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RESEARCH ARTICLE

The Auto-infection Trap with the Native Entomopathogenic Fungus, *Beauveria Bassiana* for Management of Coffee Berry Borer (*Stephanoderes Hampei* Ferrari) in the Northwest Region of Vietnam

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ABSTRACT

Among important crops in Vietnam, we have to mention the coffee crop as the main cultivated crop in northwest area of the country, then we have to deal with the negative factor, or insect, coffee berry borer (CBB), which cause losses and damages for farmers with negatively significant impact. In previous studies, we isolated and identified five *Beauveria bassiana* strains of entomopathogenic fungus that damaged on coffee berry borer in local coffee fields of Son La province, Vietnam. The objective of this study continued to choose one of them for management CBB by autoinoculation trap. The result show that the virulence against CBB of the $Bb_5(MCB_1)$ strain was highest (71.3 % confirmed mortality). Although 62.5% of CBB females that exposed to the $Bb_5(MCB_1)$ fungus product with an average of 0,47 x 10^{12} conidia.gram^{-1} were able to penetrate the coffee berry exocarp, only 3.7% reached the endosperm. An autoinoculation trap containing the entomopathogenic fungus Bb was designed for the management of the CBB (trap TBU-AIT). Traps TBU-AIT baited with methanol and ethanol (1:1 ratio) mixtures at 868 mg day-1 attracted more insects than those traps baited at 452, 715 and 1050 mg day-1. We found a statistically-significant difference in average confirmed mortalities by fungus and by different field conditions in the period between 0 - 63 days setting traps TBU-AIT. Altogether, these results and relatively low production costs these auto-infection system can be recommended for integrated pest management on coffee auto-infection trap.

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Introduction

Coffee crop, just like any other agricultural crop, there is problem need to be resolved. As Damon (2000) stated that in many countries producing coffee, borer - CBB (coffee berry) originating from Africa and spreading now to be a vital coffee pest in many areas in the world.

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As Wilkinson (1929) found out in 1928, we recorded in details the first CBB infestation in Kenya, with the rate at low level 0.7-7.8%. Also Murphy et al (1987) the highest infestation around 80% was recorded at the season peak of coffee crop, so CBB became vital pest.

Waterhouse and Norris (1989) also mentioned that CBB attacked and has caused 96% losses in coffee crop in Africa.

And Oliveira (2013) specified that CBB also caused big losses over 300 million USD. Then, Jaramillo et al (2010) said

it is harder to control with chemical inputs or biological materials because berry borer live most of its time in coffee.

Villacorta (1984) pointed that in Brazil and more recent in State of Paraná, we recorded berrey borrer in coffee called Beauveria basiana, as the entomophathogenic fungus.

And Castrillo et al (1995) said that scientists find out the rate of survival of conidia under conditions of field is low when they use this fungus for controlling CBB biological, and applications of *B. bassiana* presented certain constrains to perform economically with proper management strategies.

The appearance of the coffee berry borer had been recorded in Son La, causing damage to the coffee bean and resulting in 10% coffee yield (Nguyen Duc Thuan et al.,2004). Methidathion has been highly effective to control this beetle (Vu Hong Trang, 2013) and it has been widely used in Son La, with some success, but is currently being phased out in Viet Nam due to it's high mammalian toxicity and environmental damage. The fungal pathogen *Beauveria bassiana*, as well as the fungus *Metarhizium anisopliae* have also been tried for coffee berry control with only low effect (Vu Hong Trang, 2013). Cultural control remains the main line of defense against this pest in coffee growing regions throughout the country. Traps have been used in many countries to monitor CBB, and in some cases, to manage these populations by "Mass-trapping" to reduce females lead to reduce next generations.

