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EFFECTS OF LOCAL ENTOMOPATHOGENIC *BEAUVERIA BASSIANA* ISOLATES AGAINST *SITOPHILUS GRANARIUS* (COLEOPTERA)

SUMMARY

The granary weevil *Sitophilus granarius* L. is one of the most damaging grains pest in many parts of the World and Turkey. Chemical insecticides have been widely employed for the control of stored grain pests. This has caused such problems as insecticide resistance along with contamination of foodstuffs with chemical residues. Thus, there is a growing interest in using pathogenic control agents as an alternative.

This study aimed to evaluate the efficacy of entomopathogenic fungus *Beauveria bassiana* isolates (F-52, F-53, and F-56) on adults of granary beetle *S. granarius*. Five different doses, including 1×10^3, $1x10^5$, 1×10^7, $1x10^8$, and 1×10^9 conidia/ml for insect dipping, had been used. The experiment was laid out in a completely randomised block design with five replications and replicated two times.

Mortalities were recorded on the 1st, 3rd, 5th, and seventh days of incubation. The highest mortality rate of 70% was observed at the end of the five-day incubation period with isolate F-53. Mortality increased with increase in the incubation period, and the highest mortality was observed after seven days of incubation period.

Although the results indicated that isolate F-53 was effective against *S. granarius* and resulted in a high mortality 98% at the end of seventh day incubation period at 1×10^9 conidia/ml and followed by isolates F-52 and F-56 with 94% mortality. LC50 values confirmed that *S. granarius* was more susceptible to the isolate F-52 than the other two isolates F-53 and F-56 where the LC50's were $1x10^5$, $2x10^5$, and $5x10^5$ conidia/ml respectively. Mycosis was observed in all the treatments except the control.

Our study indicates that all the isolates could be used as potential biological control agents. Further studies are ongoing for determination of the efficacy of this isolate under storage conditions.

Keywords: Biological control, entomopathogen, *Beauveria bassiana*, granary weevil

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INTRODUCTION

Stored grains and their by-products are infested by insect pests causing approximately 10-25% losses worldwide. Losses caused by insects include not only the direct feeding damage resulting in loss of weight but they also severely reduce nutrients, lowering percentage of seeds germination, reducing grade and lowering their marketing value due to the accumulation of waste, webbing and insect cadavers (Hill, 1990). Most of these are coleopterans (Vinuela et al. 1993). Among them, the granary weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae) and is known for its economic importance. The damages consist of the reduction in weight, quality, commercial value and seed viability (Hill, 1990). Residual insecticides have been employed to control insect pests of stored grains, but alternative control strategies are desirable because of the loss of insecticides due to pest resistance and consumer desire for pesticide-free grain (Arthur, 1996).

The most significant impetus for the growth of biopesticides comes from the growing awareness by farmers of the value of integrated pest management as a more environmentally sound, economical, safer and a selective approach to crop protection (Menn, 1996). Therefore, it is necessary to find out safer alternative control strategies such as the use of microbial control agents against stored–product insect pests. Using fungi and selected insecticides can potentially reduce the use of chemical insecticides and subsequently their residues and side effects in agriculture. *Beauveria bassiana* and *Metarhizium anisopliae* are naturally occurring entomopathogenic fungi with a broad host range (Sheeba et al. 2001, Wakil and Ghazanfar, 2010, Sewify et al. 2014).

The entomopathogenic fungus *B. bassiana* bears a considerable potential for the control of the different stored product pests. Cherry et al. (2005) suggested that *B. bassiana* is a potential microbial control agent against some stored product pests. Nowadays, several *B. bassiana* formulations (Boverosil ®, Mycotrol® ES, Mycotrol® 22WP, Naturalis® SC) are commercially available and are registered for use in storage facilities. Entomopathogenic fungi within stored food products can be employed to treat empty stores to control residual pests before the new harvest is brought in or may be applied as the direct admixture of conidia to grain, either as preventative or curative treatments of bulk grain. The present work aimed to evaluate the efficacy of the local entomopathogenic *B. bassiana* isolates against the granary weevil *S. granarius.*

MATERIAL AND METHODS

Test Insect Rearing: Tested stored product insect species *Sitophilus granarius* L. were obtained from laboratory cultures reared for several generations at the Department of Plant Protection, Agricultural Faculty, Gaziosmanpasa University, Tokat/Turkey. The insects were reared on whole wheat grains. Insect cultures were maintained in glass jars (2 litres) covered with a muslin cloth. All insects were reared under laboratory conditions of 24±2 °C and 75±2% R.H.

