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Pathogenicity of entomopathogenic fungus *Beauveria bassiana* and bacterium *Bacillus thuringiensis* var. *kurstaki* against the lesser grain borer *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) under laboratory conditions

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Abstract: A laboratory strain of *Beauveria bassiana* (Balsamo) Vuillemin and a commercial formulation of *Bacillus thuringiensis* var. *kurstaki* were assayed against the adult of *Rhyzopertha dominica* F. under laboratory conditions. Four concentrations of *B. bassiana* (5 $\times10^6$, 5 $\times10^7$, 5 $\times10^8$ and 5 $\times10^9$ conidia /ml) and four of *B. thuringiensis* (1, 1.5, 2 and 2.5 mg/ml) were used alone against the adult of *R. dominica*. The mortality was recorded after 1, 3, 5 and 7 days of treatment. Regardless of the pathogen, mortality increased with concentration. Results showed that *R. dominica* was more sensitive to *B. thuringiensis* than to *B. bassiana*. Also, *B. thuringiensis* caused the highest mortality (93%) at 2.5 mg/ml. In case of *B. bassiana*, the LC_{50} value was 5.48 x 10^7 conidia /ml while the LT_{50} was 5.45 days at 5 $\times10^9$ conidia /ml, whereas the LC_{50} value of *B. thuringiensis* was 1.19 mg/ml and the LT_{50} was 5.05 days at 2.5 mg/ml. These results also showed that the strain of *B. bassiana* AUMC 3847 isolated from the insect *Galleria mellonella* could infect the lesser grain borer *R. dominica* which means no specificity of infection among the strains of *B. bassiana* and the host insect.

Keywords: Pathogenicity, *Beauveria bassiana*, *Bacillus thuringiensis*, lesser grain borer.

Introduction

The lesser grain borer, *Rhyzopertha dominica* is the essential insect pest of stored cereals (Hagstrum and Flinn 1994). Previous studies considered the best way to control this pest be in the adult stage or controlling the young stage of larvae before they enter a grain of wheat. Whereas females lay eggs on the surface of the grain and these eggs hatch into larvae that enter the grain and remained inside until complete their development (Neethirajan *et al.* 2007 and Ozkaya *et al.* 2009).

A number of entomopathogenic fungi have been reported in various studies effectively as biological control agents of stored product insect pests (Gillespie 1988, Wakefield *et al.* 2002, Akbar *et al.* 2004, Michalaki *et al.* 2006 and Wakil and Ghazanfar 2010**).** *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) is a widely distributed fungal species and has the potential to infect several important stored product insects pest (Michalaki *et al.* 2006**).**

Bacillus thuringiensis used for insect control become known active as insect pesticide safe to natural enemies; it does not cause adverse effects on mammals and less harmful environmental impact (Entwistle *et al.* 1993).

Various strains of *B. thuringiensis* indicate that they are comparatively efficacious versus the rice weevil, *Sitophilus oryza* L., the granary weevil, *S. granaries* L., the lesser grain borer, *Rhyzopertha dominica* and the Angoumois grain moth, *Sitotroga cerealella* (Olivier) Steinhaus and Bell (1953). The Initial determination of the pathogenicity of *B. thuringiensis* in which a dose of 250 ppm caused 86% fatality rate to the *R. dominica* adult reared on cracked wheat (Mummigatti *et al.* 1994). Previous studies showed that the integration between *B. bassiana* and *B. thuringiensis* leads to increase the proportion of death in many types of beetles (Wraight and Ramos 2005).

The purpose of this study is to estimate pathogenicity of the fungus *Beauveria bassiana* and the bacterium *Bacillus thuringiensis* against the adult stage of lesser grain borer, *Rhyzopertha dominica* under laboratory conditions.

Materials and Methods

1-Insect rearing

Adults of *Rhyzopertha dominica* was collected from the public wheat storages and reared on whole wheat grains. Culture was maintained in plastic jars (1 liter) covered with muslin cloth. Insects were reared under condition of 27 ±1°C and 75 ± 2% R.H. in the laboratory of Plant Protection Department, Faculty of Agriculture, South Valley University, Qena, Egypt**.**

2-Source of pathogens

2.1- Fungus, *Beauveria bassiana*

B. bassiana AUMC 3847, isolated from *Galleria mellonella* L., was obtained from Assiut University Mycological Center (AUMC) and kept in the refrigerator till using for conidiospores production.