Mendonza (1991) figured out that CBB management can be done with Methanol and ethanol lured traps as they demonstrate the efficiency of the synergistic effects of this mixture in getting CBB. Silva et al (2006) showed study trying to improve beetle attraction with design of some type of traps. There are many factors such as conditions including: climate, spacing, shade, cultivar, plant age, wind direction, speed, etc that affect trapping efficiency. However, traps haven't been studied on the slope fields in Northwestern part of Vietnam. An IPM program should be involved in various practical indicators for pest control based on the circumstance in the regions. In the case of Son La, which is located in Northwestern Vietnam, the budget for the pest control was too tight due to a low crop price. Therefore, we tried to make much of a high practicality and a reasonability to practice with ease. Refinement in trap characteristics might contribute to reducing trap costs and improved competiveness.

In this paper we report the responses of CBB to an autoinoculation trap containing local native *B.bassiana* fungal, which were strained on sterilized rice grains, that was incorporated in the trap baited with ethanol and methanol. The *H. hampei* adult females are contaminated the fungus before they leave the trap. Once attracted and infected, females can be anticipated to return to their habitat, which speeds up dissemination of the fungus. Next, the field performance was tested and we also test the effects of shading conditions on the autoinoculation traps.

Materials and Method

Insect

The CBB adults were collected from a coffee farm in the Son La province. The beetles were fed using Fernando E. Vega (2011) artificial diet, with the following modifications: 21 g of Agar, 0.6 g of cholesterol, 0.23 g of sorbic acid, 0.5 g of Vanderzant vitamin mixture for insects, 1.2 g of Kali sorbate and 350 mg of streptomycin and 500 ml distilled water. Another difference is that the CBB was reared in sterile clear glass vials (25 mm o.d, by 58 cm height, an opening was pierced through the rubber cap attached using polypropylene Micropipette tip in 200 µl size). We conduct in the experiments in which beetle were transferred into sterilized glass since their appearance, vial using a fine paintbrush and covered with PVC film and were immediately used in the experiments.

Isolation of Beauveria Bassiana

The *Beauveria bassiana* were isolated from infected *Stephanoderes hampei* Ferrari and *Coccuss hesperidum* Linnaeus samples which were collected from coffee plantations in the Son La province. Fungi were grown on PDA media at 27^0 C. The B. *bassiana* isolates were identified based on phenotypic and molecular data.

Selection of Beauveria Bassiana Isolates

We tested five *B. bassiana* isolates which were collected from some coffee fields in the Son La province, three isolates from infected coffee berry borer *Stephanoderes hampei* Ferrari sampled, two isolates from infected soft brown scale *Coccuss hesperidum* Linnaeus sampled. In the study we put and raise the entomopathogenic fungi in environment with growth media, in time of 10 days to grow and for sporulation, containing 20 g Agar, 20 g Glucose, 10 g Peptone, 5 g Bacto TM yeast Extract, and 1000 mL distilled water and placed in an environmentally controlled chamber (27^0 C, 85% RH). The completely randomized design (CRD) with four replicates of 20 CBB adult females each (n = 80 per isolate) was conducted. The CBBs were placed to walk on petri dishes (9 cm diameter and 2 cm height) lined with sterile filter paper that was inoculated with 1 ml of a *B.bassiana* $1.1x10^7$ viable conidia.mL^{-1} suspension in Tween 80 (0.05%). The number of conidia in suspension was countered by a Neubauer chamber. The sterile filter paper placed on control petri dishes were sprayed with 1 ml 0.05% Tween 80. The treated insects were individualized in sterile clear glass vials with a piece 1 cm x 1 cm sterilized artificial diet after 24h and kept in an environmentally controlled chamber ($27\ ^0$C, 85% RH, and 24h dark). CBB mortality was assessed daily for 15 days. We in the test, taking dead insects and use 80% ethanol to sterilize the surface and next, we test and check the cause whether mortality by *B. bassiana by* immersing in sterile distilled water.

Cultivation of B. Bassiana on Cooked Rice

The isolated Bb_5MCB_1 was selected to be inoculated on the cooked rice (autoclaved for 30 minutes at 120 °C) using a two stage production system which include liquid culture and solid substrate (Krishan, 2013).