Fungal Isolates: The entomopathogenic fungi, *B. bassiana*, isolates F-52, F-53, and F-56 were initially isolated from the meadow soil at Kelkit Valley. The fungi were grown on Potato dextrose agar (PDA) medium. The media was autoclaved at 121 °C for 15 minutes and poured into Petri dishes (9 cm diameter x 1.5 cm). The fungal isolates kept at 25 ±2 °C and 85± 5 % RH. The fungal isolate was sub-cultured every 14 – 30 days and kept at 4 °C.

Bioassay: The isolates F-52, F-53, and F-56 were grown on Potato Dextrose Agar (PDA) medium at 27 ^{o}C for four weeks to get sporulation. The conidia were harvested under sterile conditions by flooding the plate with 10 ml of sterile distilled water containing 0,02% tween 80 then scraping the colony with a sterile glass hockey stick. The spore suspension was filtered through four layer sterile cheesecloths to remove mycelia. The concentrations of conidial suspensions were determined, using a Neubauer hemocytometer. The conidial suspensions were stored at 4 ^{o}C for up to 1 week until used in the assays. The viability of conidia was determined by spread-plating 0.1 ml of the suspension on the PDA plates. A sterile microscope coverslip was placed on each plate. Plates were incubated at 27 ^{o}C and examined after 24 h. The percentage of germination was determined by counting 100 spores for each plate, and over 95% of the spores germinated. For the dose-mortality test, *Sitophilus granarius* healthy adults were randomly selected and used for bioassays. Conidial suspensions of fungal isolates were prepared as described above, and a series of dilutions was prepared as $1{\times}10^3$, $1{\times}10^5$, $1{\times}10^7$, $1{\times}10^8$, and $1{\times}10^9$ conidia/ml. Ten adults were dipped in conidia suspension for 5 seconds than transferred into the glass wails containing ten wheat grains as food. The experiment was laid out in a completely randomised block design with five replications and replicated two times. The control insects treated with sterile distilled water containing 0,02% tween 80. Mortalities were recorded on the 1st, 3rd, 5th, and seventh days of incubation. Dead insects were transferred in humid sterile 90 mm glass petri dishes for 14 days to determine the mycosis rates. The percentage mortality and LC50 were estimated.

Statistical Analysis

The data were analysed by analysis of variance (ANOVA) and the means compared by Tukey's multiple comparison test. All statistical analyses were carried out using the SPSS Release 16 packet program. The lethal concentration (LC50) was calculated using probit analysis. (Finney 1978).

RESULTS AND DISCUSSION

Several studies documented the high potential of entomopathogenic fungi for the control of insect pests in stored grains and their byproducts (Moore et al., 2000, Cox et al. 2003 and 2004, Cherry et al. 2005). The insecticidal efficacy of *B. bassiana* is highly influenced by several factors such as characteristics of host insects, and physiology of pathogen fungi (enzymes and toxins) (Fargues et al., 1996, Cox et al., 2004). So, the testing of different isolates of *B. bassiana* against different storage pests is needed. In the present study it was found that three *B.*

bassiana isolates tested (F-52, F-53, and F-56) were pathogenic in *S. granarius* adults.

The percentages of mortality varied from 76% to 84% at $1x10^8$ conidia/ml concentration 7 day after inoculation (Table 1, 2, and 3). The control group had 8% mortality.

Table 1. Mortality of *Sitophilus granarius* exposed to different doses (1×10^3, 1×10^5, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) of *Beauveria bassiana* isolates F-52 and controls over seven days after treatment.

	Mortality±SEM* (%)			
Doses(conidia/ml)	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
$1x10^3$	4,00±5,47a***	16,00±5,47b	32,00±4,47b	44,00±5,47b
$1x10^5$	2,00±4,47a	24,00±5,47bc	36,00±5,47b	48,00±4,47b
$1x10^7$	2,00±4,47a	32,00±4,47cd	38,00±4,47bc	66,00±5,47c
$1x10^8$	4,00±5,47a	34,00±5,47cd	46,00±5,47c	76,00±5,47c
$1x10^9$	4,00±5,47a	36,00±5,47d	56,00±5,47d	94,00±5,47d
Control	0,00±0,00a	2,00±4,47a	2,00±4,47a	8,00±4,47a

* SEM: Standard error of the mean;
** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different ($P < 0.05$).

