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The role of entomopathogenic fungi in controling the coffee berry borer (*Hypothenemus hampei* Ferrari) at various altitudes of Arabica coffee plantations

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ABSTRACT

A study was conducted to evaluate the effect of altitude on the presence and effectiveness of entomopathogenic fungi in suppressing the development of the coffee berry borer (CBB) (*Hypothenemus hampei* Ferr.) in Arabica coffee plantations in Aceh Tengah District, Aceh Province, Indonesia. We found that only the fungus of the genus *Beuveria* infects CBB pests in the coffee plantations. We also found that the infection rate of this fungus against this pests in coffee fields located at an altitude of 900-1,100 m was higher than in coffee fields located at an altitude above 1,100 m. At first observation showed that the attack rate of entomopathogenic fungi against CBB at an altitude of 900-1,100 m; 1,100-1,300 and 1,300-1,500 m were 6%, 3% and 1%, respectively. In the second observation, the attack rate at an altitude of 900-1,100 m, 1,100-1,300 m and 1,300-1,500 m, were 8%, 2% and 1%, respectively. This indicates that the higher the temperature around the coffee plantation, the higher the infection rate (attack) of the *Beuveria* fungus on CBB. The correlation analysis also showed that an increase in CBB attack on coffee berries was generally followed by an increase in entomopathogenic fungi attack. This indicates that the *Beuveria* sp fungus has played a role in suppressing and balancing the development of CBB pests in Arabica coffee plantations in Aceh Tengah District.

Key words: Arabica coffee plantation; CBB pest; altitude; *Beuveria* sp; natural enemies; attack rate.

1 INTRODUCTION

One of the largest centers for Arabica coffee production in Indonesia is Aceh Tengah District, Aceh Province, which is part of the Gayo Highlands region. The natural conditions of this area are hills and mountains that stretch from an altitude of 600 m to 1,700 m above sea level. This area has been a growing area for Arabica coffee since the Dutch colonial era. The area for growing Arabica coffee is increasing from year to year, in connection with the increasing world market demand for Gayo Arabica coffee. Until now, it is estimated that the area of coffee cultivation in this region has reached 49,030 hectares, Central Aceh District Plantation and Forestry Service (2015). Initially the Arabica coffee growing area in this region was only at an altitude of 1,100-1300 m, but recently Arabica coffee has been planted from an altitude of 600 to 1600 m.

The expansion of the planting area will certainly increase the production of coffee beans and the welfare of the community, but on the other hand, due to the expansion of the planting area, it will also have an impact on the development of pests and diseases of coffee plants, especially in areas below an altitude of 1000 m. Generally, these plant-disturbing organisms will thrive in areas with warm temperatures and high humidity.

At this time the pest that is considered very disturbing by coffee farmers in Aceh Tengah District is the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). The presence of this pest has caused a decline in the production and quality of coffee beans in Aceh Tengah District. According to information from the Agriculture Service of Aceh Tengah District, as well as the recognition of coffee farmers, this CBB pest has in the last 10 years even attacked coffee beans in plantations located at an altitude of 1500 m. This may occur due to global warming, so that it has an impact on the expansion of the ideal area for the development of this CBB pest. As a result of an increase in temperature around the world, the areas located in the highlands will also have an impact on increasing temperatures. Insect pests generally like warm temperatures for their growth. According to Silva, Costa and Bento (2014) this CBB pest is able to live at temperatures between 15-35 °C, but the optimum temperature for its development is between 22-33 °C.

Research on the effect of altitude on the level of CBB pest attack in Indonesia has been carried out in various places (Wiryadiputra, 2006; Syahnen; Asmar; Siaahan, 2010; Sitanggang, 2017). All the results of these studies indicate that the level of CBB pest attack is strongly influenced by the altitude of the coffee plantation, where the higher altitude, the lower CBB attack rate.

