



Insecticidal Activities of Four Native Entomopathogenic Fungus *Beauveria bassiana* Bals. (Vuill) Isolates Against *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) Adults Under Laboratory Conditions

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ARTICLE INFO	ABSTRACT
<p>[#]This study was presented as an oral presentation at the 5th International Anatolian Agriculture, Food, Environment and Biology Congress (Tokat, TARGID 2020)</p>	<p>In this work, control capacity of the four isolates (GN22-1, HP15, HP5-2, HP3-1) of entomopathogenic fungus <i>Beauveria bassiana</i> Bals. (Vuill) were evaluated against <i>Tribolium castaneum</i> (Herbst, 1797) (Coleoptera: Tenebrionidae) adults under laboratory conditions. To test the effect of each of the isolate on adults of <i>T. castaneum</i> were dipped into 1×10^8 conidia/ml suspension of each isolate. The data for mortality was recorded after 3rd, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th, 21st, and 23rd day. Thirteen days after application, isolates were listed as GN22-1 (72.85%), HP3-1 (48.88%), HP15 (47.37%) and HP5-2 (30.43%) based on the mortality rate they caused. Mortality rate was 83.52% at the end of the 23rd day with isolate GN22-1. While HP3-1 (53.74%) and HP15 (52.24%) caused more than 50% effect at the end of 23 days incubation period, the effect of HP5-2 remained only 32.51%. In addition, LT₅₀ and LT₉₀ rates were also determined. We arrive to the conclusion that especially GN22-1 isolate can has a potential in the control of this insect and may serve an alternative to chemical insecticides.</p>
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Introduction

Insect pest infestation can cause up to 40% loss in stored products worldwide. Red flour beetle, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) is a widespread and most destructive pest of stored products throughout the world. Both adults and larvae feed in a variety of materials containing flour, cereals, pasta, biscuits, beans, and nuts. Besides the product losses caused by feeding, this insect imparts a nauseous smell and taste to the infested material decreasing its nutritive value (Karunakaran et al., 2004). Attempts to control of stored products pests relies on physical control and mostly fumigants, such as methyl bromide or phosphine. Research has shown that synthetic insecticides are extremely toxic to non-target species and have harmful environmental impacts. Also, harmful insects have developed resistance to many pesticides. Hence, alternatives and more safer control methods should be developed (Upadhyay and Ahmad, 2011). Biological control efforts are the most important issue for achieve this goal. This method involves

the use of natural enemies such as parasites, predators, and pathogens.

Entomopathogenic fungi (EPF) are widespread in terrestrial ecosystems and play an important role in the regulation of insect populations. There are about 90 genera and 700 species with entomopathogenicity (Roberts and Humber, 1981). Insect fungal pathogens have a wide variety of hosts, can be mass-produced simply, rabidly, economically, and can be used with the same technological means as traditional contact insecticides. In this context, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarrhizium anisopliae* (Metschn.) Sorokin 1883 are the most studied fungal species for the control of stored-product insect species (Rumbos and Athanassiou, 2017). The anamorphic entomopathogenic fungus *B. bassiana* is natural enemies of a wide range of insects and arachnids and has a cosmopolitan distribution. This fungus has been documented to occur naturally in more than 700 species of insect hosts (Imoulan et al., 2017).

Beauveria bassiana has been tested against most of the major stored-product insect species (mostly coleopteran pest and only a few species lepidopteran pest), under various types of conditions and product in the world (Rumbos and Athanassiou, 2017). Some studies were performed to assess the ability of *B. bassiana* as a bioinsecticide for various stored product pests in Turkey. Most of these studies have mostly focused on coleopteran pests (Sevim et al., 2015; Er et al., 2016; Atay et al., 2017; Ak, 2019; Özdemir et al., 2020). This study was conducted to assess the efficacy of four entomopathogenic fungus *Beauveria bassiana* Bals. (Vuill) isolates (GN22-1, HP15, HP5-2, HP3-1) of 1×10^8 conidia/ml concentrations against *Tribolium castaneum* adults under laboratory conditions.

Material and Methods

Insect Culture

Insects from a stock culture of *Tribolium castaneum* (Tokat Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection in Tokat, Turkey) were obtained that reared in the laboratory on broken wheat at $28 \pm 2^\circ\text{C}$ and 65–75% R.H.

Fungal Isolates

For current study, 4 isolates of *B. bassiana* (GN22-1, HP15, HP5-2, HP3-1) were chosen from the fungal culture collection of the Mycology Laboratory at the Tokat Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection in Tokat, Turkey. These isolates were originally isolated from naturally infected *Hypera postica* (Gyllenhal 1813) (Coleoptera, Curculionidae) and *Gonioctena fornicata* (Brüggemann 1873) (Coleoptera, Chrysomelidae) adults collected from alfalfa fields in Tokat Province, Turkey.

Inoculum Preparation from Entomopathogenic Fungal Isolates

Fungal isolates were cultured on Potato Dextrose Agar (PDA) in Petri dishes and incubated at $25 \pm 2^\circ\text{C}$ with a 16/8 (L/D) photoperiod. Spores were harvested from 17 day-old cultures with 10 ml of sterilized water containing 0.02% Tween 80. The conidial suspensions were filtered through 3 sheets of sterile cheesecloth to eliminate particles and then spore suspension from each isolate was adjusted to 1×10^8 (Saruhan et al., 2017).

