EFFECT OF *Beauveria bassiana* AGAINST COFFEE BEAN BORER, *Hyphothenemus hampei* (FERRARI) IN SMALL SCALE FIELD TRIALS

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ABSTRACT

Coffee Berry Borer (CBB), *Hyphotenemus hampei* (Ferrari), is well known as scolitin beetle and a worldwide most important pest of coffee. This beetle has caught attention to entomologists worldwide to intensively study and search for the best strategy for controlling this insect. *Beauveria bassiana* is the most widely implemented biological control agent for CBB population, and has been mass-produced commercially in many countries. We studied the use of two local isolates of *B. bassiana*, *Cf-Bb* (isolated from naturally infected sweet potato weevil, *Cylas formicarius*) and *Hh-Bb* (isolated from naturally infected *H. hampei*). Small scale field efficacy study with 1 x 10^9 ml^{-1} spore concentration was conducted in a complete randomized block design, with four and one plants as the experimental units, replicated 5 and 10 times, for the first and second trial, respectively. Attacked berries and infected CBB were observed from young and mature berry clusters. The results revealed that means numbers and percentages of mature and young damaged berries varied from medium to high. Means numbers and percentages of attacked mature berries were lower in *Hh-Bb* than those in *Cf-Bb* treated and control plants. The attacked berries of younger fruits were significantly lower in plants treated with *Hh-Bb* and *Cf-Bb* isolates than those on control plants. Mean of death CBBs from *B. bassiana* infection was higher on attacked berries of treated plants than on those of controls. Both isolate reduced berry damage but the effects are weak. The highest reduction in the percentage of attacked berry was 33.93% and 76.47% for mature berry and young berries, in the first trial and 69.03% of mature berries in the second trial, respectively. The highest CBB mortality was 48.33%. The application of *B. bassiana* isolates potentially to reduce the infestation of the CBB attacked on coffee berry.

Key words: *Beauveria bassiana*, *Hyphothenemus hampei*, local isolates, robusta coffee

ABSTRAK

Pengorek Buah Kopi (PBK), *Hyphotenemus hampei* (Ferrari), adalah kumbang scolithin dan terkenal sebagai perosak tanaman kopi yang paling penting di dunia. Kumbang ini telah menarik minat ahli entomologi seluruh negara untuk membuat penyelidikan dan mencari strategi terbaik untuk mengawal perosak ini. *Beauveria bassiana* adalah entomopatogen yang paling banyak digunakan untuk kawalan biologi populasi PBK, tetapi hasilnya telah dicampur. Walau bagaimanapun, *B. bassiana* telah dihasilkan secara besar-besaran dan dipasarkan di banyak negara untuk kawalan PBK. Kami mengkaji penggunaan isolasi tempatan *B. bassiana*, *Cf-Bb* (isolasi dari kumbang *Cylas formicarius*) dan *Hh-Bb* (isolasi dari *H. hampei*). Keberkesanan medan berskala kecil dilakukan dengan menggunakan kepekatan spora 1 × 10^9 ml^{-1}. Rawatan (dua isolasi *B. basssiana* dan kawalan) disusun mengikut reka bentuk blok rawak lengkap. Unit eksperimen pada ujian pertama menggunakan empat tumbuhan dan pada ujian kedua menggunakan satu tumbuhan. Rawatan diulang lima kali pada ujian pertama dan 10 kali pada ujian kedua. Pemerhatian terhadap buah yang diserang PBK dilakukan terutama dari buah tua pada kedua-dua ujian, dan juga pada buah muda pada ujian pertama. Hasil kajian menunjukkan bahawa jumlah dan peratusan buah-buahan yang terkena PBK bervariasi dari sederhana hingga tinggi. Purata dan peratusan buah tua yang diserang oleh PBK lebih rendah dalam rawatan *Hh-Bb* berbanding dengan rawatan dan kawalan *Cf-Bb*, dalam kedua-dua ujian. Buah kopi muda yang diserang oleh PBK lebih rendah dalam rawatan kedua-dua isolasi daripada kawalan. Purata kadar kematian PBK yang dijangkiti *B. bassiana* lebih tinggi dalam rawatan isolasi *B. bassiana* berbanding dengan kawalan. Kedua-dua isolasi dapat mengurangkan kadar serangan pada biji kopi, tetapi kesannya lemah. Pengurangan tertinggi dalam ratusan buah yang diserang PBK adalah 33.93% dalam buah matang dan 76.47% dalam buah muda, dalam ujian pertama dan 69.03% buah matang dalam ujian kedua. Kematian CBB yang tertinggi ialah 48.33%. Aplikasi isolasi *B. bassiana* isolates berpotensi menurunkan infestasi CBB menyerang ke atas biji kopi.

Kata kunci: *Beauveria bassiana*, *Hyphothenemus hampei*, isolate lokal, kopi robusta

INTRODUCTION

Coffee Berry Borer (CBB), *Hyphotehenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolitinae), is the most important coffee pest that often causes a decline in production and quality of coffee worldwide (Aristizábal et al. 2017; Benavides et al. 2012; Damon 2000; Jaramillo et al. 2006; 2011; Vega 2004; Vega et al. 2015), including in Indonesia (Wiryadiputra 2014). The publications related to this species are numerous (Pe´rez 2015), not include those in the last five years. Results of research in several locations in Indonesia showed that coffee berry damage varied. For example, Lila et al. (2011) reported 30-60% berry damage from coffee field with integrated control for the beetle in Enrenkang, South Sulawesi. Wiyadiputra (2014) observed berry damage of 0-69.7% form sampling in Banyuwangi, East Java. Swibawa and Sudarsono (2011) reported berry damage of 28-32% from coffee agroforestry in two municipalities in Lampung. Sitanggang et al. (2017) reported range of 10-90% berry damage from several coffee area in North Sumatera. Vega (2004) mentions that berry damage of >50% is considered very high. Losses due to CBB attacks vary between countries, regions, and season, and according to Vega et al. (2015) reach >500 million $ US per year globally. In Indonesia, losses from CBB attacks have been estimated to be $ 6.7 million (Wiryadiputra et al. 2008).