2.2- Bacterium, *Bacillus thuringiensis*

The wettable powder (WP) commercial formulation (Biotect) containing *B. thuringiensis* var. *kurstaki* bearing a density of active toxin 9.4% and other inert material 90.6% with the potency of 32000 x10^6 I.U. was provided by Organic Biotechnology Corporation, Albuhayrah, Egypt.

3-Suspesion preparation of *B. bassiana*

The fungus was grown on Potato Dextrose Agar (PDA), which consisted of 250 g potato, 25 g dextrose, 20 g agar and 1000 ml distilled water. The medium was autoclaved at 120°C for 20 minutes, then the medium was poured in Petri dishes under sterilized conditions. The fungus was inoculated under these sterilized conditions and then incubated at 25°C in the dark for 15 days. Conidia were obtained by swilling with sterile distilled water containing 0.05% Triton x-100 and then washed twice in sterile distilled water by centrifugation at 5000 revolutions / minute for 5 minutes. A haemocytometer was used to determine the concentration of conidia in the premiered suspension. Subsequent alleviation was then made to get the required concentration of the conidia (Nazir *et al.* 2007).

4-Bioassay

4.1- *B. bassiana*

Four concentrations of conidial suspension ($5x10^6$, $5x10^7$, $5x10^8$ and $5x10^9$ conidia /ml) from *B. bassiana* as well as control treatment were prepared. Ten adults of *R. dominica* insect were treated by dipping them for 10 seconds in the conidial suspension in 50 ml-conical flask at room temperature. Thereafter the adults were transmited into a Petri dish with wet filter paper, then kept for 7 days under conditions of 25±1 ºC and 80-90% RH. Each treatment was replicated three times with 10 insects per replicate.

4.2- *B. thuringiensis*

Four concentrations from *B. thuringiensis* var. *kurstaki* (1, 1.5, 2 and 2.5 mg/ml) were used against the adult of *R. dominica* in the laboratory under controlled temperature of 25±1 ºC. In Petri dish, 10g of broken wheat were sprayed by 50 ml from tested concentrations of *B. thuringiensis* var. *kurstaki* and left to dry under laboratory conditions. Each bioassay treatment was conducted with ten adults of *R. dominica*. Insects were fed on treated broken wheat with tested concentrations of *B. thuringiensis*. Control treatment was made by feeding the adult on untreated broken wheat. Each treatment was replicated three times with 10 insects per replicate.

5-Statistical analysis

The mortality data was recorded after 1, 3, 5 and 7 days of treetment. Data were analyzed by probit analysis using SPSS program to calculate LT_{50} and LC_{50}, values.

Results and Discussion

1- **Effect of fungal entomopathogen *B. bassiana* on the mortality of adult *R.dominica.***

The results in table (1) revealed that mortality was noted in the control and in all concentrations one day after treatment. The percentage mortality was regularly increased with concentration and with the elapse of time so that the highest mortality (77%) was induced by the highest concentration ($5x10^9$) after 7 days. Mahdneshin *et al.* (2009) cleared that the application of *B. bassiana* had a great impact on the adult of *R. dominica* that achieved a significantly high cumulative mortality percentage averaged 89.35% after 7 days. When using *B. bassiana* at the lowest rate of 0.05 g mixed with long grain rice the mortality percentage averaged 80% to the rice weevils within seven days of treatment (Hendrawan and Ibrahim 2006). Tahira, *et al.* (2011) determined a correlation between the conidial concentrations of *B. bassiana* and mortality percentages of the lesser grain borer *R. dominica*. Nevertheless low concentrations of the fungus *B. bassiana* achieved high performance in the mortality rate of *R. dominica*, *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* (Lord, 2001). EL-Sebai (2011) indicated that the mortality rate recorded for the adult phase insect of *R. dominica* increased with increasing concentrations of *B. bassiana* seven days after the treatment. Adane *et al.* (1996**)** tested the pathogenicity of ten different isolates of *B. bassiana* on the maize weevil and found that one of these concentrations ($1x10^4$ conidia /ml) has caused the death rate of 88% during the eight days of treatment, which confirmed that the very pathogenic isolates lead to a high rate of death even in low concentrations.

