
Application Frequency of *Beauvaria bassiana* Isolates Against Antestia Bugs (*Antestiopsis intircata*: Pentatomidea, Hemiptera) Management

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Abstract: The production and productivity of coffee is affected by many insect pests and Ethiopian farmers get below 0.636 tons per hectare. Among insect pests Antestia bug is the major coffee insect pest affecting coffee productions. Therefore, the study was carried out to determine the frequency of promised Entomopathogenic fungi isolates against antestia bug. The experiment was done in Jimma Agricultural Research Center, Entomology and Pathology laboratories. Used entomopathogenic fungi isolates were brought from Ambo Agricultural Research Center. Two isolates of *Beauvaria bassiana*, PPRC-44BC and PPRC-27J isolates applied at 1×10^8 conidia ml^{-1} and three times were used for the experiment. Completely randomized design with three replications and probit analysis were used for data analysis by using SAS software version 9.3. PPRC-44BC and PPRC-27J isolates killed all the tested Antestia bugs in exposure time. The isolates applied three times reduced median lethal time by 42.13 and 38.89%, respectively as compared to with their respective one time application. The correlation result also showed that there was strong negative correlation between application frequency of isolates and median lethal times (LT_{50} and LT_{90} were $r=-0.811$ and $r=-0.714$, respectively). The study indicated that the more frequently applied isolates the shorter the median lethal time. This showed promising result in the microbials based insect pest management methods and need further investigations under field conditions and the effect of these isolates against natural enemies of the pest.

Keywords: Thrice, Laboratory, PPRC-44BC, PPRC-27J, LT_{50}

1. Introduction

Coffee productions take 5.21% of cultivated land in Ethiopia. Its production is concentrated mainly in Oromia (68.68%) and Southern Nations, Nationalities and Peoples' Regional (28.09%) states. Coffee produced by 6,312, 486 peasant holders on 785, 523.29 hectare of land and with 0.636 tons/hectare productivity [1]. In Ethiopia coffee yields remain low at 0.636 tons per hectare while in Brazil, the largest producer of Arabica coffee, are nearly double Ethiopia's at 1.5 metric tons per hectare [2]. Many authors reported that the production of coffee is affected by many insect pests [3, 4]. Similarly, Musoli *et al.* [5] reported that poor management practices and losses due to damage by

insect pests and diseases are the major factors contributed for the lower productivity of the crop. Additionally, Mugo *et al.* [6] indicated that increasing infestation of pests and their consequent control and management have significantly constrained economical production of coffee.

The most serious insect pests can cause coffee farmers to lose up to 20% of a crop and reduce the coffee value by 30 to 40% [7]. However, 900 species of insects have been reported on coffee in the world, less than 20 of the arthropod insect pests constitute major constraints to coffee production [8, 9]. But, five coffee insect pests are particularly important on Arabica coffee in East Africa. From these antestia bugs (*Antestiopsis spp.*) is the major insect pests which affect significantly the production and

productivity of the crop [10, 11].

In Ethiopia, the antestia bugs is the major ones causing considerable damage and recorded 9% yield loss and 48% coffee bean darkening [12-15]. The adults and immature are the damaging stages of pest and feed mostly on immature green berries, from which they suck the sap, causing the fruits to shrink [8]. Due to the damage caused by both immature and adults feeding on the berries, it results the young berries to drop and the production of soft or rotten beans by the bigger berries [16]. And they can feed on shoots and leaves of coffee plants but prefer to attack unripe coffee cherries [17]. Additionally, antestia bugs believed as vector of pathogens causing infection of coffee cherries with bacteria and fungi [18]. Mekasha [19] reported that branches of coffee trees infested with four pairs of the bug caused the highest number of damaged Coffee flower bud (1.2%), 54.1% of berry fall, 90.2% of bean damage, and the lowest yield (0.41 kg/tree) of red cherry.

Chemical insecticides recommended for Antestia are considered for its management but due to the adverse effects of chemicals, priority should be given for non-chemical control options. However, biopesticides based on microbial control agents are one of the potential options for insect pest management and development of integrated pest management. Belay *et al.* [20] evaluated different entomopathogenic fungi isolates and PPRC-44BC and PPRC-27J isolates showed promising results from the study against antestia bug. Even though, two isolates of these showed promising results, there is no sufficient and current information on the effect of application frequency of the isolates on the antestia bugs. Therefore, the study was initiated to evaluate the application frequency of *Beauveria bassiana* isolates against antestia bugs under laboratory condition.

2. Materials and Methods

The experiment was conducted in Jimma Agricultural Research Centre, Entomology and Pathology laboratories. It is located at around 7° 46' N latitude and 36° E longitude, and at an elevation of 1750 meters above sea level [21].

2.1. Antestia Bugs Rearing

Antestia bug was collected from coffee infested areas of Jimma and reared in Entomology laboratory. Fresh coffee twigs bearing large green berries were provided for the insect at 2-3 days intervals [22]. The combined coffee leaves and coffee green berries were used as rearing substrate [23].

