm$ 2.06a	81.20 $\pm$ 1.96b	5.77 $\pm$ 0.05f
P 9	2.50 $\pm$ 0.08a	4.10 $\pm$ 0.08a	5.47 $\pm$ 0.05a	6.70 $\pm$ 0.08a	54.83 $\pm$ 1.43b	69.67 $\pm$ 1.25a	93.5 $\pm$ 1.22a	11.20 $\pm$ 0.36c

**Figure 2.** Conidia viability of *B. bassiana* at 10, 24 and 48 hours of incubation (Scale bar = 10 $\mu\text{m}$ )

The medium with different types and concentrations of chitin have varying amounts of conidia. The variations of conidial number were occurred within the same chitin source but in different concentrations. Baretto (2004), adding chitin powder to the growing medium makes it more compacted and trigger the fungi to form conidia in a larger amount. Herlinda et al. (2006) indicated that the addition of chitin flour with the concentration above 0.5% has the ability to inhibit the formation of conidia due to the accumulation of metabolites and spur. Furthermore, the formation of enzymes capable of inhibiting the metabolism of entomopathogenic fungi. In addition, Agus et al. (2015), confirmed that the addition of chitin to the medium has a direct influence on conidia production and viability of the *Penicillium* sp. if the type and concentration of chitin are added appropriately.

### Conidia viability

based on the results, addition of chitin on the growing media changes the viability of *B. Bassiana* that expressed in increasing the percentage of certain parameters. The highest germination of 57.7% at 10 hours incubation was P8. The germination rate in control reached 37.17% while the lowest of 35.20% was shown in P7. Therefore, at 24 hours of incubation, the highest germination rate of 69.67% was P9, while control treatment attaining 53.4% and the lowest germination of 52.83% was shown by P4. Afterward, the highest germination rate of 93.5% at 48 hours incubation was shown by P9 and at this period, the control treatment was at 70.20%. The lowest germination of 68.37% at 48 hours was in P6. The detailed profile of conidia germination percentage is shown in Table 2, while the performance of the highest and the lowest are in Figure 2. The germinated conidia showed its ability to grow and develop with favorable environmental factors, hence, the percentage and periods of germination both play a vital role in determining the virulence of the fungus.

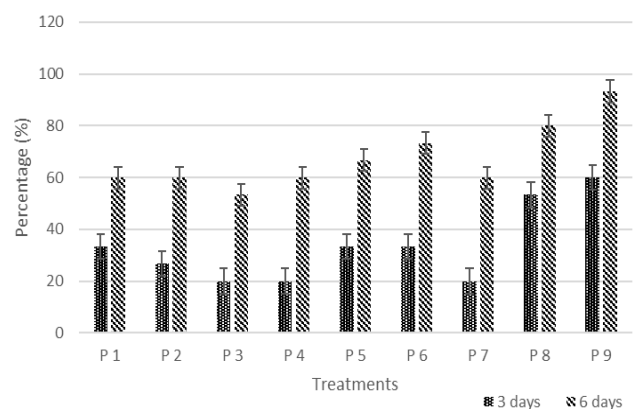
### Mortality of *Cylas formicarius*

The effectiveness of efficacy on the insect is shown by the number of dead insects measured after the infection. The lowest mortality was 21.67% in *C. formicarius* on day 3 after being infected was found in P3 (*G. assimilis* 1%), followed by P4 (*O. smaragdina* 0.5%), while the control was at 33.3% at day 3. The highest insect mortality of 66.67% was found in P9. In day 6 after infection showed a similar pattern with the lowest mortality of 53.3% was in P3, the control reached 61.7%, while the highest mortality of 91.67% was obtained in P9 as shown in Figure 3. The treatment P9 gave the highest mortality considering it has high conidial density,  $11.4 \times 10^7/\text{mL}$ , and highest conidia viability compared with other treatments. Prayogo et al. (2005) reported that the number of conidia will determine the effectiveness of entomopathogenic fungi in controlling insects. The low density of conidia at P3 ( $5.3 \times 10^7/\text{mL}$ )