In addition, *H. hampei* might also transmit ochratoxin producing fungi that infect coffee grain in storage, such as *Aspergillus ochraceus*, *Aspergillus carbonarius*, *Aspergillus niger* and *Penicillium trichophyton*. Ochratoxin contamination might be high in CBB infested coffee beans and may cause health problems to people who consume coffee (Velmourougane et al. 2010), and as such, it is subjected to strict quarantine rules for international trade (Bhat et al. 2010). Based on fruit damaged from CBB, Fernandes et al. (2011) incorporated percentages of the affected coffee berries and the decreased quality due to CBB attacks and set economic injury level (EIL) at 4.3% damaged. Whereas, Wegbe et al. (2003) set CBB EIL even lower, 2.34% for the production of 800 kg ha^{-1} coffee bean.

Control of CBB in many coffee producing countries has been practiced with insecticide (Damon 2000; de Souza et al. 2013; Infante 2018; Ruiz-Cárdenas & Baker 2010), pathogens (especially *Beauveria bassiana*) (Ruiz-Cárdenas & Baker 2010) and trapping of adult beetles with liquid attractant containing methanol - ethanol mixed (Aristizábal et al. 2015; Dufour & Frérot 2008; Fernandes et al. 2015; Wiryadiputra 2014). Introductions of some parasitoid species also were reported, but the results were often unsatisfactory (Jamarillo et al. 2009). Some ant species were reported as an important component of predators that can suppress CBB infestation (Armbrecht & Gallego 2007; Gonthier et al. 2013), although their role is still ignored, especially in Indonesia. The results of the research of Laila et al. (2011) emphasized the importance of Integrated Pest Management (IPM) by implementing, sanitation, pruning of shade trees, the use of *B. bassiana*, and CBB trapping.

For smallholder farmers, insecticide application is not or rarely practiced and they rely more on using low-energy inputs (Avelino et al. 2011). Various natural enemies of CBB have been reported, and *Beauveria bassiana* (Ascomycota: Sordariomycetes: Hypocreales: Cordycipitaceae) is the most widely studied and implemented for CBB biological control (Escobar-Ramírez et al. 2019), and also on other pest species (Ginting et al. 2020). In Bengkulu, local farmers do not control CBB (D. Apriyanto; personal interviews with farmers at several locations in Kepahyang and Rejang Lebong districts), let alone with the low price of robusta coffee. CBB control in Bengkulu was inconsistently practiced by a small part of farmers by using *B. bassiana* or CBB trapping but does not resolve the problem, as it was only a form of participation of regional or central government programs that do not cover the entire coffee area (all farmers) and only practiced temporarily. Furthermore, evaluation of the success of the program has never been undertaken and it was not sustained by farmers, and as such, there is no available formulation of *B. bassiana* in the local market in Bengkulu. The objective of the study was to determine the effectiveness of local isolates of *B. bassiana* to suppress the CBB damage on robusta coffee. Research has been conducted in farmer's coffee plantation, using two local isolates of *B. bassiana*.

MATERIALS AND METHODS

Time and Site

The study was conducted from August 2018 – April 2019, in Tangsi Duren, Kabawetan, Kepahiang municipality, Bengkulu Province, Indonesia at 900 m asl., 3º35'07"S and 102º25'37.4"E. Two experimental trials were set within the coffee farm of ±10-year-old. The experimental plots were inside of ±1.5 ha of an intentionally selected coffee farm. The coffee stands within the farm were relatively uniform, grown in 2 m × 2 m spacing and well-maintained from weed, but were not or rarely fertilized after the growth of coffee enters the reproduction phase. All coffee stands are grafted plants, forming a canopy umbrella at height of 1.5-1.75 m. The fruiting period occurs throughout the year, with main harvesting season falls within May-July. There are coffee berries in various growth phases in the field in the same plant throughout the year (Figure 1). The pickings of coffee berries outside the main harvest season are done in an irregular interval between 1-3 months. Coffee harvests are practiced for the mature (green-red) ones. Most coffee farmers in Bengkulu harvest coffee when a small portion of mature berries turn to yellow red; rarely do they wait until the majority of berries turn to red, mainly due to security reason, e.g. avoiding crime of harvesting by unresponsible.

The culture of *B. bassiana* isolates used in field experiments, isolation, and observation of the beetle number and fungus infection on berry samples were done in Biological Control Laboratory, University of Bengkulu, Indonesia

Figure 1. Coffee berries with various age (berry development phase). a) Mature berries (red and yellow); b) young berries (green and smaller size); c) young berries (early development phase) and d) dried flower (fertilization has occurred)

Beauveria bassiana Culture

Pure culture local isolates of *B. bassiana* was established from collections previously isolated from the sweet potato beetle, *Cylas formicarius* (Fabricius), and from CBB that have been maintained in the Biological Control Laboratory, University of Bengkulu, Indonesia. The fungi isolates were grown on corn-based media and kept in 2 kg plastic bags; in-room condition maintained at $\pm$27 ^{o}C. Spores were harvested for field trial 3 weeks after inoculation. The viability of conidia before used in the study was above 80%.

The local isolate of *B. bassiana* from *C. formicarius* had been tested and the result was more effective than those isolated from *Nezara viridula* (L.) and from soil, and *Metarhizium anisopliae* isolated from soil (Apriyanto & Nadrawati 2019). Local isolate from *H. hampei* was obtained from around research site, but have not been tested in the laboratory. The later isolate was also culture in the procedure on corn-based media, similar to that of Apriyanto & Nadrawati (2019). For simplification, in this research, local isolates were coded *Cf-Bb* and *Hh-Bb*, respectively.

Field Efficacy

Two field trials were performed in two coffee farms located adjacent to each other, only separated by the agricultural road (production road), but with different experimental units and replications. The first trial was conducted from August - October 2018, whereas the second trial was from November 2018 - April 2019. The two coffee farms are part of a much larger coffee ecosystem own by many farmers in the location and all are grown under the shade of mixed of different trees species but are dominated by *Gliricidia sepium*.

Conidia (spore) suspension prepared at a concentration of 1×10^9 ml^{-1} was used in both trials. The suspension was added with 0.5% Tween 80 as a surfactant. Using backpack sprayer was not practical due to overlapping coffee canopy of adjacent trees that complicated application of spore spraying. For the sake of convenience, spraying of suspension was carried out using a hand sprayer (volume 500 ml). The suspension of conidia was sprayed upon all berry clusters of experimental plants.

The treatments (two *B. bassiana* isolates and control) were arranged in a randomized complete block design (RCBD). All treatments, including control, were replicated five and 10 times in the first and second trials, respectively, using four plants in the first trial and one plant in the second trial, as the experimental units (plots). The use of block is only intuitively may be better than otherwise if we used the one without blocking. We did not or it was impossible to detect any different plant growth or environment condition that would affect the CBB behavior so that would be resulted in blocking the experimental plots.