Besides abiotic factors, another thing that affects the development of pest populations is the presence of natural enemies. One of the natural enemies that play a role in suppressing the development of CBB pests is the entomopathogenic fungus group. In nature, CBB pests can be infected by various entomopathogenic fungi, particularly *Beauveria bassiana* (Ball,-Criv.) and *Metarhizium anisopliae* (Metsch.) Sorok. (Mnyone et al., 2012; De La Rosa et al., 2000; Johnson et al., 2020).

The role of entomopathogenic fungi in controlling CBB pests has been reported in several coffee producing countries, such as Colombia (Benavides et al., 2012, Brazil (Silva; Mascarin, 2013), Hawaii (Wraight et al., 2018; Hollingsworth et al., 2020). The effectiveness of CBB control is further increased when the application of the Beuveria bassiana fungus is combined in an integrated pest control program (Silva; Mascarin, 2013; Hollingsworth et al., 2020).

Although research on the effect of altitude on the level of CBB pests has been widely carried out in Indonesia, there are still very few studies that examine the presence of entomopathogenic fungi in coffee plantations located at various altitudes. Until now there is almost no information about the presence of entomopathogenic fungi that attack CBB in Arabica coffee plantations at various altitudes in Aceh Tengah District. Therefore, this study was conducted to explore the presence of various species of entomopathogenic fungi at various heights of coffee plantations, as well as to determine the magnitude of the role of these fungi in suppressing the development of CBB pests in Aceh Tengah District.

2 MATERIAL AND METHODS

2.1 Experimental area

This research was conducted in a smallholder coffee plantation in Aceh Tengah District, Aceh Province, Indonesia. The method used in this study is a survey method with a purposive sampling pattern. Sampling was carried out at several altitude ranges, namely 900-1,100 m, 1,100-1,300 m and 1,300-1,500 m. At each altitude, two research plots were determined. The area of each research plot is 1 ha.

2.2 Meteorological data

In this study, meteorological data were not monitored, but based on a search from the accuweather.com web.

2.3 Sampling technique

In each altitude range, 40 coffee plants were determined as sample trees, thus the total number of sample trees was 120 coffee plants which were chosen at random. Sampling of coffee cherries was carried out twice with an interval of one month (sampling 1 and sampling 2). From each sample tree, 100 coffees were taken randomly. The coffee cherries that were sampled were berries that had yellowish color (hardened endosperm).

2.4 CBB infestation

To calculate the attack rate of CBB, the coffee berries taken from the field are then sorted in the laboratory. Coffee berries that were attacked by CBB were characterized by the presence of drilled holes at the bottom of the coffee berries (discus) (Figure 1).

Figure 1: Hole drill made by female coffee berry borer on the discus of the coffee berry.

2.5 Entomopathogenic fungal attack rate on CBB

The attack rate of entomopathogenic fungi on CBB pests was calculated based on the presence or absence of entomopathogenic fungal mycelia in the CBB borehole. The presence of fungal mycelia covering the insect's body can be noticed in insects that die after becoming infected with entomopathogenic fungus. Each genus of entomopathogenic fungi has a unique mycelium shape and color, such as the white mycelia of the genus *Beuveria* (Permadi; Lubis; Sari, 2018; Bayu; Prayogo; Indiati, 2021; Qayyum et al., 2021), the green or greenish yellow mycelia of the genus *Metharizium* (Sanjaya; Ocampo; Caoili, 2013). The first signs of entomopathogenic fungus infection in insects include weakness and decreased feeding activity, which leads to eventual death (Pertiwi et al., 2016; Altinok; Altinok; Koca, 2019; Kova; Lackovi; Pernek, 2020). Insects that die from being afflicted with fungi become hardened like mummies (Pertiwi et al., 2016; Bayu; Prayogo; Indiati, 2021).

2.6 Identification of entomopathogenic fungi

Coffee berries that were perforated by CBB and there were fungal mycelia covering the holes (Figure 2) were isolated (Figure 3), to identify entomopathogenic fungal species that infect CBB pests that live in the coffee berries.

2.7 Data analysis

Data analysis was performed using analysis of variance (Anova). if there is a significant difference between treatments, then a further test is carried out using the Duncan Multiple Range Test (DMRT) at a level of 0.05. All data analysis was performed using Microsoft Excel 2010 program.