Stage 1, the entomophathogenic fungi were produced in liquid Media containing 20 g Glucose, 10 g Peptone, 5 g Bacto TM yeast Extract, and 1000 mL distilled water. Two pieces 0.5 cm diameter culture discs were released into a 500ml conical flask containing 300ml of liquid media. The flasks were plugged with non-absorbent cotton wood bungs and cover with aluminum. The inoculated flasks were placed on the shaker at 160 rpm, laboratory condition with temperature 27 ± 20 C for 5 days

Stage2, inoculation of rice with liquid Media was conducted. The fungi entomophathogen was cultured on rice using plastic bags (Francisco, 2008). Grains of rice were steeped in water for one hour before autoclaving at 121^0 C for 30 minutes. Each plastic bag contained 200g of cooked rice inoculated with 10ml *B. bassiana* inoculum grown in liquid media by syringe. Then we supply, equally, the inoculum over all the rice grains by massaging the bag from outside. We also left the phase of solid substrate of mass production to settle for 3 first days in an environmentally controlled chamber (27± 2 ^{0}C, 85 ± 10% RH, and 24 h dark). Shaking the solid substrate was conducted from outside the bag once a day from 4 days left. The conidiation process was complete and conidia have been formed over the whole surface of the solid substrate for about 15 days. To dry the cultures the plastic bags were opened in a laboratory condition and allowed to dry using FujiE Dehumidifier HM-616EB in 2 days. *Beauveria bassiana* products were vacuum packaging, low-temperature storage in fridge. Then we use product of the fermentation, the whole, for application directly into the autoinoculation trap.

The Capability of Infected S. Hampei with B. Bassiana to Enter Coffee Berries

We had carried out the experiment in the laboratory of plant protection, Tay Bac university after the Bb_5MCB_1 fungus products were made completely. The completely randomized design (CRD) with four replicates of 20 CBB adult females each and two treatments: CBBs exposed to the Bb_5MCB_1 fungus product with an average of 0,47 x 10^{12} conidia.gram^{-1} (simulating passage through the trap) and without the fungus in control (n=80 per treatment). The CBBs were placed individually in a flat bottomed glass tube (4.9cm x 2.5cm) containing three fresh coffee berries 120 days after bloom and covered with moistened hydrophilic cotton. They were kept in a temperature-controlled chamber (27± 2^0C, 85 ± 10% RH, and 12 h light: 12h dark).

Killed CBB (outside the berries) was collected and kept individually in a flat bottomed glass tube with moist cotton daily for 10 days to check appearance or symptom of *B. bassiana* infection. After 10 days, the coffee berries were dissected to check number of insect dead and the position of CBB adults (exocarp, mesocarp, endocarp or endosperm).

Design of TBU Auto-infection Trap

The TBU auto-infection trap (TBU-AIT) was designed to attract *Stephanoderes hampei* females, contaminate them with *B. bassiana* conidia, and disperse the entomophathogen to the environment after the beetles run away from the trap. The home made traps were formed with 1.5 litter old transparent plastic bottles (originally used as a soft drink container). Make a rectangular (4.0 x 10 cm) opening into the each side of container for *S. hampei* females entry. Heat a small piece of metal to make the hole easily on center of bottom of the bottle. Put a wire from the bottom of the bottle to suspend the lure at 15 cm above the funnel that top part of bottle was use to separate the upper and bottom compartments where insects are contaminated. A plastic jar (7.5 ϕ x 9.0 cm high), where *Beuveria bassiana* will be placed, is inserted into the bottom compartment of the trap. This part could be removable for change Bb product. One exit holes (1 cm ϕ) is opened on each side of plastic jar. The plastic cylindrical tube (9 cm ϕ x 15 cm high) is fixed around the plastic jar for reducing the effect of the rain on the entomopathogenic products.