In the first trial, there were total of 15 experimental units (plots), each plot consisted of four plants, the treatments were replicated five times. There were total of 10 main rows of coffee trees. Each block was a two rows of coffee trees across 10 main rows. Thus, each plot consisted of four plants adjacent to each other within two rows and main rows. The treatments (*Cf-Bb*, *Hh-Bb* and control) were arranged randomly assigned in each block.

In the second trial, we used three rows of coffee trees within five that appeared similar in appearance, 10 tree per row. The treatments were arranged randomly in each of selected plant across rows. Thus, there were 10 blocks, each of three plants. The three treatments were randomly assigned within the three coffee trees in each block.

The field application (spraying) of spores was carried out at 07.00-08.00, three times in the first trial (5$^{th.}$ September, 2$^{nd.}$ October and 23$^{rd.}$ October 2018) and four times in the second (6$^{th.}$ November 2018; 30$^{th.}$ November 2018; 8$^{th.}$ January 2019 and 6 February 2019). The application of entomopathogen was done before 08.00 am to avoid too much UV.

Data Collection to Measure the Efficacy of *Beauveria bassiana*

The efficacy of CBB control was determined from several observed variables: 1) number and percentage of damaged (drilled) berries (infestation rate of CBB), 2) the number of un-attacked berries (free from CBB damage), 3) number of CBB found in the samples of attacked berries and 4) the percentage of died CBBs infected by *B. bassiana* in attacked berries. Determination of the level of infestation of CBB was performed by observing four berry clusters taken from different branches on each treated coffee trees following Pulakkatu-Thodi et al. (2017).

The observation of CBB incidence was done once every two weeks, but some observations were delayed to the third week due to technical constraints, especially rain. The observation of the attacked and healthy berries was done directly in the field by counting the number of infested and un-infested ones. The presence of CBBs inside of infested berries was observed from 20 attacked berries per experimental unit, from 2 branches/twigs of opposite directions. All samples were opened one by one and the existing CBBs of all stadia were counted.

Observations of *Beauveria bassiana* Incidence

Observation of *B. bassiana* infection in experimental plots was performed by taking samples of damaged (attacked) berries. Samples were taken haphazardly from each experimental unit (plot). Samples from each plot were separated between ones that showed growth of mycelial and ones that did not. Samples that did not show mycelial growth were washed with 5% formalin solution and rinsed with sterile water before being incubated for five days to allow mycelial growth of the *B. bassiana* if any. Samples were spread in Petri dishes (of 19 cm diameter) and covered with wrapping plastic. Micro-openings were made with a needle to avoid water condensation inside the Petri dishes. The occurrence of *B. bassiana* infected CBBs was confirmed from the growth of white mycelial covering the CBB body. After five days, all samples were opened and the beetle's presence was examined and recorded, both the living and the dead ones. The dead beetles were preserved in the petri dish until the growth of fungus covering the dead body was clear indicating *B. bassiana*.

Data Analysis

All data obtained (number and % of un-attacked and attacked berries and number of CBB inside attacked berries, number and percentage of *B. bassiana* infected and healthy CBB) were analyzed by analysis of variance (ANOVA) with Sx.8 (Analytical Software; Tallahassee Florida) to see if *B. bassiana* isolates have effects on the dependent (observed) variables. If so, the treatment means were separated with least significant difference (LSD).

RESULTS AND DISCUSSION

CBB Incidence

CBB infestation in the experimental plots are depicted in (Figure 2-4); the levels were medium to high, both on the *B. bassiana* treated and control plants. The number of attacked berries before *B. bassiana* application did not change much until the second application. However, the number and percentage of the attacked berries on plants treated with *Hh-Bb* (*B. bassiana* isolated from *H. hampei*) continued to decline until the end of the experiment in both trials. Whereas, those on *Cf-Bb* treated and control plants did not showed any reduction.

In the first trial, CBB infestation (number and percentage of infested) on mature berries was relatively stable, but it tended to decrease on *Hh-Bb* treated plants after the third application (Figure 2). Mean reduction in the percentages of attacked berries was 22.24%, ranging from 0.56% ($25^{th.}$ September 2018 observation) to 33.93% ($16^{th.}$ October 2018 observation).

Statistical analysis subjected to $16^{th.}$ October data showed highly significant different between treatments (F=10.37; df=2; P=0.006). The mean number of damaged berries was significantly lower on *Hh-Bb* treated plants than those on *Cf-Bb* treated and control plants. While damaged berries on *Cf-Bb* did not differ significantly with those on control. These results indicate that *B. bassiana* isolated from *H. hampei* was more infective than that isolated from *C. formicarius*.