To determine the relationship between the level of CBB attack on coffee berries and the level of entomopathogenic fungi attack rate on CBB, correlation analysis was carried out. The correlation calculation was performed using a simple correlation analysis technique (Pearson) with the Microsoft Excel 2010 application program.

Figure 2: Coffee berry borer hoist holes on coffee cherries covered by fungus mycelium.

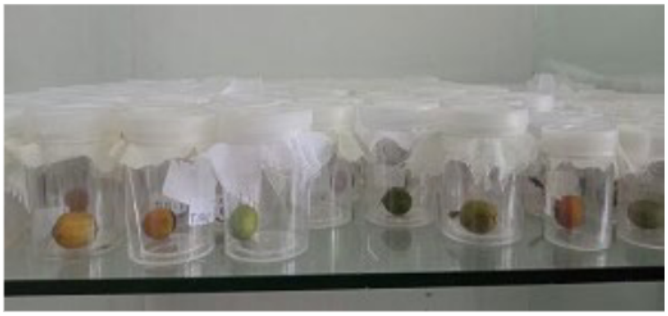

Figure 3: Coffee berries that were perforated by coffee berry borer and there were fungal mycelia covering the holes were isolated in a specimen bottle.

3 RESULTS

3.1 CBB attack rate

It was observed that in both coffee berries sampling the highest attack rate occurred in coffee plantations located at an altitude of 900-1100 m. In the first observation, it was seen that the attack rate of CBB at an altitude of 900-1,100 m, 1,100-1,300 m and 1,300-1,500 m were 33%, 27% and 15%, respectively. Meanwhile, in the second observation, the attack level also showed the same tendency. At altitudes of 900-1,100 m, 1,100-1,300 m and 1,300-1,500 m the attack rates are 34%, 24% and 15%, respectively (Figure 4).

3.2 Entomopathogenic fungus attack rate

Observations on the level of entomopathogenic fungus attack on CBB pests are also strongly influenced by altitude, where the higher the location of the coffee plantation the lower the *B. bassiana* fungus attack rate. Observation I showed that the attack rate of entomopathogenic fungi against CBB at an altitude of 900-1,100 m; 1,100-1,300 and 1,300-1,500 m were 6%, 3% and 1%, respectively. In the second observation, the level of fungal attack on CBB pests also showed the same trend, namely at an altitude of 900-1,100 m, 1,100-1,300 m and 1,300-1,500 m, were 8%, 2% and 1%, respectively (Figure 5).

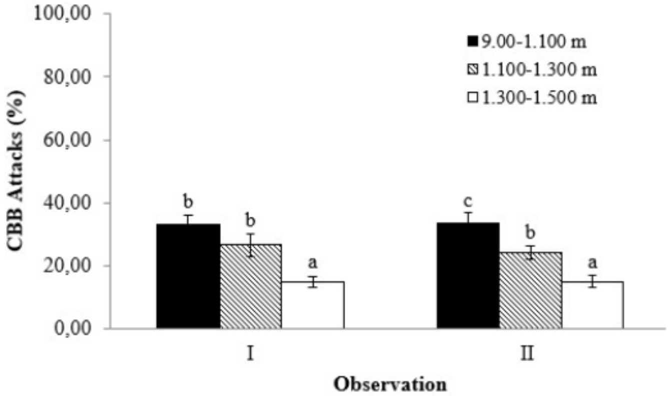

Figure 4: The attack rate of coffee berry borer at various altitudes of coffee plantations. The vertical line above the bars indicates the standard error. The same letters above the bars indicate not significantly different by DMRT. (P < 0.05).