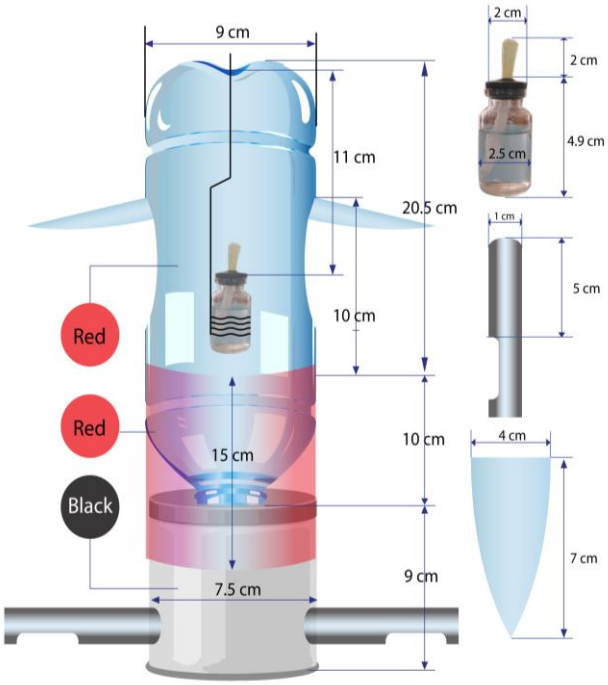

Fig. 1. Design of infection system

Evaluation Effect of the TBU-AIT Trap in the Field

Field trials were conducted on a mountainside located in in the Phong Lai village (21^0 34'41''N, 103^0 35'38''E), Thuan Chau district, Son La province. The climate is sub-tropical, with rainy season extending from April to October and a mean annual temperature of 21^0C. The Cartimor variety was cultivated on terraced lands (one coffee row per terace) with intensively managed high density - 1 ha coffee in the full sunlight system (monoculture) and 1 ha coffee intercropped with fruit trees (long, plum). The plants were 10 years old and about 1.7 m high.

- ***Response of CBB to Alcohol Release Rates***

The first experiment to determine the optimal release of Methanol: Ethanol mixtures (1:1) to attract *H.hampei* into traps. Lure was a 20 ml glass bottle with a vial rubber cover. The different mean release rates of methanol and ethanol (1:1 ratio) were 0, 452, 715, 868 and 1050 mg day-1 using different sizes of wick which was inserted through the vial rubber cover (0.5 x 8 mm; 1.0 x 8 mm; 1.5 x 8 mm; 3 x 8 mm respectively). In this case, approximately 150 ml water with 2ml liquid detergent was placed in the bottom part of the trap instead of the *B. bassiana*. 12 m within a block is distance between traps, and 15 m is between blocks. Plots were laid out in a randomized complete block design with three replicates of one release rates, and three traps per treatment. Water with liquid detergent and alcohol were replaced every week. We then remove water for counting with dead insects in our assessments, and the vial weighted to determinate mean volatile release rates. Traps were installed on November 28 and assessed the number of CBB on December 05, 12, 19 and 26, 2019 on 1 ha coffee in the full sunlight system.

- ***Percentage of CBB Infected by Bb from Auto-inoculation Trap in the Field***

In the second experiment, the percentage of the female coffee berry borer infected by *B. bassiana* were evaluated weekly using modified autoinoculation traps that two plastic exit tubes are inserted to exit holes on contamination jar. The randomized complete block design (RCB) experiment with two treatments (1. Modified autoinoculation trap with *Beauveria bassiana*; 2. Modified autoinoculation trap without *Beauveria bassiana*) and four replicates of three traps each was conducted in the in the coffee full sunlight system and coffee intercropped with fruit trees system. The trap were hung on branches of coffee tree at 1 m hight - three per plant line and 15 m away from one another. In this experiment, the number of the CBB's female in the modified autoinoculation trap was counted once a week. These insect were removed to plastic bag for evaluations were determined 7, 14, 21, 28, 35, 42, 49, 56, 63 days after setting the traps.

Air temperature and relative humidity were record at setting auto-infection place both below shade tree and in open condition by mini environmental quality meter equipment

We also, in our experiment, we use Petri dishes to place the CBB (80mm ϕ x 15 mm high) with some piece sterilized artificial diet. And then the confirmed mortality was determined following to methodology described above.