Table 1: Percentage mortality of *R. dominica* adult as affected by different concentrations of *B. bassiana.*

Concentration (conidia/ml)	% Mortality			
	1 day	3 days	5 days	7 days
Control	0	0	0	0
5×10^6	0	0	10	37
5×10^7	0	3	17	50
5×10^8	0	7	23	60
5×10^9	0	10	33	77

The LC_{50} value for *B.bassiana* was 5.48×10^7 conidia /ml for the adult of *R. dominica* (Table 2).. Searle and Doberski (2007) mentioned that the LC_{50} and LC_{90} values of *B. bassiana* against *R. dominica* adult were 5.40×10^7 and 8.11×10^7 conidia /ml respectively, whereas Abdel-Raheem *et al.* (2015) recorded that the LC_{50} value was 1.20×10^5 conidia /ml after 11 days treatment by *B. bassiana*.

The efficacy of the conidia of *B. bassiana* against the adult stage was expressed in terms of LT_{50} (Table 3) which revealed that LT_{50} values were 7.80, 7.19, 6.48 and 5.48 days for the concentration of 5×10^6, 5×10^7, 5×10^8 and 5×10^9 conidia /ml respectively.

The difference in the time necessary to kill 50% of insects exposed to the highest and lowest concentrations of *B. bassiana* was 2.32 days. Results of treatment with *B. bassiana* announced that the effectiveness of the tested entomopathogenic fungus in killing the adult insects is depending on both the fungus concentration and the time elapse after treatment, i.e., the same percentage of mortality could be obtained either when the insects were treated with high concentration for shorter time or treat with low concentration for longer time. Exterior white mycelial outgrowth from all deceased bodies was apparent within 48-72 hours of dying. After the death of the insect mycelial growth proves that the fungal pathogen is the causal agent of insect's death. These results are consistent with Ramlee *et al.* (1996) who announced that later transplantation of *B. bassiana* to the

insect body, the hyphae insinuate to the cuticle into entire body hole up to the fat bodies, neural and muscle tissues destroying them. As well, it connects to malpighian tubule, epithelial cells and eventually settles in the gut lumen. Over time the infective insects becomes lifeless.

Similar results were obtained by Batta (2008) who recorded that LT_{50} value was 5 days for the lesser grain borer *R. dominica* inoculated with *B. bassiana*. Quesada-Moraga *et al.* (2006) demonstrate that the performance of *B. bassiana* begins its influence to emerge after 48 hrs. After infection and the hyphae infiltrate, the internal membrane of the trachea and the epithelial and epidermal cells. The mortality rate of up to 100% in the treatment insects with this fungus after 96 hours, whereas during this period corruption occurs for all the fatty tissues of the body of the insect, which leads to certain death in the end. Vassilakos *et al.* (2006) reported that LT_{50} values were 6.34 and 5.25 days for *B. bassiana* in combination with diatomaceous earth (DEBBM) respectively when they were applied against lesser grain borer *R. dominica* and *Sitophilus oryzae* on stored wheat. Batta (2005) explained that the insect *R. dominica* achieved the highest percentage of mortality after 7 days of treatment with *M. anisopliae.* The differences are attributed to the effect of different isolates of the same species of fungus to many factors, including the species of insect, the circumstances in which was conducted the experiment, the materials used in the experiment and finally the pathogenicity effect of each isolate of the fungus.

Table (2): LC_{50} values of *B. bassiana* and *B. thuringiensis* against the adult of *R. dominica*

Analysis program	LC_{50}	Confidence limits		Slope ± S.E
Pathogen		Lower	Upper	
B. bassiana	5.48×10^7 (conidia/ml)	3.53×10^6	2.71×10^8	0.34 0.11
B.thuringiensis	1.19 (mg/ml)	0.92	1.38	4.11 0.89

Table (3): LT_{50} Values of *B. bassiana* against the adult of *R. dominica*.

Analysis program	LT_{50} (days)	Confidence limits		Slope ± SE
Concentration (conidia/ml)		Lower	Upper	
5×10^6	7.80	6.79	12.48	6.90± 2.25
5×10^7	7.19	6.20	10.04	5.27± 1.41
5×10^8	6.48	5.62	8.35	4.93±1. 19
5×10^9	5.48	4.84	6.37	5.50±1 .13

2-Effect of *B. thuringiensis* var. *kurstaki* on the mortality of adult *R.dominica*

Four different concentrations of the bacterium (1, 1.5, 2 and 2.5 mg/ml) were tested against the adult of *R. dominica*. No mortality was noted in the control. Percentage mortality of adult was dose dependent and increased with increasing concentration. Mortality rate of *R. dominica* adult treated with *B. thuringiensis* var. *kurstaki* ranged from 40% to 93% after 7 d (Table 4).