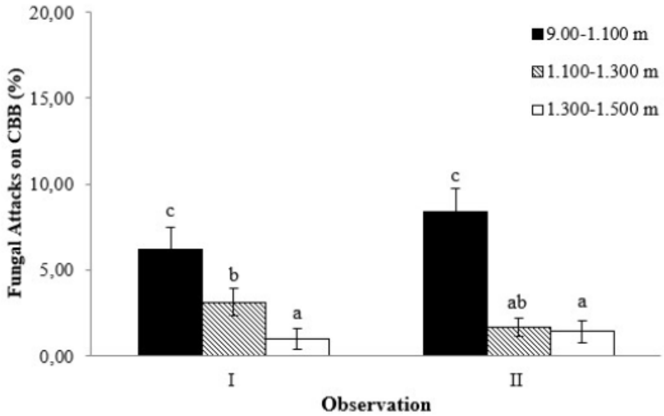

Figure 5: The attack rate of *Beuveria* fungus on coffee berry borer pests at various altitudes of coffee plantations. The vertical line above the bars indicates the standard error. The same letters above the bars indicate not significantly different by DMRT. (P < 0.05).

3.3 Identification of entomopathogenic fungi

Identification of entomopathogenic fungi found on coffee berries that were attacked by CBB was carried out based on macroscopic (Figure 6) and microscopic morphology (Figure7).

As for the color of the fungal mycelia that we found on coffee cherries attacked by CBB, all of them were white. The mycelia covered the borehole (Figure 8a). If the coffee cherries that are covered with fungal mycelia are split open, the coffee beans are found to be intact (Figure 8b). In the split coffee cherries, dead CBB was found and the entire body surface was covered with white fungal mycelia (Figure 8c). This indicates that the *Beauveria* fungus has played an important role in inhibiting the attack and development of CBB pests on Arabica coffee plantations in Aceh Tengah District.

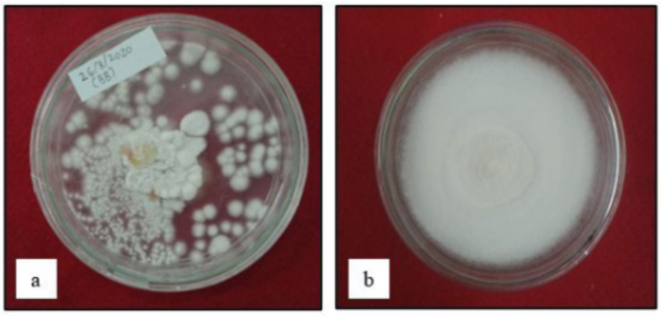

Figure 6: Macroscopic morphology of the fungus *Beauveria* sp. (a) age 7 days (b) age 14 day.

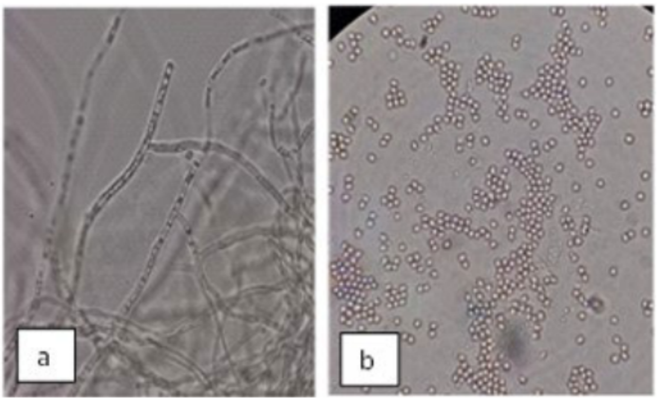

Figure 7: Microscopic structure of the fungus *Beauveria* sp. Magnification 100x (a) hyphae (b) conidia.

Figure 8: Coffee berry borer pests infected with *Beauveria* sp. (a) *Beauveria* sp mycelia covering the CBB hole, (b) intact coffee beans (c) CBB body covered with *Beauveria* sp mycelia.

3.4 Correlation between CBB and entomophatogenic fungus attack rate

Based on the results of correlation analysis showed that, there was a correlation (r2 = 0.924) between the level of CBB pest attack and entomopathogenic fungal infection at an altitude of 900-1,100 m (Figure 9). Likewise at an altitude of 1,100-1,300 and 1,300-1,500 m there was a correlation between the level of CBB pest attack and entomopathogenic fungal infection, namely: $r^2 = 0.928$ (Figure 10) and 0.764 (Figure 11).

