



## Research Article

# Efficacy of Biopesticide Be-Bas against Sweet Potato Weevils (*Cylas formicarius* Fabricius) in Tidal Land

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## ABSTRACT

In Indonesia tidal land is very wide and quite potential for sweet potatoes, but an obstacle for this cultivation is sweet potato weevil (*Cylas formicarius*) causes loss of 100%. This research aimed to evaluate the efficacy of biopesticide Be-Bas contains *Beauveria bassiana* entomopathogenic conidia to *C. formicarius* mortality at tidal lands. This study was conducted on June–November 2016 in South Kalimantan. The research consisted of five treatments and five replications. Results showed that the application of biopesticide Be-Bas in the planting hole and stem base was effective to cause mortality of 17 and 15 larvae per tuber, respectively. However, the application by soaking cutting was able to kill 3 larvae per tuber. The application of biopesticide Be-Bas in the planting hole and stem base were also effective to suppress the number of eggs laid by *C. formicarius* in the tuber, hence there was no damage found on the tuber. Meanwhile, the application of conventional insecticide was less effective because a total of 17 eggs and 3 larvae of *C. formicarius* were still found in each tuber. It caused tuber damage up to 17%, thus the tuber cannot be consumed. Therefore, the application of biopesticide Be-Bas in the planting hole or stem base was considered capable to suppress tubers damage caused by *C. formicarius* better than that of conventional insecticide.

Keywords: application method, biological control, *Beauveria bassiana*, *Cylas formicarius*, entomopathogenic fungi

## INTRODUCTION

Indonesia estimated has 33,393,570 ha tidal land which consists of 20,096,800 ha (60.2%) of tidal land and 13,296,770 ha (39.8%) of non-tidal swamp land (lebak) spread over three islands: Sumatra (32%), Kalimantan (40%), Irian Jaya (20%), and the remaining 5% is in Sulawesi (Wahyunto & Mulyani, 2011; Wahyunto *et al.*, 2012). Most of the land has not been fully utilized, thus it is a great opportunity to be advanced considering that productive land on Java becomes limited. Swiadikarta (2012) and Alwi (2014) reported that the type C tidal land in South Kalimantan mostly used for plantation and crops.

Sweet potato is one of the commodities cultivated in tidal land, including in Barito Kuala Regency. Sweet potatoes are local varieties that have potential productivity up to 25 tons/ha (Rina & Syahbudin, 2013; Galib, 2014). One of the obstacles of sweet potato production is tuber borer, *Cylas formicarius*, as reported in various other countries (Reddy *et al.*, 2012; Devi *et al.*, 2014). Yield loss caused by this pest reaches 100 %, in consequence of infested tuber that is not suitable for human consumption due to

toxins that can cause bitter taste (Maingi *et al.*, 2002). Control of *C. formicarius* using conventional insecticides has not shown delighted results (Korada *et al.*, 2010; Leng & Reddy, 2012). Because of the chemical compound cannot reach the larvae live inside the tuber under the soil. Various efforts yet to develop to manage *C. formicarius* (Reddy *et al.*, 2012; Taye & Tadesse, 2014; Hue & Low, 2015), one of which is biological control method using entomopathogenic fungi (Shams *et al.*, 2011; Khosravi *et al.*, 2015).

*Beauveria bassiana* is an entomopathogenic fungus to infect almost all orders and various stages of insects, thus it is quite prospective to be used as an alternative for conventional insecticides (Reddy *et al.*, 2014). The control of tuber borer using entomopathogenic fungi *B. bassiana* in Indonesia is not yet developed, because there has not been found the right application method to obtain optimal efficacy in the field. Stafford and Allan (2010) studied that *B. bassiana* applicated on the leaf surface is quite effective in controlling pests from mite. Ali *et al.*, (2009) indicated that *B. bassiana* applicated on the

ground is more effective than other methods. Meanwhile, the efficacy of *B. bassiana* is more determined by the type and behavior of the target pests (Boyle & Cutler, 2012; Rashki & Shirvani, 2013).