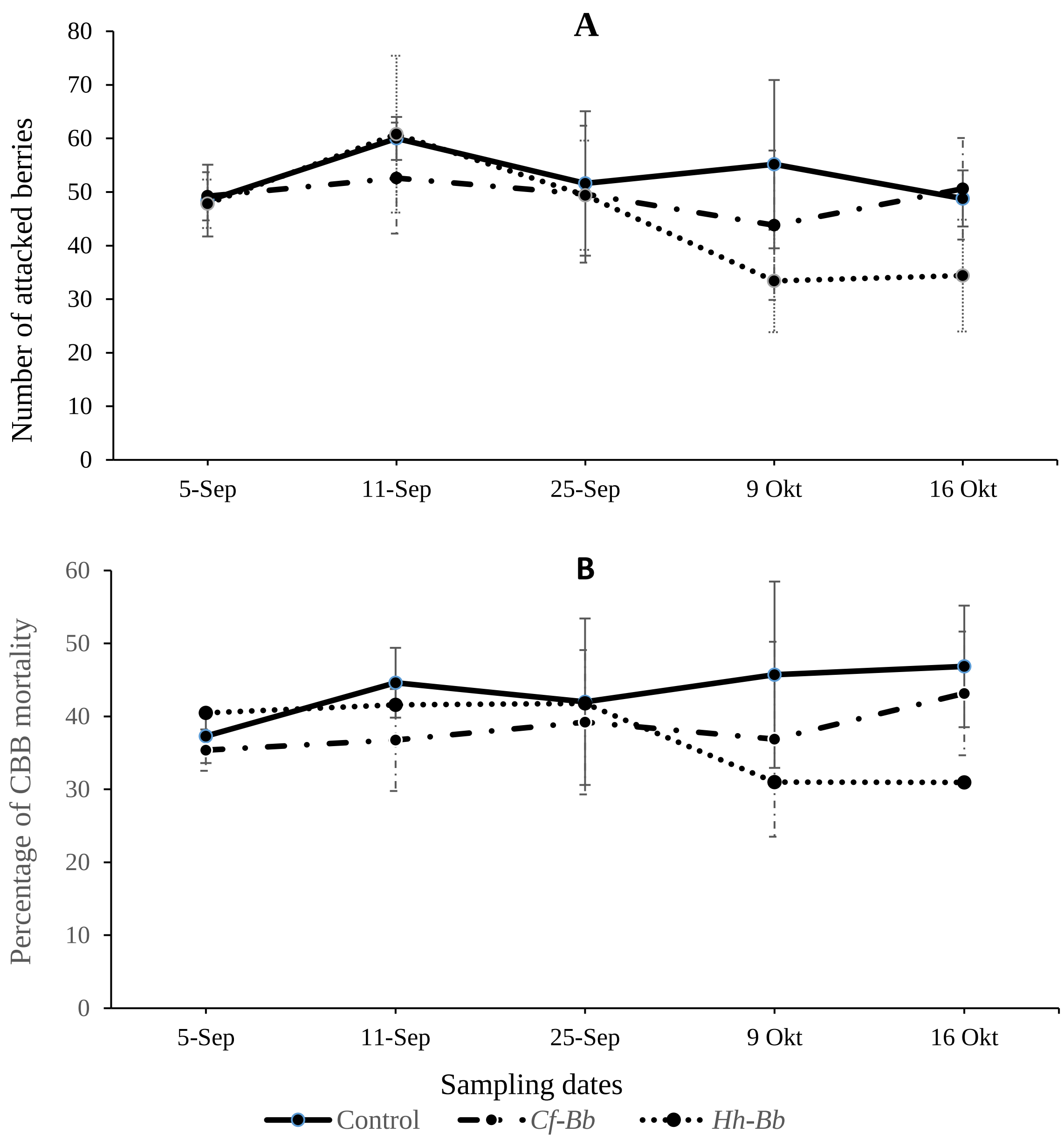

Figure 2. Fluctuation of number (A) and percentage (B) young coffee berries attacked by CBB before and after *Beauveria bassiana* application - first trial

Similar pattern of CBB incidence was apparent in the second trial. Control plants which showed the lowest CBB infestation at the first four observations, increased and it occurred after the *B. bassiana* treatment plots were applied with the fungus for the second time. In contrast, the number and percentage of mature berries of plants with *Hh-Bb*, but not with *Cf-Bb*, treatment was declined after the third application until the last observation (Figure 3). Statistically, they differ significantly only at the observations on March 6 (F=5.37; P=0.0149) for the number of attacked berries and highly significant (F=15.06; P<0.001) for the percentage of attacked berries.

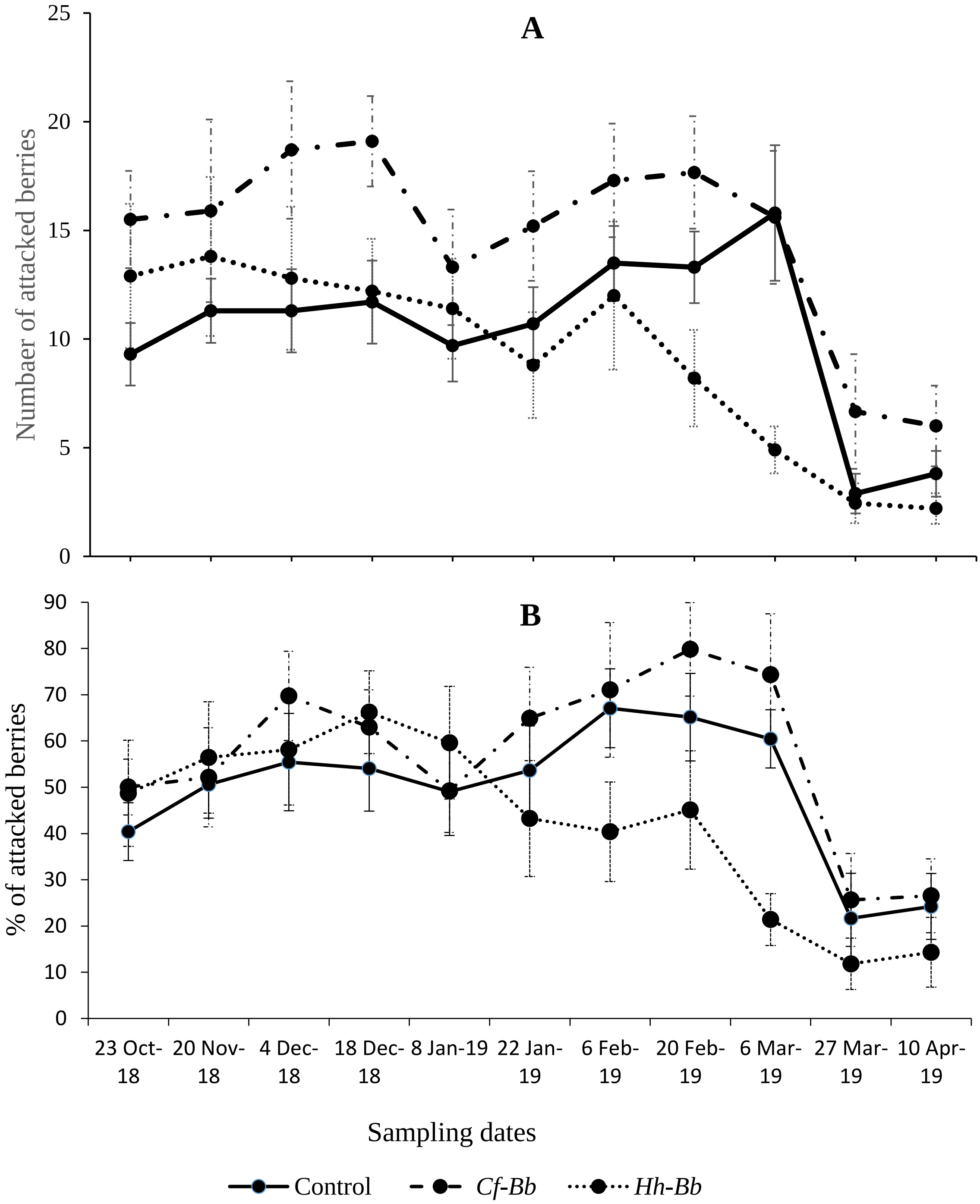

Figure 3. Fluctuation of number (A) and percentage (B) mature coffee berries attacked by CBB before and after *B. bassiana* application - the second trial

Reduction in the percentage of damaged berry from CBB infestation in plant applied with *Hh-Bb* ranged from 0.7% (10$^{th.}$ April 2019) observation to 69.03% (24$^{th.}$ April 2019) with mean of 38.55 from 7 observation within which the infestation level was lower than those of control.