4 DISCUSSION

The temperature range for each study location is as follows, according to a search on the accuweather.com website: The temperature ranges from 25 to 28 °C at a height of 900-1,100 m (Celala Sub District), 1,100-1,300 m (Silih Nara Sub District), and at an altitude of 900-1,100 m (Silih Nara Sub District) the range of temperature is 25-28 °C, and at an altitude of 1,300-1,500 m (Bies Sub District) the temperature range is 20-23 °C.

The conditions are thought to be extremely favorable for the establishment of this pest because the high attack of CBB occurs at an altitude of 900-1,100 m where the temperature is warmer. On the other hand, at an altitude of 1,300-1,500 m, the attack rate of CBB is always low because at that altitude the temperature is relatively cold, so it is not conducive to the development of CBB. Silva, Costa and Bento (2014) stated that the optimum temperature for the development of CBB pests is 22-33 °C. At a temperature of 15 °C or at a temperature of 35 °C, adult females often fail to pick up coffee cherries, although some are able to make holes in them but cannot lay eggs. Barerra (2008) also stated that the CBB life cycle is strongly influenced by temperature, where the lower the temperature, the longer the insect life cycle. At a temperature of 27 °C the life cycle of this insect is 21 days, at a temperature of 22 °C it is 32 days and at a temperature of 19 °C it is longer to 63 days. Temperature has also a significant impact on the rate of metabolic processes, feeding behaviors, and the growth of insects (Thomson; Macfadyen; Hoffmann, 2010; Wardani, 2018).

According to Geiger (1950), height and temperature are closely associated, at a lower altitude, the temperature will be higher, particularly during the day. From this study, it is clear that the level of pest attack decreases with increasing the altitude of coffee plantations. At an altitude of 900-1,100 m the attack rate of CBB ranges from 33-34%, while at an altitude of 1,100-1,300 m it ranges from 24-27%, while at an altitude of 1,300-1,500 m the attack rate of CBB is 15% (Figure 4). Several studies conducted in several other coffee-producing areas in Indonesia showed that the altitude of the coffee planting area also greatly affected the level of CBB pest attack. At altitudes below 1000 m, the CBB attack rate is generally very severe, while at altitudes above 1400 m the attack rate is still very low (Syahnen; Asmar; Siaahan, 2010; Sitanggang, 2017).

Besides various abiotic factors, the presence of natural enemies also affects the development of pest populations on agricultural land. The presence of diverse natural enemies is also influences lowering the CBB attack's intensity (Vega et al., 1999). One of the natural enemies that is assisting in slowing the expansion of CBB populations is entomopathogenic fungus.

Our identification of the fungal isolates from that area shows that only Beauveria sp. fungi have been found to infect CBB pests in Aceh Tengah District coffee farms. This fungus has macroscopic characteristics in the form of white colonies resembling cotton, a collection of mycelia like flour. According to Tantawizal, Inayati, and Prayogo (2015), Priyatno et al. (2016), Shanmugam and Seethapathy (2017), and Qayyum (2021), the *Beauveria* fungus is known for its white colony surfaces that resemble cotton, its white base color, and its equally distributed colony distribution pattern. This fungus is also known as white muscardine because of its white mycelia and conidia (Pu'u, 2009).

Based on morphological observations under a microscope, the *Beauveria* fungus shows characteristics such as hyphae of hyaline (clear), branched and insulated, while conidia are round and oval, the distribution pattern of conidia is clustered on conidiophores in the form of zig zag and clustered so that it is identified into the genus *Beauveria* sp. (Figure 8). This is in accordance with the opinion of Barnett (1960), Sari and Rosmaita (2020), Herlinda et al. (2020), that *Beauveria* has branched, insulated, hyaline and thick hyphae. Then the conidia are round or oval, one-celled, formed solitary at the tip of the conidiophores and attached to the short sterigma with alternating growth patterns (zig zag).