*B. bassiana* isolates had been explored in 2010 from various locations in Java and among those, there were three effective isolates cause adult and larvae mortality up to 100%, and unhatched eggs of *C. formicarius* (Prayogo, 2012; 2013). In advance research, *B. bassiana* was formulated in the form of powder with the product name Be-Bas under patent number P00201605992 (Prayogo, 2016). Powder formulation is expected to make application simple in the field yet effective. Therefore, to verify the consistency of the efficacy of Be-Bas formulation in controlling tuber borer, this biopesticide needs to be validated in various locations, especially at the *C. formicarius* endemic centers of sweet potato plantations. The aim of this study was to validate the efficacy of Be-Bas biopesticide (*B. bassiana*) against sweet potato borer *C. formicarius* in tidal land, South Kalimantan.

## MATERIALS AND METHODS

This study was conducted in Sidomulyo Village, Wanaraya District, Barito Kuala Regency, South Kalimantan Province, from June to November 2016. The study was arranged in Randomized Block Design (RBD) with five replications. The treatments were: P1 (Be-Bas applicated in planting hole), P2 (Be-Bas applicated at the base of plant stem), P3 (sweet potato cuttings soaked in Be-Bas for 60 minutes before planting), P4 (full protection using chemical insecticides starting at the age of 30 DAP and 2-week interval), and P5 (non-controlling treatment).

### Land Selection

The research location was selected in the center production of sweet potato which is endemic to tuber borer. An observation on *C. formicarius* existence was done prior to research to ensure that the land was truly endemic to tuber borer. Observation *C. formicarius* was conducted by exposing fresh tubers of local varieties on the surface of the land at several points taken diagonally, then the tubers were covered with tilled-soil and left for approximately 30 days. The exposed tuber was taken, observed and calculated the number of egg masses, larvae, and adults of *C. formicarius*. Land indicated as endemic

to tuber borer if all of the exposed tubers were infested by *C. formicarius* with damage level of  $\geq 50\%$ . If the land was not indicated as tuber borer endemic, then the artificial infestation has to be employed using *C. formicarius* reared in the laboratory.

### Land Preparation

The soil was tilled until it was loosened and then made a mound (400 cm in long and 75 cm in wide). The soil then amended with mixed of 2 tons/ha of limestone and 5 tons of manure/ha at the time of tillage. The dosage of fertilizer given was as follows: 100 kg N, 150 SP36, and 100 KCl/ha. All dosages of fertilizer were given during planting time, while 2/3 doses of fertilizer were given at the age of 1 month. Sweet potato cuttings used in this study were local varieties.

### Preparation of Be-Bas Biopesticide

Entomopathogenic fungus *B. bassiana* isolate used in this study were obtained from Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Laboratory collection of Biopesticides, formulated in powder under Be-Bas trademark. The efficacy of this product has been tested to kill larvae and adults of *C. formicarius* with mortality level reach 100%. Preparation of Be-Bas formulations was as follows: *B. bassiana* isolate was grown on a medium made from sweet corn plus 5% of chitin flour from clamshells (*Scylla serrata*). Media then put into a plastic bag containing 200 g media and then sterilized in an autoclave at 121°C for 30 minutes. Media then inoculated with *B. bassiana* isolate, and after 21 days post-inoculation (DPI). Each culture bag was put into Erlenmeyer, mixed with 1000 ml of water then shook using a shaker for 60 minutes. Furthermore, the conidia suspension was filtered using gauze to separate conidia from the substrate and then 3 ml/l of Tween 80 was added and shaken so that conidia mixed homogeneously because it is hydrophobic. The conidia suspension was mixed with a carrier material (Kaolin powder), then dried in an aseptic laminar flow cabinet.

The application of the conidium suspension to the planting hole and to the base of the stem was 600 l/ha using conidia density of  $10^7$ /ml by taking 5 g of Be-Bas product put in water and stirring until homogeneous. The application of the conidia *B. bassiana* suspension on sweet potato cuttings was carried out by soaked cuttings in conidia suspension

for 60 minutes before planting. Full protection treatment (P4) was used a conventional insecticide with the active ingredient of lambda-cyhalothrin at the age of 30 DAP (day after planting) with an interval of every week. Non-controlling treatment (P5), plants were sprayed only with water since the plant was 30 days old with an interval of every week.