A sharp decline in number and percentage of CBB incidence of the last three sampling dates that occurred in all treatments, including control, was due to harvesting of mature berries so that observation was done only on scarce mature fruits. This declining pattern of CBB infestation at harvest was also reported by Aristizábal et al. (2017). The *Cf-Bb* isolate was reported to cause the highest mortality than other *B. bassiana* and *M. anisopliae* isolates in laboratory assay (Apriyanto & Nadrawati 2019), whereas *Hh-Bb* has not gone through laboratory assay.

Observation on the sample of young berries demonstrated that the CBB incidence was lower in plants treated with *B. bassiana* for both isolates than in control plants (Figure 4). The differences of the number attacked berries between means of *B. bassiana* treated and control plants were significant, except for the third observation (F=5.27, P=0.0346; F=4.73, P=0.0441; F=5.17, P=0.0362, for first, second, and third observation, respectively). The means of percentage of attacked berries were different between treatments, except in the third observation (F=7.35, P=0.0154; F=7.04, P=0.0173; F= 3.19, P=0.0956, for first, second and third observation, respectively).

Reduction in percentage of attacked berries ranged from 31.48% ($23^{rd.}$ October 2018) to 60.65% ($6^{th.}$ November 2018 observation), averaged 52.85% in plant applied with *Hh-Bb* and from 45.11 to 58.62%, averaged 50.31% in plant applied with *Cf-Bb*. The probability that CBB females are exposed to *B. bassiana* spores applied in the field would be higher for those attacking younger berries, mainly due to CBB position that still outside of the developing seed (endosperm) (Hollingsworth et al. 2020). The higher efficacy of *B. bassiana* on young coffee berries was also reported in other studies in relation to application on the early fruiting season to target green berries in which the position of dispersing female CBBs infesting berries are still outside the endosperm, and therefore it is still more reachable by the *B. bassiana* spore (Hollingsworth et al. 2020).

The advantages of targeting young berries are that females of CBB attacking young berries do not lay eggs (Hollingsworth et al. 2020). Dispersing female CBBs to new un-attacked berries would be most likely exposed to the fungus (Pereira et al. 2012). CBB attack on the young berries is not followed with the behavior of female beetles to settle and lay eggs. Nevertheless, they still cause damage to the berries. Young coffee berries attacked by CBB usually turned yellow and fallen off prematurely.

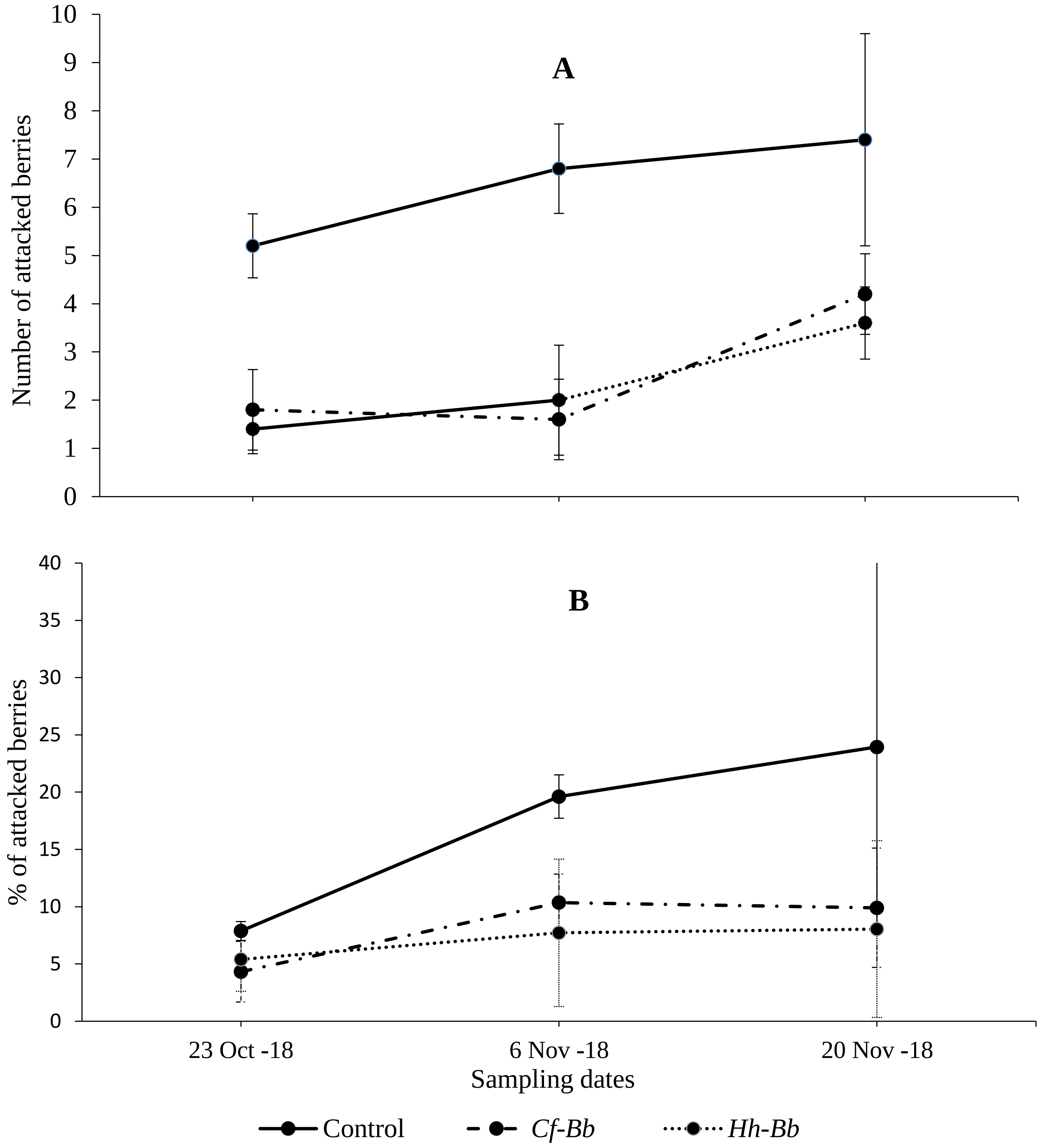

Figure 4. Mean number (A) and percentage (B) of young berry (±20% dried weight) attacked by CBB after application of *B. bassiana* - first trial

CBB is more vulnerable to *B. bassiana* infection when the position of female is still outside the endosperm because they are still close to the surface and therefore spores of pathogenic fungus such as *B. bassiana* can get into contact with the insect.