Data Analysis

The binomial model were used to analyze the mortality data from the virulence test of *B. Basssiana*. When significant differences were detected, One-way analysis of variance (ANOVA) was performed followed by Tukey test (P<0.01) to compared means (Tukey, 1949). Pearson's Chi-squared test was used for statistical independence between variables.

And then we express in percentage, showing the sum of the experiments replicated at four different time periods (n = 80) to recognize the infestation of coffee berries by *H. hampei* females contaminated by *B. bassiana* as well as the number of conidia produced.

Next we transform the number of *H. hampei* captured data into a logarithmic (x + 1) for normalization and we perform variance analysis, and we use the Tukey test to compare averages (P < 0.01).

Two-way analysis of variance (ANOVA) was performed followed by Tukey test (P<0.01) to find out if local native Bb and field condition (open and shade condition) have an effect on average number coffee berry borer capture and percentage of CBB infected by Bb from auto-inoculation trap in the field.

We use R software to carry out all analyses and the expresses all results as mean ± standard error.

Result

Selection of Beauveria Bassiana Isolates

All the 5 fungal isolates tested were pathogenic to adult females of CBB with mortalities mean of at least 38.8 ± 2.4 (isolate $Bb_1(RCB_1)$) by 15 days of the experiments. Mean beetle mortality in the control was 11.25 ± 1.25%. Mortality caused by *B. bassiana* was a significant difference from the respective control when a concentration of 1 x 10^7 conidia mL - 1 was applied (F = 62.54; P = 9.59e-11; x2 = 186.55) (Table 1). The highest mortality rates were observed on the isolate $Bb_5(MCB_1)$ (77.5 ± 3.2%). Furthermore, the confirmed mortality also differ between the treatments (F = 58.39; P = 1.71e-10; x2 = 287.09) and the fungal sporulation presented in the cadavers varied from 31.3 ± 2.4 to 71.3 ± 4.3%. The isolate $Bb_5(MCB_1)$ was showed that is also highest confirmed mortality. Based on these results, the fungal $Bb_5(MCB_1)$ isolate were selected for further studies.

Table 1. Results and data of coffee berry borer, Total and confirm mortality rate, caused by infection with *Beauveria bassiana* isolates using concentration of 1 x 10^7 conidia mL^{-1}

Isolates	Isolate host	Mean cumulative mortality rate 15 days (%)	Mean cumulative confirmed mortality rate after 15 days (%)
$Bb_1(RCB_1)$	*Coccuss hesperidum* Linnaeus	38.8 ± 2.4^b	31.3 ± 2.4^b
$Bb_2(RCC_1)$	*Coccuss hesperidum* Linnaeus	67.5 ± 4.3^a	61.3 ± 4.2^a
$Bb_3(MCC_1)$	*Stephanoderes hampei* Ferrari	51.3 ± 3.2^b	40.0 ± 3.5^b
$Bb_4(MCD_1)$	*Stephanoderes hampei* Ferrari	43.8 ± 2.4^b	33.8 ± 3.2^b
$Bb_5(MCB_1)$	*Stephanoderes hampei* Ferrari	77.5 ± 3.2^a	71.3± 4.3^a
Control	-	11.3 ± 1.3^c	0^c

Averages (± SE) followed by the same letter in the columns are not different by turkey's test (P < 0.05).

The Capability of Infected S. Hampei with B. Bassiana to Enter Coffee Berries

On average, 88.8% of the CBB females: able to successfully infest the coffee berries, that had not been exposed to the fungus, while only 9.9% of the beetles exposed to the fungus. Whereas 37.5% of the females exposed to the Bb died before infestation coffee berries, the other 57.6 % penetrated the exocarp but died reaching the endosperm (Table 2).