### Observation

The variables observed were the number of dead larvae infected by *B. bassiana*, number of live larvae in tubers, number of eggs in each tuber base, tuber damage level (%), and weight tuber of each plant. Observations were made at harvest on tuber of five plants per treatment which were taken randomly.

### Data Analysis

The results were analyzed using the Minitab program 14. Any significant differences between treatments will be followed by Duncan Multiple Range Test (DMRT) at  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

### Larval Mortality of *C. formicarius*

Be-Bas biopesticide was effective in killing sweet potato borer larvae and adults, but the efficacy of the fungi was influenced by the application technique was carried out. This was characterized by the presence of dead larvae and adults infected by *B. bassiana* as bioinsecticide in treatment P1 (application of conidium *B. bassiana* suspension in planting holes), P2 (Be-Bas conidia suspension sprayed to the base of plant stem), and P3 (application through soaking sweet potato cuttings into the conidium *B. bassiana* suspension for 60 minutes before planting). The number of dead larvae and adults of *C. formicarius* infected by *B. bassiana* was higher in P1 (17), then P2 (15) (Table 1). However, P3 (3) was less effective

compared to P1 and P2 refer to the larval and adult mortality.

The died larvae or adults of *C. formicarius* infected by *B. bassiana* from the field at P1, P2, and P3 did not show any colonization of *B. bassiana* mycelium. Colonization was seen after the body of the dead insect was incubated in Petri dishes for 98 hours with the presence of white mycelium covering the entire insect body (Figure 1). Colonization of *B. bassiana* mycelium in *C. formicarius* larvae was indicated by mass of white conidia as inoculum or source of infection to host insects. The died instars I and II of Coleoptera order due to *B. bassiana* infection are characterized by colonization of white mycelium covers the entire body of insects in a short time (Ansari & Butt, 2012; Malekan *et al.*, 2015; Erler & Ates, 2015). This conidia produced by mycelium colonization is a media to spread the disease in the body of host insects (Gracia-Munguia *et al.*, 2011; Lopes *et al.*, 2011; Svedese *et al.*, 2013).

Referring to the mortality of *C. formicarius*, conidia suspension of *B. bassiana* applied in planting holes or sprayed at the base of plant stems was quite effective to kill *C. formicarius* larvae and adults compared to soaked cuttings and conventional insecticide applications. Because of conidia applied in the planting hole or around the stem of the plant established and colonized plant root area where tubers grow. Thus tubers have been protected by the conidial mycelium *B. bassiana*. According to Du *et al.* (2013), Quesada-Moraga *et al.* (2014), and Swiergiel *et al.* (2015), the conidia suspension of entomopathogenic fungi *B. bassiana* applied to the soil is able to suppress the population of several types of underground pests. *B. bassiana* is an entomopathogenic fungus that is endophytic, parasitic, and saprophytic that establish in the soil hence it can be used for pest control agents in the soil (Lopez *et al.*, 2011; Brownbridge

Table 1. The larval and adult mortality of *Cylas formicarius* infected by *Beauveria bassiana* every five clumps of plants

No.	Treatment	Mortality (larvae or adults)
1.	P1 (Be-Bas applicated in planting hole)	17.0a
2.	P2 (Be-Bas applicated at the base of plant stem)	15.0b
3.	P3 (sweet potato cuttings soaked in Be-Bas for 60 minutes before planting)	3.0cd
4.	P4 (chemical insecticide)	4.0c
5.	P5 (non-controlling treatment)	0.0e

Remarks: Values followed by different letter were not significantly different according to DMRT ( $\alpha = 0.05$ )





Figure 1. *Cylas formicarius* larvae colonized by *Beauveria bassiana* after 98 hours incubation

*et al.*, 2012; Greenfield *et al.*, 2016). This study informed that the pests living around the base of plant stems and soil can be controlled using the biological agent *B. bassiana*.