CBB Mortality Due to *Beauveria bassiana* Infection

Infected CBBs by *B. bassiana* was apparent from mycelial growth covering the body of the dead beetles from 1-7 days of incubation in the laboratory (Figure 5). The position of infesting beetle whether it is still outside or inside the endosperm was mentioned by Aristizábal et al.

(2017), see also Hollingsworth et al. (2020). Mean percentage of dead CBBs infected by *B. bassiana* was lower on the attacked berries sampled from the control plants compared to those taken from treated plants with either *B. bassiana* isolates (Figure 6 and 7).

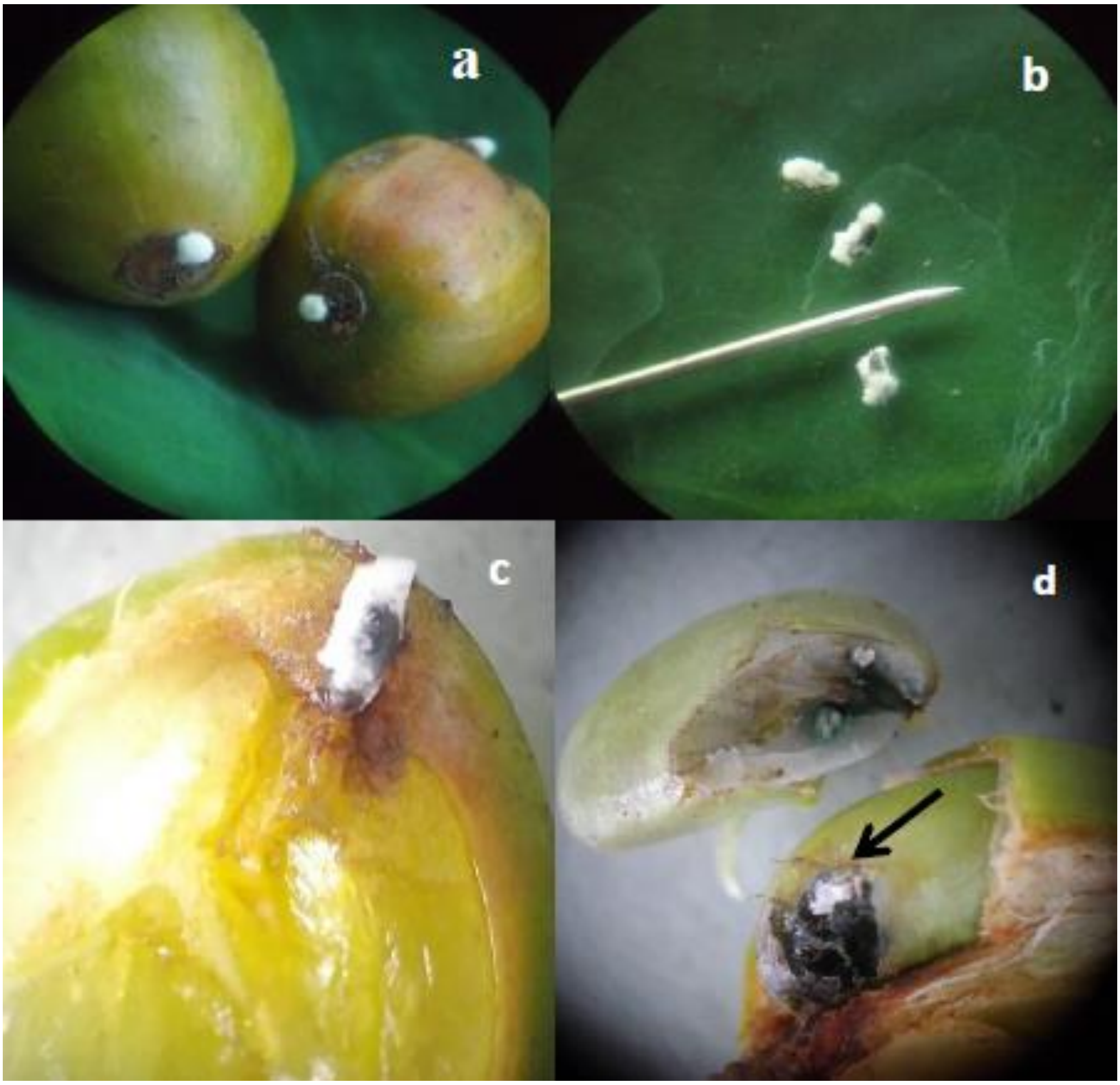

Figure 5. *Beauveria bassiana* infected CBB which was collected from infested berries of treated trees with the fungus: a) mature coffee berries with colony of *B. bassiana* on adult beetle, CBB has not entered the endorsperm (seed), b) *B. bassiana* colony grows completely covering CBB, c) female CBB is covered by *B. bassiana* outside the seed and d) *B. bassiana* infected female, CBB at the position inside the endorsperm (seed)

In the first trial, the means of percentage of infected CBB differed significantly (F=6; P=0.0256) between treatments, in the first sampling date (18$^{th.}$ September 2018) and highly significant (F=11.61; P=0.0043), in the second sampling dates (25$^{th.}$ September 2018). However, the means of infected CBB were low (Figure 6). Female CBBs made shallow drilling and remained outside the endosperm without laying eggs on young berries so that the probability that the beetle makes contact with the spore was greater than those on more mature berries, in which female CBB drill further into endosperm to lay eggs (Hollingsworth et al. 2020).