Table 2. Infestation of Coffee Berry Borer Female 10 Days after Exposed to *B. Bassiana*

Insect position	**CBBs exposed to the Bb (%)**	**CBBs not exposed to the Bb (%)**
Dead outside the berry	37.5	11.2
Dead outside the berry – the penetration of the exocarp begins	13.8	0
Dead partly inside the berry – Has not yet reached the endosperm	43.8	0
Alive – produced a gallery in mesocarp	6.2	8.8
Alive – produced a gallery in the endosperm	3.7	80

Responses of CBB to Alcohol Release Rates

We found a statistically-significant difference in average number of adult coffee berry borers caught weekly per trap by difference release rates of Methanol: Ethanol (1:1) that were 0, 452, 715, 868 and 1050 mg day^{-1}. The Tukey test revealed significant pairwise differences between 868 mg day^{-1} and another release rates. When the release rate of methanol and ethanol (1:1 ratio) were 868 mg day^{-1}, the captures were highest.

Table 3. Mean number (± SE) of adult coffee berry borers caught weekly per trap with different release rates of Methanol: Ethanol (1:1) mixture. Son La, (November 28 to December 26, 2019), Vietnam.

Rate mg day^{-1}	**Mean number of adult CBB (CBB/trap/7days)**				
	December 5th	**December 12**	**December 19**	**December 26**	**Total**
1050	40.0 ± 6.6^{bc}	89.3 ± 11.7^{ab}	77.5 ± 16.5^{b}	39.6 ± 9.6^{a}	247.3 ± 11.2^{b}
868	58.4 ± 5.6^{a}	100.1 ± 7.9^{a}	136.3 ± 7.9^{a}	26.7 ± 2.9^{a}	310.4 ± 13.0^{a}
715	43.5 ± 3.6^{ab}	60.1 ± 7.8^{b}	58.6 ± 12.4^{b}	27.7 ± 2.2^{a}	189.9 ± 5.8^{c}
452	23.2 ± 2.6^{c}	68.5 ± 8.5^{ab}	66.7 ± 5.0^{b}	31.2 ± 5.9^{a}	189.9 ± 9.6^{c}
Control (Water)	0.9 ± 0.4^{d}	0.6 ± 0.2^{c}	0.2 ± 0.1^{c}	0.6 ± 0.2^{b}	2.2 ± 0.6^{d}
$F_{4,40}$	119.0	56.9	261.1	65.0	383.6
p	<2e-16	5.66e-16	<2e-16	<2e-16	<2e-16

Different letters in the same column indicate statistical differences according to Tukey test with P < 0.05, n = 9.

Percentage of CBB Infected by Bb from Auto-inoculation Trap in the Field

We found a no statistically-significant difference in average captured insects by Bb fungus (F= 0.37, p = 0.56) and by different field conditions (F =1.18, p = 0.39), and the interaction between these terms was not significant (F = 1.07, p = 0.32). There were found a statistically-significant difference in average confirmed mortalities by fungus (F = 8464.9; p = 1.82E-18) and by different field conditions (F = 10.2, P = 0.008) in the period between 0 - 63 days setting traps TBU-AIT. The number CBB attracted by TBU-AIT with the fungus were dead caused by Bb higher than TBU-AIT without the fungus, and the number CBB attracted by traps placed at intercrop condition were dead caused by Bb higher than those placed at open condition (Table 4).

Table 4. Mean number (± SE) of adult coffee berry borers caught and confirm mortality rate of adult CBB females 7 days after expose to *Beauveria bassiana* spores in traps TBU-AIT under field conditions (from May 29 – July 31, 2020)

