Pathogenicity of *Beauveria bassiana* against *Chnootriba similis* Thunberg (Coleoptera: Coccinellidae) under laboratory and screen house conditions

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ABSTRACT

The herbivorous ladybird beetle, tef epilachna *Chnootriba similis* Thunberg (Coleoptera Coccinellidae), is a serious pest of cereal crops in many African countries. In Ethiopia, it was reported as a pest of tef (*Eragrostis tef* (Zuccagni) Trotter (Poaceae) and caused severe damage to all cereal crops in the country. This study was designed to investigate the pathogenicity of different *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) isolates ($1x10^8$ conidia mL^{-1} concentration) against egg, larvae and adults of *C. similis* under laboratory and screen house conditions. All isolates of *B. bassiana* showed pathogenicity in all life stages of *C. similis*. Among the tested isolates, PPRC-56 caused the highest mortality in eggs (76%), first instar larvae (36%), fourth instar larvae (57%) and adults (78%) of *C. similis*, 11, 6, 8 and 16-days post treatments, respectively. The first and fourth instar larvae were more susceptible than the eggs and adults of *C. similis*. Our study showed that all *B. bassiana* isolates were highly effective at killing all stages of *C. similis* in both laboratory and screen house conditions. The study suggests using the entomopathogenic fungus *B. bassiana* as a biocontrol agent to control *C. similis* is very impressive.

Keywords: Tef epilachna, susceptibility, *Beauveria bassiana,* Biocontrol, pathogenicity

MS History: 30.06.2024(Received)-01.11.2024(Revised)- 10.11.2024 (Accepted)

Citation: Amlsha Mezgebe and Ferdu Azerefegne. 2024. Pathogenicity of *Beauveria bassiana* against, *Chnootriba similis* Thunberg (Coleoptera: Coccinellidae) under laboratory and screen house conditions. *Journal of Biopesticides*, **17**(2): 86-91. **DOI: 10.57182/jbiopestic.17.2.86-91**

INTRODUCTION

The herbivorous ladybird beetle, *Chnootriba similis* Thunberg (Coleoptera Coccinellidae) is a major pest of cereal crops in many African countries and Yemen (Tuey and Port, 2001). It has been reported as a pest of tef (Eragrostis tef) and other cereal crops in different areas of Ethiopia (Beyene *et al.,* 2007). It is also known to transmit the most economically important diseases of rice in Sub-Saharan Africa, the Rice Yellow Mottle Virus (RYMV) (Woin *et al.,* 2007). At the times of high population density, growers use the effective insecticides registered in Ethiopia to control *C. similis* include: endosulfan, chlorpyrifos and lambda-cyhalothrin (Beyene *et al.,* 2009). However, repeated use of synthetic insecticides against the pest could create severe ecological problems, including harmful effects on non-target arthropods, environmental pollution, exaggeration within the food chain, and may lead insecticide resurgence by the pest. In addition, human beings are affected by direct contact. Entomopathogenic fungi (EPF) have the potential to be used as one of the mechanisms of integrated pest management against insect pests (Hassan *et al.,* 2019). Fungal pathogens are being developed worldwide for the control of numerous agricultural important pests. The cosmopolitan anamorphic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a well-accepted entomopathogen known to infect hundreds of host species belonging to numerous insect orders and control over 70 insect pests of several crops (Nancy Shophiya *et al.,* 2014, Majesh Tomson *et al.,* 2021).

Vulnerability of numerous ladybirds (Coleoptera: Coccinellidae) to *B. bassiana* has been well proved (Hassan *et al.,* 2019, 2021). Observations made by Beyene *et al.* (2007) on field survey indicated that; the phytophagous ladybird beetle *C. similis* were infected with limited EPF. However, there is no scientific investigations on the

pathogenicity of *B. bassiana* against the phytophagous ladybird beetle *C. similis* sub family Epilachna. Therefore, the present study was aimed at investigating the effectiveness of *B. bassiana* extracted from different host arthropods on the mortality of egg, larvae and adults of *C. similis* under laboratory and screen house conditions.

MATERIALS AND METHODS

Rearing of the tef epilachna *Chnootriba similis*

Adults of *C. similis* were collected from infested barley fields and along nearby rivers at Wolaita Zone (6°54′N latitude, 37°45′E longitude and 1600-2100 meter above sea level), Southern Ethiopia. Adults were reared on barley (*Hordeum vulgare* L.) seedlings planted in pots on screen house at average diurnal temperature of 21.5^{0}C and 65-80% relative humidity. Seedlings were placed under insect rearing cages and maintain adults to mate and lay eggs to gain different stages of *C. similis* for the trail.