2.2. Preparation of Fungal Isolates

Two *Beauveria bassiana* isolates (PPRC-44BC and PPRC-27J) were taken from Ambo agricultural research center. The initial cultures of isolates were stored at -5°C and sub-culturing was made as appropriate. The isolates were cultured on sabouraud dextrose agar and potato dextrose agar.

Two to three weeks old cultures were harvested in 10 ml of sterile distilled water containing 0.05% Tween-80% for use in the laboratory bioassays.

2.3. Experimental Design and Treatments

Beauveria bassiana isolates with negative and positive controls replicated three times and laid out in Completely Randomized Design (CRD) (Table 1). Five days old adult antestia bugs and five adults per petri dish were used for the experiment.

Table 1. List of treatments used for the experiment.

Treatments used	Application frequency of the isolates
PPRC-44BC	One time
	Two times
	Three times
PPRC-27J	One time
	Two times
	Three times
Negative control	Distilled water
Positive control	Fenitrothion

Five day old adult antestia bugs were transferred from rearing cage into container and covered with nylon mesh. Insects were allowed to settle on the green berry and then sprayed with 10 ml/ container of aqueous suspension of each fungal isolate at a concentration of 1×10^8 conidia ml^{-1} using syringe. The suspensions were sprayed in equal quantity in each box containing these bugs and uniform distribution of the biopesticide on the insect was ensured. The control groups were sprayed with sterile distilled water with 0.05% Tween-80 and Fenitrothion. Mortality data were corrected for the corresponding control mortality by the formula:

$$\text{CM (\%)} = \frac{(T (\%) - C (\%))}{(100 - C (\%))} \times 100$$

Where, CM is corrected mortality, T is mortality in treated insects and C is mortality in untreated insects [24].

2.4. Application of Isolates Against Antestia Bugs

PPRC-27J and PPRC-44BC isolates were applied one time at first day, two times at first and third days and three times at first, third and sixth days of the initial isolate applications. It was conducted under laboratory condition against adult Antestia bugs.

2.5. Median Lethal Concentration Time (LT₅₀)

Median lethal time (LT₅₀) was determined by taking into account the time required at which the inocula of fungus caused 50% of the mortality on adult Antestia bug population. Lethal time (LT₅₀) required to achieve 50% mortality per replicate were obtained from Probit analysis.

2.6. Data Analysis

Percentage insect mortality data were corrected by using Abbott formula [24]. Collected mortality of the insect pests was analyzed using the SAS version 9.3 [25]. Tukey's test at 5% level was used to compare treatment means.

3. Results

3.1. Application Frequency of EPF Isolates Against Antestia Bugs

PPRC-27J and PPRC-44BC isolates applied once, twice and thrice against antestia bugs showed significant difference (Table 2). After three days treatments applications, positive control recorded complete mortality and showed significant difference from evaluated treatments (Table 2). On this day after application, PPRC-27J and PPRC-44BC applied thrice registered mortality while other isolates didn't resulted in to mortality. After six days of application, positive control showed significant difference from tested isolates frequency. On this day, except negative control mortality was registered by all tested treatments.

PPRC-27J and PPRC-44BC applied twice and thrice

showed non-significant difference from the positive control but showed significant difference from isolates applied once and negative control after 9th days of application (Table 2). After 12th and 15th days of application, positive control showed non-significant difference with all tested isolates frequency except PPRC-27J applied once and negative control. PRC-27J applied thrice and PPRC-44BC applied twice and thrice recorded complete mortality similar to positive control on the same days after application. After 18th days of application, all evaluated isolates frequency showed non-significant difference from positive control but with negative control. All the tested isolates frequency recorded complete mortality against antestia bug on the same days after application. Also similar trends achieved after 21st days of application (Table 2).

Table 2. Mean mortality of Antestia bug over exposure time.

Treatments	Days of exposure						
	3 rd	6 th	9 th	12 th	15 th	18 th	21 th
PPRC-27J (once)	0 (0.707) ^b	0.27 (0.880) ^{cd}	1.93 (1.559) ^{ab}	5.27 (2.402) ^b	7.94 (2.905) ^b	10 (3.240) ^a	10 (3.240) ^a
PPRC-27J (twice)	0 (0.707) ^b	1.66 (1.470) ^{bcd}	7.94 (2.905) ^{cd}	9.31 (3.132) ^a	9.66 (3.188) ^a	10 (3.240) ^a	10 (3.240) ^a
PPRC-27J (thrice)	0.61 (1.052) ^b	1.64 (1.462) ^{bcd}	9.31 (3.132) ^d	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a
PPRC-44BC (once)	0 (0.707) ^b	2.54 (1.739) ^{bcd}	3.76 (2.064) ^{bc}	8.62 (3.020) ^a	8.62 (3.020) ^a	10 (3.240) ^a	10 (3.240) ^a
PPRC-44BC (twice)	0 (0.707) ^b	3.76 (2.064) ^b	9.66 (3.188) ^d	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a
PPRC-44BC (thrice)	0.61 (1.052) ^b	3.23 (1.932) ^{bc}	8.98 (3.079) ^d	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a
Negative control	0 (0.707) ^b	0 (0.707) ^d	0.27 (0.880) ^a	1 (1.225) ^c	1 (1.225) ^c	1.31 (1.344) ^b	1.31 (1.344) ^b
Positive control	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^d	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a	10 (3.240) ^a
Tukey's test	0.2586	0.7154	0.5198	0.2553	0.2537	0.1259	0.1259
CV	13.5	24.5	12	5.3	5.1	2.4	2.4

Means within a column followed by the same letter are not significantly different by Tukey's HSD multiple range test at 5% level. Square root ($\sqrt{x+0.5}$) transformed value in the parenthesis along with mean mortality values.