The mortality was enhanced by increasing concentration and time. At first concentration of *B. thuringiensis*, the mortality was 40%, the second concentration was 63%, the third concentration was 80% and the fourth concentration was 93% respectively after seven days post treatment as shown in (Table 3).

Table 4: Percentage mortalality of *R. dominica* as affected by different concentrations of *B. thuringiensis* var**.** *kurstaki*.

Concentration (mg/ml)	% Mortality			
	1 day	3 days	5 days	7 days
Control	0	0	0	0
1	0	0	13	40
1.5	0	0	23	63
2	0	0	33	80
2.5	0	7	43	93

The results obtained from this study are consistent on the whole with the result of Phillips and Throne (2010) who observed that 93.3% mortality of different instars of lesser grain borer *R. dominica* treated by various strains of *B. thuringiensis*. Similarly**,** Zhang *et al.* (1995) recorded a maximum mortality (92.88%) of lesser grain borer *R. dominica* one day old adult in a formulation containing *B. thuringiensis* var. *kurstaki*. Also, a significant correlation between the increase in the proportion of death in insects and its association with increased ratio of the concentrations used. Kausarmalik and Rizwana (2014) determined a correlation between the concentrations of *B. thuringiensis* and mortality percentages of the red flour beetle *Tribolium castaneum*. Brenda *et al.* (2011) declared the relationships between the virulence of *B. thuringiensis* and the concentrations of the bacterium toward coleopteran storage pests.

The LC_{50} value for *B. thuringiensis* was 1.19 mg/ml for adult of *R. dominica*. High slope value indicated that small variation in bacterium concentration promoted large variation in the mortality of adult *R. dominica* (Table 2).

Consistent with the results obtained from former bioassays of *R. dominica* adult, it was observed that the extent of toxicity recorded for *B. thuringiensis* was from 1 to 5 mg/ml (LC_{50}) relying on when the assays were estimated from 5 days to 2 weeks (Herrnstadt *et al.*

1986). Brenda *et al.* (2011) illustrated that the LC_{50} value for *B. thuringiensis* was 1.18 mg/ml for the adult of *R. dominica*.

The LT_{50} values were slightly decreased with increasing concentration. The difference in the time needed to kill 50% of insects exposed with the highest concentration (2.5 mg/ml) of *B. thuringiensis* and the lowest one (1 mg/ml) was only 2.53 days indicating a relatively intensive effect of *B. thuringiensis* on the adult of *R.dominica* (Table 5).

Table (5): LT_{50} Values of the *Bacillus thuringiensis* var. *kurstaki* to the adult stage of *Rhyzopertha dominica*.

Analysis program	LT_{50} (days)	Confidence limits		Slope ± S.E
Concentration (mg/ml)		Lower	Upper	
1	7.57	6.61	11.12	6.59± 2.00
1.5	6.28	5.71	7.18	8.04± 1.87
2	5.64	5.14	6.17	9.73±0. 85
2.5	5.04	4.56	5.57	8.05±0 .47

In this respect, Ahmedani *et al*. (2008) recorded that LT_{50} varied from 3 and 7 days for commercial formulations of *B. thuringiensis* when they were applied against *Tribolium castaneum* adults**.** Also, Naiema *et al*. (2013) found that the LT_{50} values varied from 96.7 hours to 188 hours for *B. thuringiensis* isolations against *Tribolium castaneum* adults. Alternatively, Elyass (2004**)** examined different isolates of *B. thuringiensis* against *Henosepilachna elaterii* and reported that LT_{50} ranged from 48.6 to 72 hours for the higher concentrations examined.

In general, the proportion of death of the treated adult increases with the passage of time, the reason for this is due to the requirement of bacteria to enough time to interact into the midgut after being swallowed. Also time needed to activate the poison produced by the bacteria, which leads to an imbalance ion stability, and finally leads it to death (Lonc *et al*., 2003).

Finding an isolate with high potentiality gives hope in controlling this devastating pest, which can live for up to three years. This insect acquired resistance to most insecticides, while the most effective ones, such as methyl bromide were banned (Ahmedani *et al*. 2008). The present result revealed that *B. bassiana* AUMC 3847 and *B. thuringiensis* are potential, effective and economically feasible organisms for controlling *R. dominica*, but the former is more efficient than the latter.

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