Preparation of *Beauveria bassiana* isolates

Three isolates of *B. bassiana* were obtained from the Ethiopian Institute of Agricultural Research (EIAR), Ambo Plant Protection Research Centre (PPRC).

Table 1. List of *B. bassiana* isolates tested

Isolate code	Host arthropod	Specific site of origin	Altitude
PPRC-56	Pachnoda (Coleoptera)	Breber	1925
9615	Spider (Arachnida)	Awassa	2450
9614	Ground beetle	Awassa	1500

Isolates of *B. bassiana* had been isolated from different arthropods in diverse agro-ecological zones of Ethiopia. Detail information of these isolates are given in table (1). Original cultures of all isolates were stored at 4°C and sub-culturing was made for the present study. The fungi were sub-cultured by conidial transfer to the PDA dishes to produce inoculum for the experiments. After obtaining sporulation, fungal conidia were collected by scratching with a scalpel. The conidial suspension was prepared by adding 10 mL of sterile distilled water containing 0.01% Tween 80. The conidial suspension was vortexed for 1-2 min and filtered through four layers of sterile cheesecloths to remove mycelial fractions. The subsequent spore suspensions were adjusted to concentrations of 1× 10^8 mL^{-1} conidia using a hemocytometer. The viability of conidia was determined by applying 0.1 mL of 10^6 conidia/ml conidial suspensions to the PDA dishes and incubated at 25°C. Percentage of germinated conidia was determined after 24 hrs by examining 100 conidia from each of three replicate dishes using compound microscope. Conidia were considered as germinated when they produced a germ tube at least half of the conidial length (Tadele and Pringle 2004 and Erper *et al.,* 2016). The viability of all isolates was greater than 90%.

Pathogenicity of *B. bassiana* under laboratory

Under laboratory condition, the concentration of 1× 10^8 mL^{-1} conidia suspension (Ozdemir *et al.,* 2020) was used to estimate the pathogenicity of all isolates of *B. bassiana* on egg and first instar larvae. Tween 80 at a concentration of 0.01 % was added to the suspension. Thirty uniform adults of *C. similis* were introduced to freely oviposit on barley leaves in treated Petri dishes lined with moistened filter paper for 24 hrs. Subsequently, adults were removed from the leaf, leaving thirty eggs of the same batch having similar age and sprayed by 0.5 mL of spore suspension. For the control treatment, eggs were sprayed with 0.5 mL of 0.01 % Tween 80 by a small hand held sprayer. Sprayed eggs were maintained in an incubator at 25^{0}C and 60±5% RH. Number of hatched and unhatched eggs was recorded daily for 11 days after treatment. The mortality of the hatched larvae from the treated eggs was recorded.

For the first instar larvae, 20 larvae were spread out individually in petri dish of 9cm diameter, containing barley leaf with moistened filter paper and sprayed with one ml of 1.0 x10^8 mL^{-1} conidia suspension. While control treatments were sprayed with one ml of 0.01 % Tween 80 using a small hand held sprayer. The larvae in the Petri dish were covered with perforated lid and maintained at 25^{0}C and 65±5% RH. Fresh leaves were provided daily to the larvae and monitored to record

mortality percentage for 10 days. Mortality of the second instar larvae, which molted from the treated first instar larvae were recorded. Dead larvae were removed and placed in a Petri dish with moist filter paper and fungal infection was confirmed after observing sporulated cadavers under stereomicroscope.

Pathogenicity of *B. bassiana* under screen house

The experiment was carried out on twenty days old potted barley seedlings grown under insect cage in the screen house. Batches of 15 mixed sex adults were introduced to each experimental pot and adults were sprayed by 300 mL of each fungus isolate conidia suspension (1×10^8 mL^{-1}) using a small hand held sprayer. For the control treatments, the plants were sprayed by 300 mL of 0.01 % Tween 80. The mortality percentage of adults was recorded after 4, 8, 12 and 16 days of post treatment.

For the fourth instar larvae, 20 larvae were added in each experimental pot and sprayed by 300 mL of each fungus isolate conidia suspension (1×10^8 mL^{-1}) with 0.01 % Tween 80. Control treatments were sprayed by 300 mL of 0.01 % Tween 80 using a small hand held sprayer. The mortality percentage of 4th instar larvae was recorded after 4, 6 and 8 days of post treatment. Dead adult and larvae were collected and located in a Petri dish with a moist filter paper and fungal infection was confirmed after observing sporulated cadavers under stereomicroscope.

Statistical Analysis

Cumulative mortality counts obtained from all experiments were corrected for natural mortality, using Abbott's formula (Abbot 1925). Mortality data were statistically analyzed by SAS program using a completed randomized design (CRD) with four replicates and the means were compared using Tukey's test at $P<0.05$.