3.2. Median Lethal Time (LT_{50} and LT_{90}) of Application Frequency of EPF Isolates

The frequent application PPRC-27J and PPRC-44BC isolates showed statistically significant difference in LT_{50} and LT_{90} against antestia bug (Table 3). The shortest median lethal time (LT_{50}) was 5.97 days by PPRC-44BC when applied three times followed by 6.08 days of PPRC-44BC when applied two times (Table 3). But, the longest median lethal time (LT_{50}) was 10.54 days by PPRC-27J applied once followed by 9.77 days of isolate PPRC-44BC applied once. On the other hand, PPRC-27 and PPRC-44BC applied three times reduced median lethal time by 42.13 and 38.89%,

respectively as compared to with their respective one time application against antestia bug. PPRC-27 and PPRC-44BC applied two times also reduced by 29.17 and 7.77%, respectively over one time application. The correlation result also showed that there was strong negative correlation between application frequency of isolates and median lethal times (LT_{50} and LT_{90} were $r=-0.811$ and $r=-0.714$, respectively). The study indicated that the more frequent applied PPRC-27J and PPRC-44BC isolates the shorter the median lethal time. This showed promising result in the microbials based insect pest management methods and need further investigations.

Table 3. Median lethal time (LT_{50} and LT_{90}) of application frequency of *B. bassiana* isolates and percent days reduced.

Application frequency	LT_{50}			Days Reduced (%)	LT_{90}		
	LT_{50}	Lower 95%	Upper 95%		LT_{90}	Lower 95%	Upper 95%
PPRC-27J (once)	10.54	9.25	11.95	-	19.51	17.42	22.20
PPRC-27J (twice)	7.36	6.42	8.39	29.17	11.45	10.03	13.24
PPRC-27J (thrice)	6.41	5.53	7.37	42.13	9.97	8.65	11.63
PPRC-44BC (once)	9.77	8.8	10.82	-	12.08	10.62	13.92
PPRC-44BC (twice)	6.08	5.23	7.02	37.77	9.46	8.18	11.08
PPRC-44BC (thrice)	5.97	5.13	6.90	38.89	9.28	8.01	10.89

* Days reduced calculated from isolates applied once versus isolates applied twice and thrice.

4. Discussions

This experiment showed that mean mortality of antestia bug increased as the application frequency of PPRC-27J and PPRC-44BC isolates increased over exposure time. This may be related with increasing of conidia concentrations due to application frequency increased. This increased the conidia concentrations attachment and penetration to the antestia bug body causing high mortality. The study is in line with Fernandes and Bittencourt [26]; Kannan *et al.* [27]; Ojeda-Chi *et al.* [28] and Fernandes *et al.* [29] who reported that the higher the concentration, the higher the possibility of causing higher mortality. Alonso-Díaz *et al.* [30] also reported that repeated treatment of Ma34 strain controlled the natural infestation of engorged female *Boophilus microplus* on cattle in the Mexican tropics. Even though there is a limitation of literatures on antestia bug, different authors [30, 31] reported on other pests that application of *B. bassiana* four to six times can suppress the population of *Empoasca sp.*, *Riptortus linearis*, and *Maruca testulalis*, but did not significantly differ when compared with the application of chemical insecticides. Application of *B. bassiana* six times led to significantly high yield (659.7 g/plot) as compared with the application of chemical insecticide (374 g/plot) [31]. Additionally, the time required to reach complete mortality of the pest also decreased with increasing exposure time [32, 33].

5. Summary and Conclusion

PPRC-27J and PPRC-44BC isolates evaluated at 1×10^8 conidia/ml concentrations against antestia bug. These isolates were applied once, twice and thrice for application frequency effect. The mortality of antestia bugs increased as application frequency and exposure time of the isolates increased. The more frequently applied isolates are associated with more mortality of antestia bugs within short period of time as compared to the single application over exposure time. The study indicated EPF isolates, application frequency, exposure time and fungal isolates rates have profound effect on mortality of antestia bugs.

PPRC-27J and PPRC-44BC isolates of *Beauveria bassiana* applied two and three times showed promising result under laboratory condition. But their efficacy under field conditions on antestia bug and its natural enemies needs further investigations. Furthermore, isolation and evaluation of entomopathogenic fungi, others microbials, entomopathogenic bacteria and entomopathogenic nematode could also be considered for further investigations. Finally, the development of microbials based management options need focus.

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