#### **Number of *C. formicarius* Eggs Found in Each Tuber**

*C. formicarius* eggs found on tuber skin from P1, P2, and P3 were only 5 eggs in each tuber (Table 2). The eggs found on the tuber at harvesting time revealed colonization of *B. bassiana* mycelium after incubation in the petri dish. Furthermore, after 4 days, the incubated eggs failed to hatch, because they had been infected by *B. bassiana* except for the eggs from P3. It was estimated that uninfected eggs by *B. bassiana* from P3 will hatch and the larvae will feed on tubers thus creating damage. Eggs from P1 and P2 were failed to hatch because *B. bassiana* was able to impede eggs (ovicidal) as reported by Nisha *et al.* (2013). Furthermore, Wu *et al.* (2015) and Peter (2015) reported that *B. bassiana* produces metabolites

from mycelium and conidium, e.i. bassionalide, oosporin, beauvericin, and beauveriolides which are toxic to aphids (aphicidal), anti-feeding, inhibit egg production and the process of oviposition. Therefore, *B. bassiana* has great potential as a biological agent to suppress the population of pests in the field due to its ability in killing egg (Lord, 2009).

The highest number of the eggs found in P5 (non-controlling treatment) was 23 eggs per tuber, in the conventional insecticide intensively applied every two weeks (P4) found 19 eggs/tubers (Figure 2). *C. formicarius* eggs found in both treatments had a 99% chance of hatching, which triggered the development of larvae and growing inside the tuber. Because of the conventional insecticide used (lambda-cyhalothrin) has no ovicidal ability (Stevenson *et al.*, 2009; Kumano *et al.*, 2011; Wang *et al.*, 2014). Previous research reported that only conventional insecticides with active ingredients of phenylpyrazoles, ethiprole, organophosphates, and carbamate were toxic to Hymenoptera eggs.

Table 2. Number of *Cylas formicarius* eggs and larvae in each tuber applied with Be-Bas and conventional insecticide

No.	Treatment	Number of eggs	Number of larvae
1.	P1 (Be-Bas applicated in planting hole)	5.30c	0.0c
2.	P2 (Be-Bas applicated at the base of plant stem)	5.25c	0.0c
3.	P3 (sweet potato cuttings soaked in Be-Bas for 60 minutes before planting)	5.50c	0.0c
4.	P4 (chemical insecticide)	19.50b	3.0b
5.	P5 ( non-controlling treatment)	23.50a	7.0a

Remarks: Values followed by different letter were not significantly different according to DMRT ( $\alpha = 0.05$ )

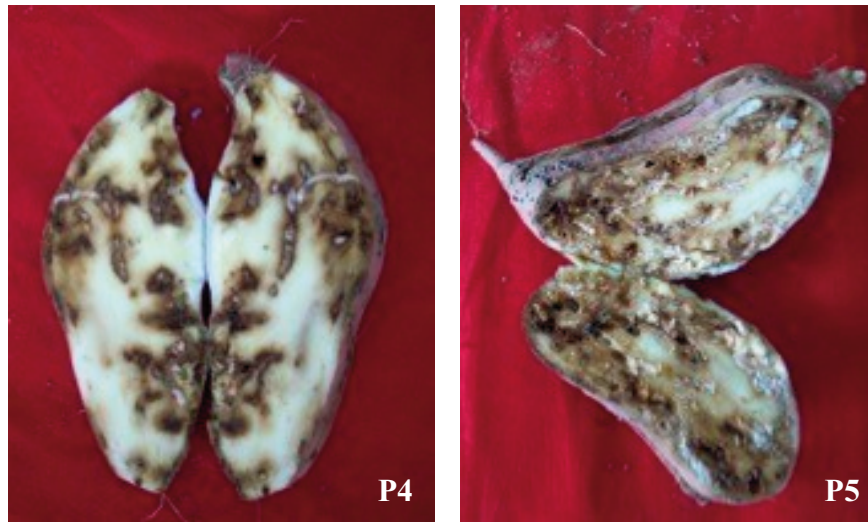


Figure 2. Damaged tuber bored by *Cylas formicarius* larvae from conventional insecticide treatment (P4) and non-controlling treatment (P5)