RESULTS

Pathogenicity of *B. bassiana* under laboratory

The fungal isolates showed significant mortality rates on *C. similis* eggs and first instar larvae emerged from treated eggs (P=0.001) (Table 2). The highest egg mortality rate was recorded at the isolate PPRC-56, followed by the isolate 9615, while the lowest mortality rate was at the isolate 9614, 7 days post treatment (Table 2).

Table 2. Pathogenicity of *B. bassiana* isolates on eggs of *C. similis* (mean ± SE)

Isolates	Egg mortality (%)	Mortality of Emerged larvae (%)
PPRC-56	76.07±0.82[a]	32.69±0.58[a]
9615	67.51±0.58[b]	23.71±1.15[b]
9614	60.68±0.58[c]	25.71±0.96[b]
Control	2.5±0.5[d]	3.42±0.5[c]

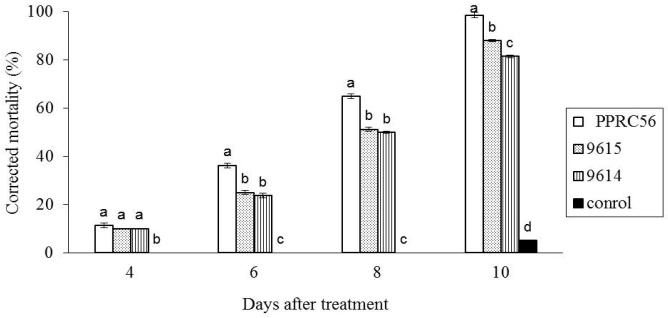

Figure 1. Percent mortality (mean ± SE) of the first instar larvae and second instar larvae (molted from the treated substrate by *B. bassiana*). Values followed by the same letter within the same date of observation are not significantly different (Tukey test, $P<0.05$).

Higher mortality of 1st instar larvae emerged from treated eggs was recorded when treated with the isolate PPRC-56. While the lowest mortality rate was recorded at the isolate 9614 (Table 2). The data in Fig 1, showed that all isolates of *B. bassiana* were found to be pathogenic to the first and second instar larvae of *C. similis*. The results also showed significant differences among isolates in their effect on first and second instar larvae (P=0.012) mortality, 10-days of treatment. All isolates caused high level of mortality on the larvae of *C. similis* and were significantly different than the control treatment (Fig. 1). Highest mortality rates of 1st instar larvae were observed when treated with the isolate PPRC-56, while the lowest was at the isolate 9614, 6 days of treatment. Highest mortality of second instar larvae was observed at the isolate PPRC-56, while the lowest was at the isolate 9614 10 days of treatment (Fig.1).

Pathogenicity of *B. bassiana* under screen house

Corrected percentage mortality fourth instar larvae of *C. similis* treated with the 3 different isolates of *B. bassiana* were showed in (Fig. 2).

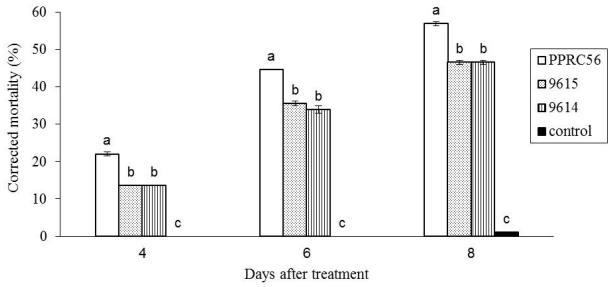

Figure 2. Percentage mortality (mean ± SE) on fourth instar larvae of *C. similis* caused by *B. bassiana* isolates. Values followed by the same letter within the same date of observation are not significantly different (Tukey test, P <0.05).

Results showed significant differences among isolates in their effect on mortality of larvae (P=0.02) after 8 days of treatment. All isolates caused high levels of mortality on the fourth instar larvae and were significantly different than the control treatments (Fig. 2). Highest mortality of fourth instar larvae was recorded when injected with isolate PPRC-56 and the lowest when injected with the isolate 9614, 8 days after treatment. Isolates of *B. bassiana* showed a significant effect on the proportion of infected adults of *C. similis*, when incubated under screen house conditions (P=0 .002). Highest mortality rate occurred when inoculated with the isolate PPRC-56, while the lowest was at the isolate PPRC-9614, 16 days after treatment (Fig. 3).