In all the experiments, mortality increased with increase in conidia concentrations and incubation periods while the highest mortality was observed after seven days of incubation at $1x10^9$ conidia/ml concentrations. The results indicated that isolate F-53 was effective against *S. granarius* and resulted in a high mortality 98% at the end of the 7th day incubation period at 1×10^9 conidia/ml.

Followed by isolates F-52 and F-56 with 94% mortality (table 1,2 and 3). Mycosis was observed in all the treatments except the control. The present study agrees with the results of previous studies where higher doses produce the highest percentage of mortality (Athanassiou and Steenberg 2007, Khashaveh et al., 2011; Magda and Mohamed, 2015).

Table 2. Mortality of *Sitophilus granarius* exposed to different doses (1×10^3, 1×10^5, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) of *Beauveria bassiana* isolates F-53 and controls over seven days after treatment.

	Mortality±SEM* (%)			
Doses(conidia/ml)	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
$1x10^3$	0,00±0,00a***	18,00±4,47b	28,00±4,47b	38,00±4,47b
$1x10^5$	0,00±0,00a	22,00±8,36b	36,00±5,47bc	46,00±5,47b
$1x10^7$	6,00±8,94a	34,00±5,47c	44,00±5,47c	66,00±5,47c
$1x10^8$	0,00±0,00a	38,00±4,47c	56,00±5,47d	82,00±4,47d
$1x10^9$	8,00±10,95a	40,00±0,00c	70,00±7,07e	98,00±4,47e
Control	2,00±4,47a	2,00±4,47a	2,00±4,47a	8,00±4,47a

* SEM: Standard error of the mean; ** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different (P < 0.05).

Table 3. Mortality of *Sitophilus granarius* exposed to different doses (1×10^3, 1×10^5, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) of *Beauveria bassiana* isolates F-56 and controls over seven days after treatment.

	Mortality±SEM* (%)			
Doses(conidia/ml)	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
$1x10^3$	6,00±8,94a***	10,00±7,07ab	20,00±7,07b	34,00±5,47b
$1x10^5$	2,00±4,47a	16,00±5,47bc	30,00±7,07bc	44,00±5,47b
$1x10^7$	2,00±4,47a	24,00±5,47cd	36,00±5,47cd	56,00±5,47c
$1x10^8$	2,00±4,47a	28,00±4,47de	44,00±5,47d	84,00±5,47d
$1x10^9$	0,00±0,00a	36,00±5,47e	56,00±5,47e	94,00±5,47d
Control	2,00±4,47a	2,00±4,47a	2,00±4,47a	8,00±4,47a

* SEM: Standard error of the mean; ** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different (P < 0.05).

Probit analysis was carried out to determine LC50. The parameters of the probit analysis and LC50 are given in Table 4. LC50 values confirmed that *S. granarius* was more susceptible to the isolate F-52 than the other two isolates F-53 and F-56 where the LC50's were $1x10^5$, $2x10^5$, and $5x10^5$ conidia/ml respectively (Table 4).

Table 4. Lethal concentrations (LC_{50}) values of the adult of *Sitophilus granarius* treated *Beauveria bassiana* isolates F-52, F-53, and F-56.

Isolates	Slope±SE	LC_{50} (95% fiducial limit)	χ^2
F-52	0,26±0,015	$1x10^5$ ($2.8x10^4$ – $4x10^5$)	91,5
F-53	0,34±0,016	$2x10^5$ ($6,0x10^4$ – $5x10^5$)	105,5
F-56	0,34±0,016	$5x10^5$ ($1,0x10^5$ – $1x10^6$)	121,9

[*] Slope value (±standart deviation) of dose-mortality respose of *Sitophilus granarius* to *Beauveria basiana* isolates F-52, F-53, and F-56.

[**] Pearson χ2 value (α=0.05)

CONCLUSIONS

It can be concluded that the isolates F-52 was more efficient against *S. granarius* than the isolates F-53 and F-56 and proved that F-52 had higher virulence than the others, All the three *B. bassiana* isolates have the potential for practical and economically feasible control of *S. granarius.*

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