Days in the field	Treatment	Monoculture condition		Intercrop condition	
		Captured insects*	Confirm mortally (%)**	Captured insects*	Confirm mortally (%)**
7	TBU-AIT with the fungus	16.0 ± 2.0	89.7 ± 2.6	25.0 ± 7.0	92.0 ± 1.8
	TBU-AIT without the fungus	12.0 ± 1.6	0.0 ± 0.0	21.0 ± 3.3	0.0 ± 0.0
14	TBU-AIT with the fungus	55.0 ± 5.4	89.2 ± 1.1	68.0 ± 4.0	94.4 ± 0.9
	TBU-AIT without the fungus	58.0 ± 4.9	0.3 ± 0.1	73.0 ± 5.4	0.7 ± 0.3
21	TBU-AIT with the fungus	47.0 ± 5.5	84.3 ± 1.2	50.0 ± 4.8	91.5 ± 1.0
	TBU-AIT without the fungus	45.0 ± 6.4	2.4 ± 0.7	50.0 ± 7.5	2.6 ± 0.6
28	TBU-AIT with the fungus	29.0 ± 2.8	93.4 ±1.4	33.0 ± 4.9	90.8 ± 1.1
	TBU-AIT without the fungus	26.0 ± 5.3	4.8 ± 1.5	34.0 ± 4.4	4.5 ± 0.9
35	TBU-AIT with the fungus	14.0 ± 2.3	89.7 ± 2.6	19.0 ± 2.3	95,9 ± 1.3
	TBU-AIT without the fungus	13.0 ± 2.1	1.5 ± 0.9	21.0 ± 3.5	1.2 ± 0.7
42	TBU-AIT with the fungus	10.0 ± 1.7	74.7 ± 3.0	16.0 ± 2.1	82.6 ± 1.8
	TBU-AIT without the fungus	8.2 ± 1.0	0.0 ± 0.0	12.0 ± 0.8	0.0 ± 0.0
49	TBU-AIT with the fungus	9.7 ± 0.9	70.5 ± 7.0	9.8 ± 0.9	78.6 ± 3.4
	TBU-AIT without the fungus	9.0 ± 0.6	0.0 ± 0.0	11.5 ± 9.3	0.0 ± 0.0
56	TBU-AIT with the fungus	7.8 ± 1.5	44.1 ± 4.9	11.3 ± 1.2	53.3 ± 2.7
	TBU-AIT without the fungus	10.1 ± 2.1	0.0 ± 0.0	12.3 ± 0.9	0.0 ± 0.0
63	TBU-AIT with the fungus	7.9 ± 0.9	26.2 ± 3.6	6.4 ± 0.6	30.4 ± 2.7
	TBU-AIT without the fungus	8.6 ± 0.7	0.0 ± 0.0	6.3 ± 0.9	0.0 ± 0.0