DISCUSSION

EPF are effective biological control agents against various insect pests (Roy *et al.,* 2006, Yanar *et al.,* 2023). The EPF *B. bassiana* has high genetic disparity among several isolates, great diversity, high capacity for vertical and horizontal dispersion without significant environmental effect and 80% of potential infection on pest populations (Jaronski, 2014; Ibrahim, 2015). All fungal isolates of *B.bassiana* showed pathogenicity to eggs of *C.similis* and the rate mortality of eggs were significantly different among isolates. The current result indicated that the isolate PPRC-56 caused the highest egg mortality. While all isolates had capability to infect and kill the first instar larvae, which emerged from the treated eggs. The current results agree with the previous studies of Abdul and Naheed (2008) and Jeffrey (2009) who reported that *B. bassiana* has an ability to attack eggs of many insect species. Neonate larvae, which escaped infection in the egg stage, may be contaminated later with fungal spores during eclosion.

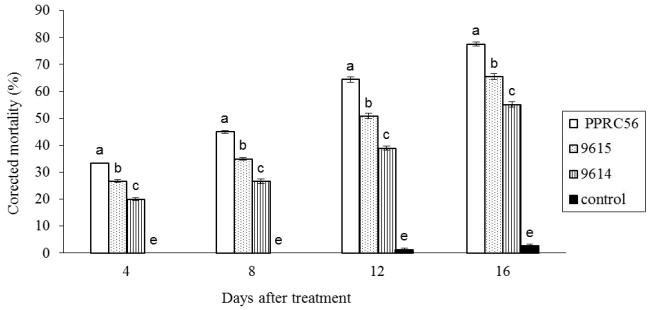

Figure 3. Percent (mean ± SE) adult mortality of *C. similis* caused by *B. bassiana*. Values followed by the same letter within the same date of observation is not significantly different (Tukey test, P <0.05)

Variations in virulence among *B. bassiana* isolates have been reported on many insects and stages of development (Todorova *et al.,* 2022). Isolate PPRC-56 killed about 99% of the first and second instar larvae which molted from the treated first ones within 10 days of treatment. Hassen *et al.* (2020) recorded 61-100% mortality in the first instar larvae of the squash beetle *Epilachna chrysomelina* after 3 days post treatment with *B. bassiana* at $1 \times 10^7 mL^{-1}$ concentration.

The results also showed significant differences among isolates in their effect on fourth instar larvae mortality after 8 days of treatment, the fourth instar larvae treated with *B. bassiana* mortality percentage reached 56.89%. Hassen *et al.* (2020) obtained 53.57% mortality of fourth instar larvae squash beetle *Epilachna chrysomelina* when treated with *B. bassiana* isolate from soil after 3 days post treatment at 1×10^7 mL^{-1} concentration. Other investigation by Topkara *et al.* (2022) on the efficacy of various *B. bassiana* isolates against the fourth-instar larvae of *T. wilkinsoni* under laboratory conditions showed that all isolates caused 100% mortality at a concentration of 1 ×

10^8 conidia mL^{-1}.The results showed a decrease in mortality percentage as larvae grew older and increased with increasing the exposure period. The findings of this study are reliable with the result of Sönmez *et al.* (2017), who found the fourth instar larvae of *T. pityocampa* was susceptible than the earlier instars. Studies on squash beetle *Epilachna chrysomelina* (Feyroz *et al.* (2021) and on pine processionary moth, *T. wilkinsoni* (Yaner *et al.,* 2023) reveals that the mortality percentage decreased with larval age. Obtained results also agree with the previous studies (Assaf *et al.,* 2011; Ozdemir *et al.,* 2020; Hassan *et al.,* 2021), who reported that the mortality percentage was low at low concentrations and duplicated by increasing the spore concentration and period of exposure. All isolates of *B. bassiana* showed pathogenicity to *C. similis* adults. However, variations were observed among isolates in the extent of mortality and time taken to kill the adults. Isolate PPRC-56 of *B. bassiana* caused the highest mortality to the adults of *C. similis* than the other strains at all times of observations. Hassen *et al.* (2020) mentioned 41.33% adult mortality of squash beetle *Epilachna chrysomelina* after 3 days post treatment of *B. bassiana* at 1×10^7 conidia/mL after 12 days. In general, *B. bassiana* caused the highest mortality rates on *C. similis* under laboratory and screen house conditions. The isolate PPRC-56 was highly virulent and potent on all tested developmental stages of *C. similis* and has the potential to be used as biocontrol agent. Follow up studies on field performance of these virulent isolates are recommended. Combination of EPF in the integrated pest management strategyfor controlling *C. similis* can reduce reliance on synthetic insecticides and increase the levels of control.

ACKNOWLEDGEMENTS

The authors would like to thank Plant Protection Research Center (PPRC), Ambo, Ethiopia for providing isolates of *B. bassiana*.

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