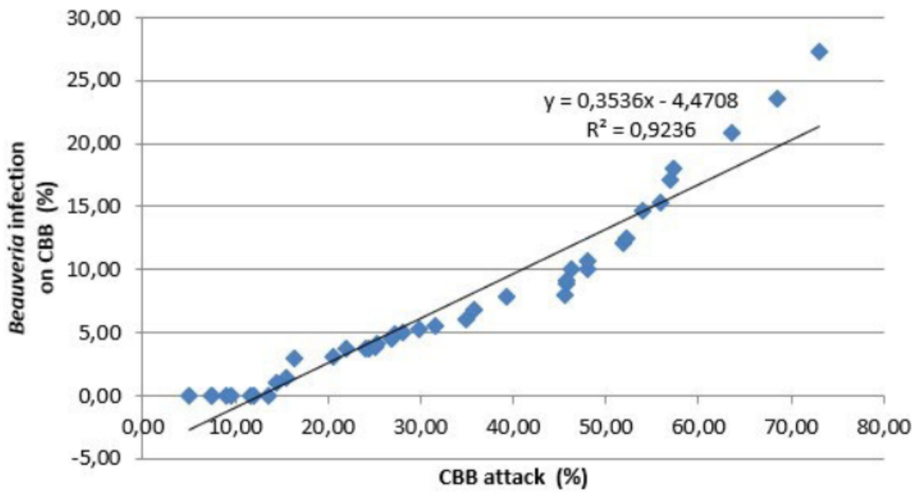

Figure 9: Correlation between coffee berry borer attack rate and *Beauveria* infection rate on CBB at an altitude of 900-1,100 m.

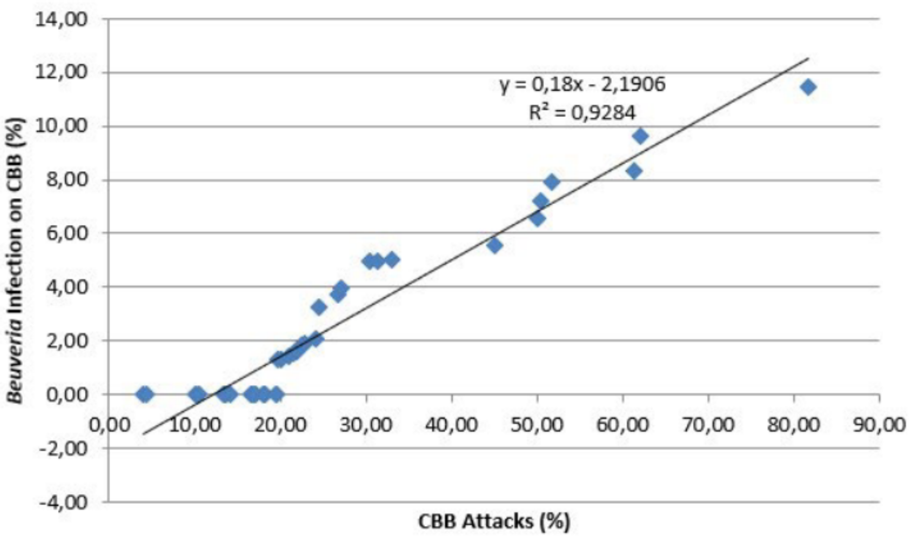

Figure 10: Correlation between coffee berry borer attacks rate on coffee berries and *Beauveria* infection rate on CBB at an altitude of 1,100-1,300 m.