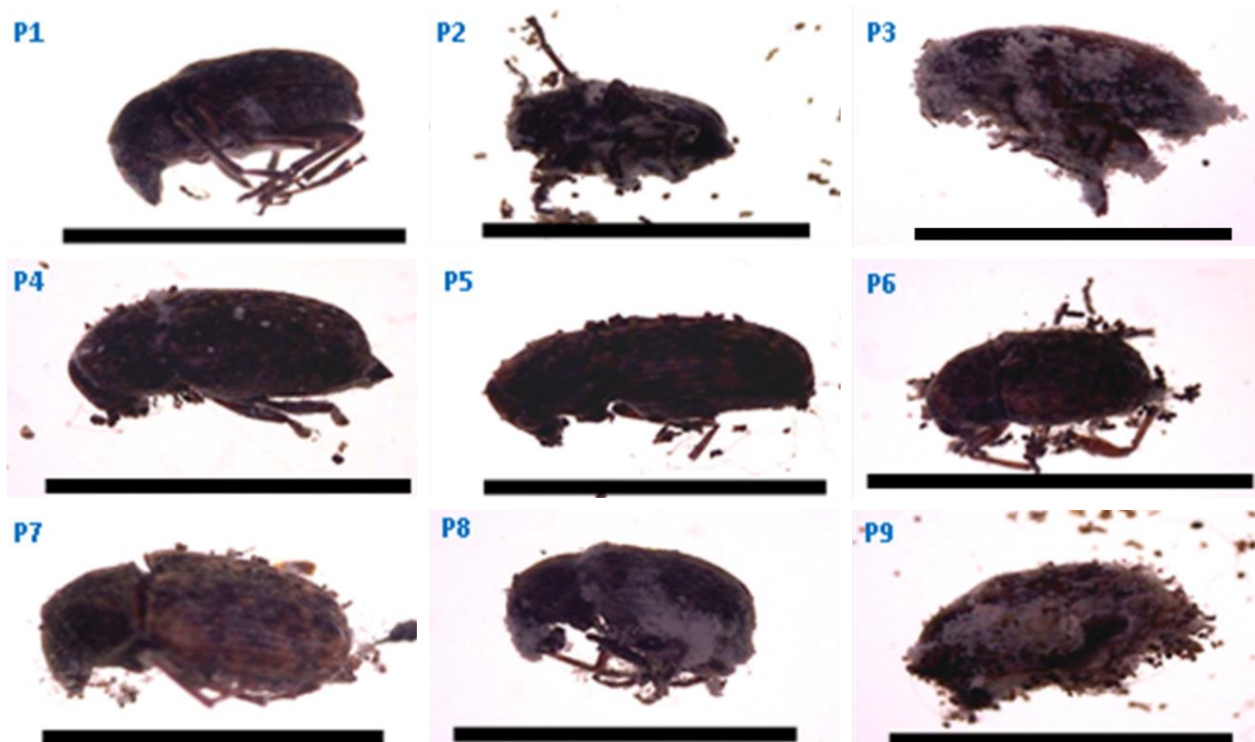
makes the fungus not to be able to penetrate at the maximum percentage, thereby resulting in low mortality.

All the treatments were able to infect *C. formicarius* which resulted in the colonization of mycelium (Fig.4). The most visible colonization was found in P9 which shows in the entire body of *C. formicarius* that fully filled and coated with white mycelium. The mortality rate of insect due to infections of entomopathogenic fungi is influenced by both internal and external factors (Anderson et al. 2011; Ricario et al. 2013). The virulent properties of entomopathogenic fungi are mainly influenced by the production of mycotoxins and the viability of conidia. Mondal et al. (2016) stated that chitin has the ability to stimulate the *B. bassiana* to increase its chitinase enzymes production, which plays an important role in the infection process and can be used to degrade the chitin layers of insects. Bacteria and fungi are the main sources of chitinases which are highly recommended for use as biological protection in several crops (Mondal et al. 2016). In line with this founding, Ortiz-urquiza and Keyhani (2013) and Akbar et al. (2004) conclude that the ability of *B. bassiana* to degrade the insect cuticle is the most critical factor to penetrating the body, spreading, and finally dispatching the insects.

The infection of the insects by the fungi started when the conidia attached itself as an infective organ on the insect host cuticle and the infected *C. formicarius* experienced a change in activity and behavior on day 3 after infection. The apparent symptoms were decrement in feeding ability and slow movements. moreover, the infected *Schistocerca gregaria* insect by *Metarhizium anisopliae* has a significant decrement on its appetite and had slower movements, before the insects died in 3 days after infection (Seyoum et al. 2002). Tefera and Pringle (2003) detailed that the decrease in consumption pattern reached 80% in *Chilo partellus* instar II insect after being infected by *B. bassiana*.