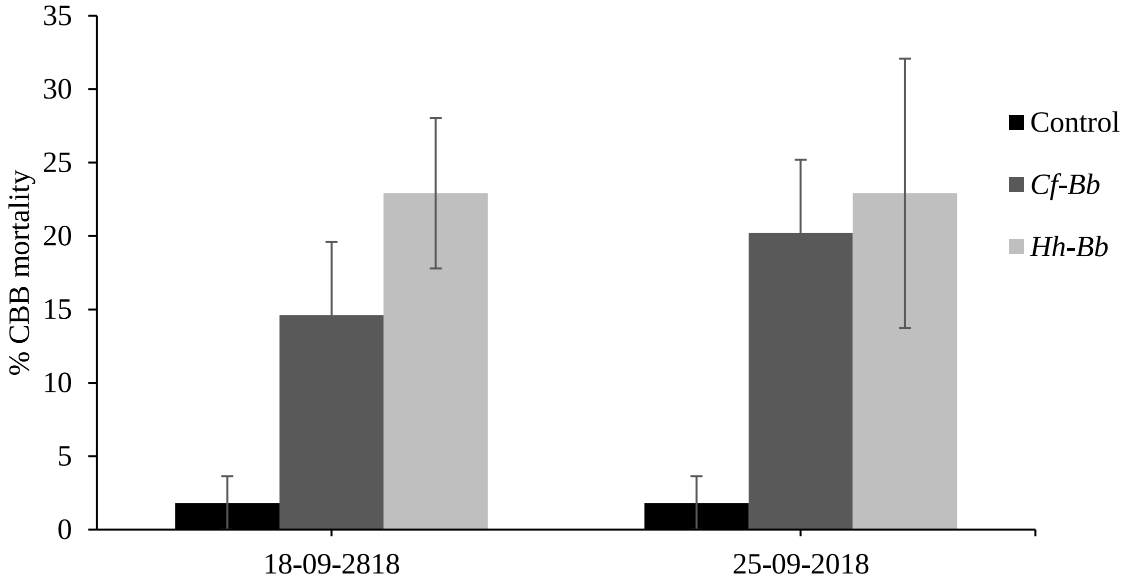

Figure 6. Mean percentage of CBB mortality due to *Beauveria bassiana* infection in trial 1, two and three weeks after the third application of spore suspension; samples were 20 mature attacked berries per experimental units of the first trial, taken haphazardly

In the second trial, where observations were done at more sampling dates, the data were more fluctuating, but in general tend to decline with the time, seems in accordance with the data of attacked berries by the CBB (Figure 7). Significant different between treatment means of percentage infected CBB occurred in the first sampling date (January 8) (F=4.80; P=0.0213) and third sampling date (February 6) (F=3.778; P=0.0425). Sampling from green younger berries of the second trial on January 8 resulted in significantly higher CBB mortality on treated plants than on untreated control (F=3.99; P=0.0367) with the treatment means of 11.17, 27.00, and 38.83% for control, *Cf-Bb*, and *Hh-Bb*, respectively. *Beauveria bassiana* infection on CBB that occurs in the control plant may be due to natural infection. We observed it occurred in a coffee farm far enough from the experiment plots that have never been treated with the fungus. The natural infection of entomopathogen, including one with cryptic life stages, such as CBB, has been frequently reported (Feng et al. 1994, Monzón et al. 2008, Wraight et al. 2018), which vary between different locations, time and altitude (Monzón et al. 2008), even though there is always an exception (i.e. some strains are more effective when applied to host species of non-origin of the isolates).

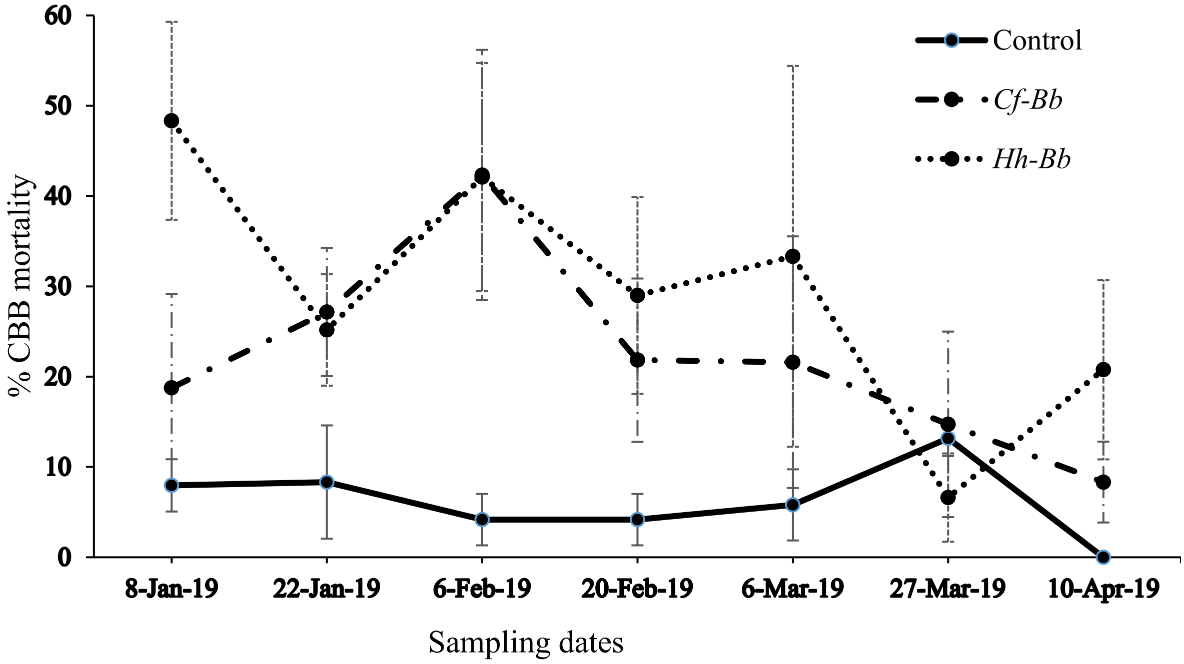

Figure 7. Mean percentage CBB mortality due to *Beauveria bassiana* infection in trial 2, samplings were initiated two weeks after the third application of spore suspension; samples were 20 mature attacked berries per experimental units of the first trial, taken haphazardly

The fact that *Bb-Hh* performed better in infecting and causing mortality to the host, maybe because of a longer time of natural parasite-host interaction (i.e. coevolution), so that it is more adapted to the host. A somewhat similar result was reported by Zayed (2003), where *B. bassiana* strain isolated from soil by trapping with greater wax moth (*Galleria mellonella* L.) was more infectious to the same insect than the one isolated directly from the soil. The variability of *B. bassiana* in germination and pathogenicity to CBB from different isolates initiated from single spore (Posada & Vega 2005).