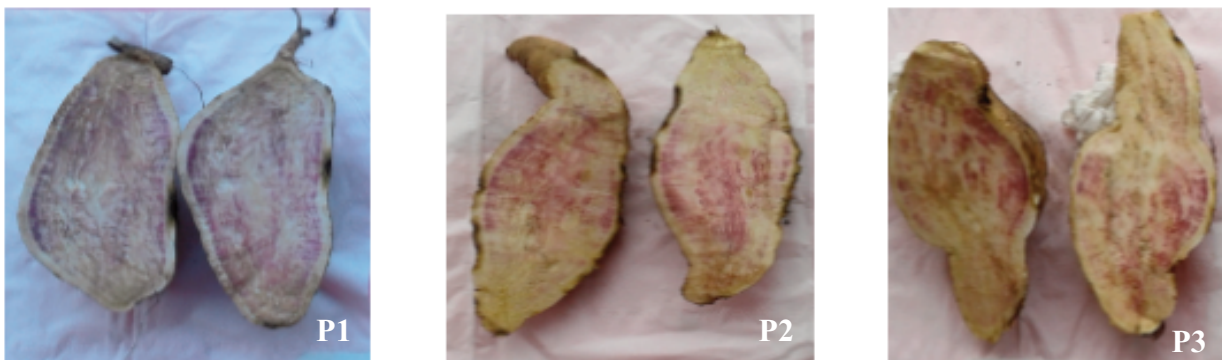


Figure 3. The tuber treated with Be-Bas. P1= (Be-Bas applied in planting hole), P2 = (Be-Bas applied at the base of plant stem), P3 = (sweet potato cuttings soaked in Be-Bas for 60 minutes before planting)

### **Number of *C. formicarius* Larvae**

There were no larvae found from P1, P2, and P3 even when the harvested tubers were cut to ascertain the existence of larvae inside the tubers. Because the habitat of *C. formicarius* larvae is inside the tubers under the soil. Observations on the number of eggs indicated that in P3 was found 5 eggs uninfected by *B. bassiana* so that they would become larvae. Furthermore, P3 showed no eggs hatched, which could be influenced by internal and external factors. Therefore, the efficacy of the P3 treatment still needs to be reviewed further to ensure the performance consistency of the Be-Bas biopesticide in protecting cuttings by soaking before planting for 60 minutes.

Larvae found from P4 and P5 were 3 and 7 per plant, respectively (Table 2). That showed that P1, P2, and P3 were quite protected the tubers because there were no larval stages in the tubers hence there was no damage to the tubers (Figure 3). The efficacy of *B. bassiana* in suppressing the development of larvae and eggs is because the fungus is capable of killing various types of pests in all insect stadia (eggs, larvae, and adults) (Hernandez *et al.*, 2012; Tangavel *et al.*, 2013; Krishna & Bhaskar, 2013; and Zhang *et al.*, 2013).

### **Damage level of the tuber of each plant**

P1, P2, and P3 showed there was no tuber damaged by *C. formicarius* larvae (Table 3). From

destructive observations, that there were no larvae alive found in each tuber. The destructive observation was carried out by cutting each tuber to examine the existence of larvae inside tuber. This observation informed that the application of *B. bassiana* in P1, P2, and P3 decreased *C. formicarius* population inside tuber. According to Akmal *et al.* (2013), the efficacy of pest control using entomopathogenic *B. bassiana* was related to insect target behavior and habitat. The application of *B. bassiana* into the soil was more able to suppress soil living pests population of Coleoptera order compared to applications on plant canopies. Because of the applied conidium suspension can establish and colonize the root area (Stafford & Allan, 2010). Thus, pests have habitat around the root area will die by fungal infection. In addition, conidia formed from dead insects in the root area become potential inoculum that will transmit the disease to another host pests (Conceschi *et al.*, 2016).

Tuber damage was only found in P4 (17.5%) and P5 (60.5%). The infested tuber by *C. formicarius* contained poison because of larval feces, hence un consumable because it tastes bitter and can cause cancer (Figure 2). Therefore, control of *C. formicarius* on sweet potatoes using conventional insecticides was considered to be less effective, because the attack symptoms on the tuber still occur so that the tuber

cannot be consumed. The average tuber damage in P5 reached 60.50% with tubers being severely damaged. Symptoms of tuber borer attack are the presence of *C. formicarius* feces found around the base of the stem (Okonya & Kroschel, 2013; Seow-Mun & Min-Yang, 2015; Zimmerman *et al.*, 2016).