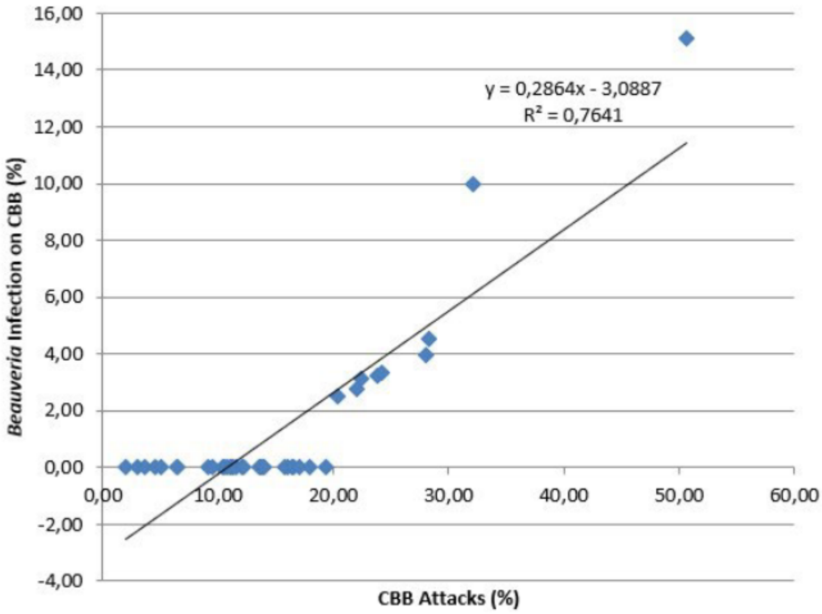

Figure 11: Correlation between coffee berry borer attacks rate on coffee berries and *Beauveria* infection rate on CBB at an altitude of 1,300-1,500 m.

The level of entomopathogenic fungi attack on CBB pests at various altitudes of coffee plantations is thought to be strongly influenced by the level of fungal conidia density in the plantations. The density of the conidia of the fungus is closely related to various abiotic factors, especially temperature and humidity. Fargues et al. (1997), James et al. (1998), Wakil, Ghazanfar and Yasin (2014) and Qayyum (2021) stated that the presence of entomopathogenic fungi in the field is strongly influenced by climatic conditions and environmental factors. In the Moroccan Argan Forests, Hallouti et al. (2020) looked at the entomopathogenic fungi connected to the Mediterranean fruit fy (*Ceratitis capitata*). They claimed that the origin, physical characteristics, and chemical composition of the soil were the main factors that significantly influenced the abundance of entomopathogen fungus. According to Wakil, Ghazanfar, and Yasin (2014), who investigated the distribution of entomopathogenic fungi in grains stored in Punjab, Pakistan, that the spread of entomopathogenic fungus has been affected by altitude, latitude, and longitude. More than 400 m above sea level is where the majority of entomopathogenic fungus isolates were discovered. Wakil, Ghazanfar, and Yasin (2014), however, did not adequately explain the suitable land altitude for the growth of entomopathogenic fungus. Another fact also shows that the distribution of the fungus *B. bassiana* is more commonly found in soil samples taken at an altitude of 400-1,000 m which is about 48% (James et al., 1998).

Entomopathogenic fungi have a wide variety of temperature requirements for growth. Temperature and humidity affect the survival rate of *B. bassiana*, the fastest germination and growth occurred at a temperature of 25-30 °C (Mwamburi; Laing; Miller, 2015) or 30-35 °C (Mishra; Kumar; Malik, 2015).

The correlation analysis (Figures 9, 10 and 11) shows that an increase in CBB attack on coffee berries is always followed by an increase in entomopathogenic fungi attack. The establishment of CBB populations and the expansion of the *Beuveria* fungus occur more favorably in coffee fields situated between 900 and 1100 m than on land above 1000 m. Temperatures at 900–1000 meters above sea level range from 28–32 °C. This temperature is suitable for the growth of CBB (Silva; Costa; Bento, 2014) and *Beuveria* fungus (Mwamburi; Laing; Miller, 2015; Mishra; Kumar; Malik, 2015).

5 CONCLUSION

In Arabica coffee plantations in Central Aceh Regency, entomopathogenic fungi of the genus Beuveria were found to infect CBB pests. Warmer temperatures in coffee plantations with lower elevations are regarded to be the primary contributor to higher CBB infections by the genus Beuveria. To determine the Beuveria species that are crucial in controlling the CBB population in the region, the fungus must be molecularly identified, so that the conservation strategy of this fungus in the field can be carried out more optimally.

6 AUTHORS CONTRIBUTION

HH contributed as coordinating the entire research activities and editing of draft scientific articles JJ contributed as responsible for the preparation of laboratory equipment, and data analysis NM contributed as responsible for collecting and sorting coffee berries.

7 ACKNOWLEDGMENTS

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