Our results indicated of weak effects of *B. bassiana* treatments on CBB infestation in the field. Similar results were reported from a study in Ecuador, where field application of the *B. bassiana* failed to show a significant difference of mean between treated and control, even though conidia viability test revealed satisfactory results (Damon 2000; de La Rosa et al. 2000). Low mortality of sprayed beetles with the aqueous formulation of the same fungus also was mentioned by Mota et al. (2017). Other researchers reported higher CBB incidence in *B. bassiana* treated plants (plots). For example, Escobar-Ramírez et al. (2019) reported reduction in attacked berry ranged from 70.1-92% with *B. bassiana* spore concentration of 1 x 10^6. Increased in CBB mortality was up to 80% compared to treatments without the fungi, when used 1x10^9 conidia concentration. Greco et al. (2018) observed high mortality (96.6%) from a commercial formulation of *B. bassiana* when applied in recommended doses (2337 ml/ha). de La Rosa (2000) stated that the *B. bassiana* infection on CBB of Ca. 49% was considered low.

Low efficacy of *B. bassiana* could be attributed to the combination of factors affecting mass-culture (fermentation), formulation, and application in field trials (including equipment and time of application), and environment conditions (de La Rosa et al. 2000). Direct exposure to sunlight may degrade *B. bassiana* spore altogether in one hour of exposure (Edgington et al. 2000).

It was possible that hand sprayer (volume 500 mL) used, may not be able to produce even distribution of conidia, due to inconsistency of pumping (by hand). CBB females coming out of the infected berries and/or started boring the coffee berries were not contaminated and therefore could not spread the applied spore *B. bassiana* isolates. Behle (2006) has recognized the weaknesses of applying entomopathogen fungus by spraying the spore to plant and expecting the target insect pest would be contaminated by the spore residue. In addition, in-field application, spores of *B. bassiana* would rapidly loss viability. *Hyphothenemus hampei* as the target insect does not feed on the surface of the plant part, making it much less effective in causing mortality on cryptic insect such as *H. hampei*.

Jaronski (2010) listed some ecological factors affecting field application of entomopathogen fungi, such as sunlight (UV), rain, temperature, humidity, leaf surface chemistry, and phylloplane microbiota. UV light had been studied to reduce the life and effectiveness of *B. bassiana* and other fungi applied in the field for biological control (Vega et al. 2015). Even though the application of the treatments in these field trials was ended before 8.00 am, which was still in the best time window for entomopathogen fungus application between 6.00 - 8.00 am (de La Rosa et al. 2000), it was still a lot of time that the prayed *B. bassiana* was exposed to UV light as the time went on. We applied *B. bassiana* with water and surfactant, without adding material that otherwise would provide spore protection. Some studies had indicated that application of fungus in the certain formulation by incorporating other materials such oil (Florez 1998; Inglish et al. 1995), or humic acid (Kaiser et al. 2018), could reduce spore degradation by UV and prolong the half life span of *B. bassiana* spore. Using different formulation and by adding Arabic gum as an emulsifier, de Souza et al. (2020) obtained result average of < 40% beetle mortality, which is considered as less effective and the reason was that the Arabic gum could not protect the spore from degradation by UV. Mota et al. (2017) introduced an autoinoculation method of *B. bassiana* by attracting the beetle to the traps that contain conidia of *B. bassiana*. Their trap design allowed the conidia contaminate the beetles when they escape/disperse to find coffee berry for egg-laying, thus facilitating spore delivery directly to target, reducing spore exposure to sunlight (UV).

Although in many coffee producing countries, commercial mycoinsecticide from *B. bassiana* with effective formulations for pest control are available in the market (Mascarin & Jaronski 2016), there is still lacking in many coffee producing areas in Indonesia, and as such, evaluating the effectiveness of local isolates is still needed. Furthermore, mass production of entomopathogen will always need the addition of field population (isolates) to keep quality stable, regardless of the availability of formulations that could stand better in field condition. Specifically, for the situation in many localities in Indonesia, a commercial formulation of the *B. bassiana* would not be appealing to coffee farmers at low prices local coffee. The price of coffee in Bengkulu is at Rp. 18, 000, 00 or lower. With current productivity of less than 1 ton per ha per year, it will not encourage farmers to spend expenditure to purchase insecticide (bioinsecticide) and exercise pest control. Increasing farmer's tolerance upon pest infestation will further decrease coffee production.

In conjunction with IPM, low efficacy of *Beauveria bassiana* might be still important as it is not the only tool which farmers depend upon. *B. bassiana* alone would not solve CBB problem unless the other technics such as cultural control and harvesting are incorporated into IPM program consistently (Laila et al. (2011). Beetle trapping with ethanol - methanol mixture currently has been introduced as part of CBB IPM (Aristizábal et al. 2017; Wiryadiputra et al. 2008). We found some reduction in the number and percentage of attacked coffee berries, especially in fruit clusters treated with *Hh-Bb* isolate, as compared to controls. The reduction was not as sharp as those reported by some researchers. The highest reduction in the percentage of attacked berry was 33.93% and 76.47% for mature berry and young berries, respectively in the first trial, and 69.03% of mature berries in the second trial. The highest CBB mortality was 48.33%.

Low efficacy in our study might be as a result of lack of spore protection form UV and/or other environmental factors rather than due to spore quality. Our study, however, provides some insight that *B. bassiana* isolated from *H. hampei* performed better than that isolated from *C. formicarius* in both field trials. Therefore, it will be further studied in the future, for example by improving methods of mass-culture and formulating the spore by adding other material for UV light protection to improve its performance (effectiveness) in killing CBB. At least until now, there is no other biological agent that demonstrates more cost-effective, effective and ecologically safe than that has been proved as with *B. bassiana*. As such, endeavor to find and improve local isolate of this fungus is worth to pursue. Cruz et al. (2005) for example, found that better result of CBB control was obtained by blending of different *B. bassiana* strains, and it was surprising that the best result was not the mixture of the best strains.

CONCLUSION

In general, the application of *B. bassiana* isolates can reduce the incidence of CBB attacked on coffee berry. However, the effectiveness of the local isolates was low and inconsistent without addition of material for spore protection from degradation by UV or low moisture, and therefore the effect to CBB was weak. *Beauveria bassiana* isolated from CBB was slightly better in causing CBB mortality and reducing coffee berry damage.

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