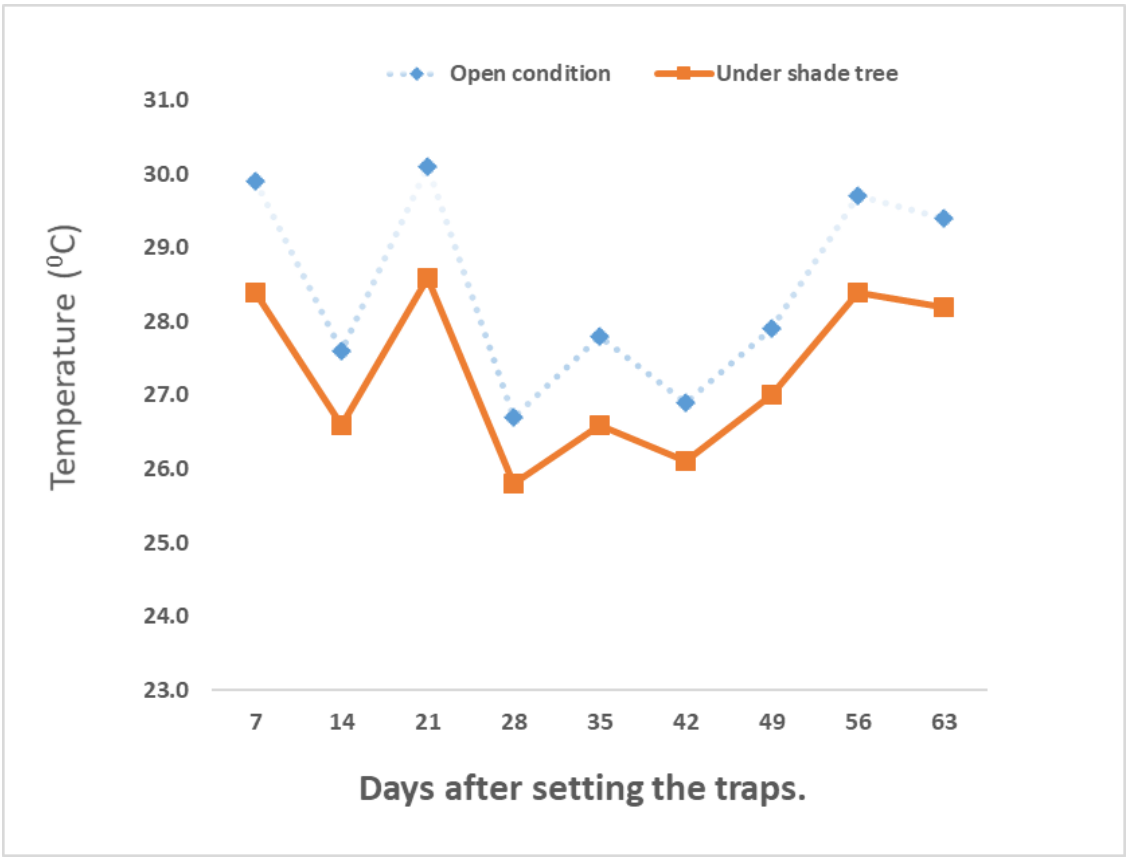

Fig. 2. Mean condition of temperature in traps at the field site

Discussion and Conclusion

When we test virulence of B. bassiana at 107 conidia mL-1, which is the field-recommended concentration, the mortality caused by B. bassiana were low (< 52%) excepted Bb2(RCC1) and Bb5(MCB1). Our results are consistent with other previous studies. Neves and Hirose (2005) mentioned that considerable H. hampei control is only achieved by spraying a high B. bassiana conidia concentration. Further, Damon (2000) also stated that the management of this pest is difficult because the most of the life cycle of them inside the coffee fruit. In addition, Beauveria bassiana was effected by the negative of environmental conditions such as UV light, moisture and temperature. Therefore, conidia sprayed in the field may not survive long time (James et al., 1995).

On the other hand, auto-infection trap (TBU-AIT) was designed here offers protection to the fungus against direct UV light; raining in the field, which may have contributed to the long survival of conidia, but high B. bassiana mortalities just in the period between 0 - 49 days setting traps. These results suggests that we should remove Bb product after 49days place inside TBU-AIT traps under field conditions similar Son La province, Vietnam.

The result showed there were statistically-significant difference in average confirmed mortalities by fungus and by different field conditions in the period between 0 - 63 days setting traps TBU-AIT. The number CBB attracted by traps placed at intercrop condition were dead caused by Bb higher than those placed at open condition, probably because the amount of survived conidia at shade condition better than open condition. This phenomenon may be explained by the temperature at place setting traps TBU-AIT. The results show that the shade trees reduced temperatures (0.8◦C - 1,5◦C) and protected traps better than open condition (Fig 2). Mi¸etkiewski et al. (2014) reported the optimum virulence of Bb at 25 ◦C and reduced virulence at 30 ◦C.

In order to prevent and avoid the coffee berry borer (Stephanoderes hampei Ferrari) is the one that affects significantly and negatively, this study was conducted. Authors via study has recommended to carry out for integrated pest management on coffee auto-infection trap, together with auto-infection system implemented to explore practical and economic values for controlling pest.

Damon (2001) mentioned in his study to emphasize method of controlling non-chemical factors and giving out suggestions of ecological and environmental elements. Johnson et al (2020) stated that for coffee planting and crops, borer (coffee berry or CBB) is the most harmful insect, seriously, causing big losses and damages up to over US$500M yearly. This will lead to big reduction in agricultural yield and decrease in coffee quality, as well as reproduction ability. Author take an example in Hawaii which shows that a successful program of pest management has been implemented in 10-year research period.

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