**Figure 3.** Mortality percentage of *C. formicarius* after being infected with *B. bassiana* in 3 and 6 days after application (DAA)



**Figure 4.** The Morphology of *C. formicarius* infected by *B. Bassiana* in 6 DAA (Scale bar = 5 mm)

## Discussions

Chitin is an important compound in increasing the virulence level of fungus. In this study, all of chitin types and concentrations added to the growth medium had a positive effect on the fungi growth rate as it can be used as the source of energy required by these fungi. Moreover, the growth of fungi in the medium supplemented with chitin resulted in conidia with better quality compared to chitin-free medium. Carbon and Nitrogen have the ability to increase the formation of mycelium and germination of conidia (Guerrero et al. 2007; Hou et al. 2013; Kim et al. 2014). The highest production of conidia in *B. bassiana* in this study was shown in P9 treatment.

The amount of conidia is vital due to its major role in the transmission and infection of entomopathogenic fungi (Trizelia, 2005). Alavo et al. (2002) demonstrated that the attachment of conidia and its germination, play a significant role in the ability of the fungi to successfully penetrate and infect the host insects. Since chitin and proteins are an enormous source of energy in insect integuments, adding both substances decreases the viability of *B. bassiana*. Several studies have been able to establish that nutrient-rich growth media significantly affect the conidia germination and viability of the fungi (Guerrero et al. 2007; Lopes et al. 2013). In addition, Kassa et al. (2015) reported that 80% of conidia germination is the minimum rate required to decide if fungi can be classified and used as biological agents. Liu et al. (2003) suggested that the conidia germination, which can be used as a biological agent should be above 90%. The germination of conidia is depended on strains of isolates and fungi species because

each isolate has different nutritional needs. Moreover, conidia size affects the germination rates, the larger the conidia, the faster to germinate. Otherwise, if the biological agent has a longer germination period, it would have a negative impact on its ability to infect the host insect. Meanwhile, the conidia would dry and then die before finding a suitable host if the temperature and humidity are less than the desired condition (Barbosa et al. 2002; Lazzarini et al. 2006).

The media supplemented with chitin from *Tellina* sp. (P8 and P9), shows the fastest germination rate. Although, further research is necessary as these two treatments can be used to increase the virulence of *B. bassiana* in infecting *C. formicarius* faster as the blood of the insects (hemolymph) would be harmed quicker by the poisons from *B. bassiana*. The toxins increase the pH in hemolymph which could damage the brain nerves or make it not work properly Shavithri (2014). Moreover, the level of moisture surrounding the insect is a key factor in forming germ tubes after the adhesion of conidia to the insect (Gaborty et al. 2014; Devi and Bai 2015). The suitable moisture for conidia germination at the penetration and infection step should be above 95% (Mwanburi et al. 2015). Fungi penetrate into the insect cuticle by producing various chitinase and proteases enzymes to degrade the coat compounds (Ortiz-urquiza and Keyhani, 2013). The mycelium absorbs nutrients in hemolymph that contain trehaloses, proteins, and fats. In addition, fungi that have protease and lipases enzymes grow faster and fill the hemocoel thereby affecting the insect blood quicker (Lobo et al. 2015; Ondiaka et al. 2015). Furthermore, the demisal

of *C. formicarius* can be attributed to the toxic compounds in the hemocoel which caused dysfunction in the nervous system and brain. Insects defend themselves under these conditions by producing hormonal or melanizing compounds, however, *C. formicarius* is unable to hold it longer if the concentration of toxic compounds is continuously increasing (Dubovskiy et al. 2013; Jaronski, 2014).

In conclusion, there are four main types of *B. bassiana* colony including Velvety, Woolly, Pellicular, and Farinaceous. The 1% Chitin from *Tellina* sp. (P9), is considered the best treatment for all parameters observed. P9 has farinaceous form with 6.7cm in diameter within 20 DAI, which was the fastest compared with others. P9 treatment increased the viability of conidia by 93.3%. Furthermore, the addition of chitin shows a positive trends in increasing the mortality of *C. formicarius*, except in addition of 1% chitin from *G. assimilis* that lower than then control. P9 gave a significant increment to the mortality of *C. formicarius* which attaining 91,67%. Based on these results, the exploration of chitin type as well as the various concentrations, gave a strong recommendation to improve the performance of *B. bassiana* in eliminating *C. formicarius*. Further research was necessary to be conducted to gave the information about the composition and the structure of chitin from *Tellina* sp.

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