#### **Weight and Number of Healthy Tuber of Each Plant**

The highest tuber weight was from P1 (575.5 g/plant) (Table 4). Tuber weight of each plant from P2 (565.3 g/plant) and P3 (565.2 g/plant) was also quite high, almost the same as P1. The tuber weight from P4 (495.8 g/plant) was not significantly different from P5 (495.9 g/plant), but in P5, the level of tuber damage was higher than that in P4.

The highest average number of tubers was also from P1 (3.4 tubers/plant). The average number of tubers from P2 and P3 were 3.3 and 3.2 tuber, respectively. P4 only produced 2.2 tubers/plant, and P5 was 2.9 tubers/plant. The number of tubers from P5 (non-controlling treatment) was higher than P4 (conventional insecticide treatment), but the tuber damage on P5 was heavier so it was not possible for consumption. This condition caused by a large number of larvae inside tuber so that the consumable tubers were low. According to Hue & Low (2015), the higher number of *C. formicarius*

Table 3. The damage level on sweet potato tubers by *Cylas formicarius* larvae every five clumps of plants

No.	Treatment	Tuber damage (%)
1.	P1 (Be-Bas applicated in planting hole)	0.0c
2.	P2 (Be-Bas applicated at the base of plant stem)	0.0c
3.	P3 (sweet potato cuttings soaked in Be-Bas for 60 minutes before planting)	0.0c
4.	P4 (chemical insecticide)	17.50 b
5.	P5 (non-controlling treatment)	60.50a

Remarks: Values followed by different letter were not significantly different according to DMRT ( $\alpha = 0.05$ )

Table 4. The weight and number of sweet potato tuber per plant treated with Be-Bas and conventional insecticide (lambda-cyhalothrin)

No.	Treatment	Tuber weight (g)*	Number of tuber/plant
1.	P1 (Be-Bas applicated in planting hole)	575.5a	3.40
2.	P2 (Be-Bas applicated at the base of plant stem)	565.3b	3.30
3.	P3 (sweet potato cuttings soaked in Be-Bas for 60 minutes before planting)	565.2b	3.20
4.	P4 (chemical insecticide)	495.8c	2.20
5.	P5 ( non-controlling treatment)	495.9c	2.90

Remarks: Values followed by different letter were not significantly different according to DMRT ( $\alpha = 0.05$ )



larvae inside the tuber, the higher damage level of tuber will obtain and even not suitable for consumption.

According to the weight of the tuber and the tuber damage level, the application of Be-Bas biopesticide in planting holes and at the base of the stem of the plant was an effective and suitable technique to suppress *C. formicarius* population in order to save yield. The method of application required an effort in such manner each planting hole could obtain an adequate initial inoculum so the pests around the planting hole will be infected by this biopesticide. Application method by soaking sweet potato cuttings into *B. bassiana* conidia suspension for 60 minutes before planting can be recommended if there was not enough labor for the application in the planting hole. During soaking, an adhesive must be added to increase the persistence of the conidium attached to the cuttings because the amount of inoculum is quite limited and to ensure the colonization of the inoculum around the stem of the plant can occur. Adhesives will increase the persistence of conidium in the field from less favorable environmental factors, especially on dry land (prevent evaporation) and on the wetland (prevent run off) (Mwamburi *et al.*, 2015; Jadhav & Patil, 2016). The application of conventional insecticide with the active ingredient of lambda-cyhalothrin has not been able to suppress the development of *C. formicarius* in tidal land which presumed as endemic area. This finding was concluded from the high larvae population because the insecticide was not able to kill *C. formicarius* eggs, so the tuber damage level was still quite high as well as the treatment without control treatment.

## CONCLUSION

Be-Bas (entomopathogenic fungus *B. bassiana*) is more effective in suppressing the development of *C. formicarius*, tuber damage, and maintaining sweet potato yield compared to the efficacy of conventional insecticide. Applying Be-Bas biopesticide in planting holes and at the base of plant stems are more effective in suppressing the development of *C. formicarius* and thus saving the yield. Soaking cuttings before planting for 60 minutes into Be-Bas biopesticide was recommended for controlling *C. formicarius* although it still needs to be revalidated. Validation of Be-Bas biopesticide in controlling

sweet potato borer *C. formicarius* need to be reviewed in various locations of sweet potato production centers and those that have endemic criteria.

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