Application of Fungal Inoculum

Tribolium castaneum adults were dipped into 1×10^8 conidia/ml suspension of each isolate for 7–8 sec. and placed in Petri dishes containing broken wheat as a food. The experiment was laid out in completely randomized design with 9 replications and 10 adults were used for each replication. In control, adults were treated with 0.02% aqueous solution of Tween 80 (v/v). The treated adults were maintained at $25 \pm 2^\circ\text{C}$ with a 16/8 (L/D) photoperiod. The mortality was recorded up to 23 day of treatment with an interval of two days. Dead insects were separated from the Petri dishes and taken to another Petri dishes with a moist filter paper and mycelium development was observed.

Statistical Analysis

The raw data was first converted to % mortality values and then exposed to Arcsine transformation. All the data were analysed using analysis of variance (ANOVA) and the means were compared with Tukey's multiple comparison test at 5% significant level. All statistical analyses were carried out by using the MINITAB Release 16 packet program. LT₅₀ and LT₉₀ values were calculated by using the Probit analysis.

Results and Discussion

The four *Beauveria bassiana* fungal isolates were tested against *Tribolium castaneum* adults at a concentration of 1×10^8 conidia/ml. Mortality of *T. castaneum* in all isolates was significantly different from each other. Table 1 shows that the entomopathogenic fungal isolates produced different insecticidal activity rates after 3 days post inoculation. Long exposure interval had a positive effect on the mortality rate of *T. castaneum*. Among the entomopathogenic fungal isolates, GN22-1 (51.07%) showed the highest insecticidal activity against *T. castaneum* at the end of day 9, followed by HP3-1 (44.37%), HP15 (40.67%), and HP5-2 (23.68%). Thirteen days after the application, isolates were listed as GN22-1 (72.85%), HP3-1 (48.88%), HP15 (47.37%), and HP5-2 (30.43%) according to the mortality rates they caused. Mortality rate of isolate GN22-1 was 83.52% at the end of the 23rd day. While HP3-1 (53.74%) and HP15 (52.24%) caused more than 50% effect at the end of 23 days incubation period, the effect of HP5-2 remained only 32.51% (Table 1).

Table 1. Mortality of *Tribolium castaneum* adults exposed to Turkish isolates of the entomopathogen *Beauveria bassiana*

Days/Isolates	Mortality \pm SEM*(%)				
	HP5-2	HP3-1	GN22-1	HP-15	Control
3	2.03 \pm 1.01 ^{b**}	13.01 \pm 0.18 ^a	6.91 \pm 0.87 ^{ab}	12.73 \pm 0.82 ^a	0.00 \pm 0.00 ^c
5	5.93 \pm 1.31 ^b	26.05 \pm 0.49 ^a	21.86 \pm 0.25 ^a	23.93 \pm 0.39 ^a	0.00 \pm 0.00 ^c
7	14.20 \pm 1.75 ^b	35.31 \pm 0.31 ^a	38.76 \pm 0.23 ^a	31.78 \pm 0.49 ^a	0.00 \pm 0.00 ^c
9	23.68 \pm 0.55 ^b	44.37 \pm 0.28 ^a	51.07 \pm 0.40 ^a	40.67 \pm 0.83 ^a	0.00 \pm 0.00 ^c
11	28.30 \pm 0.52 ^c	48.88 \pm 0.50 ^{ab}	63.62 \pm 0.40 ^a	43.98 \pm 0.99 ^b	0.00 \pm 0.00 ^d
13	30.43 \pm 0.63 ^c	48.88 \pm 0.50 ^b	72.85 \pm 0.56 ^a	47.37 \pm 1.24 ^b	0.00 \pm 0.00 ^d
15	30.43 \pm 0.63 ^b	50.05 \pm 0.65 ^b	78.37 \pm 1.69 ^a	47.37 \pm 1.24 ^b	0.00 \pm 0.00 ^c
17	31.46 \pm 0.76 ^b	50.05 \pm 0.65 ^b	81.69 \pm 1.66 ^a	47.37 \pm 1.24 ^b	0.51 \pm 0.71 ^c
19	32.51 \pm 0.97 ^b	51.34 \pm 0.91 ^b	81.70 \pm 1.66 ^a	47.38 \pm 1.24 ^b	2.03 \pm 1.01 ^c
21	32.51 \pm 0.97 ^b	51.34 \pm 0.91 ^b	82.68 \pm 1.57 ^a	48.50 \pm 1.21 ^b	3.16 \pm 1.01 ^c
23	32.51 \pm 0.97 ^b	53.74 \pm 1.08 ^b	83.52 \pm 1.30 ^a	52.24 \pm 0.81 ^b	4.53 \pm 0.91 ^c

* SEM: Standard error of the mean, ** Means in a line followed by the same letter are not significantly different (ANOVA, P<0.05; Tukey's test).

Table 2. Lethal time (LT₅₀ and LT₉₀) values (days) of *Tribolium castaneum* adults treated with isolates of the entomopathogenic fungus *Beauveria bassiana*

Isolates	Slope±SE	LT ₅₀ (95% fiducial limit)	LT ₉₀ (95% fiducial limit)	chi-square
HP5-2	0.044±0.007	28.813 (24.749-36.426)	58,043 (47.053-79.505)	78.735
HP3-1	0.043±0.007	17.186 (15.236-19.928)	46.844 (38.909-61.436)	74.579
GN22-1	0.103±0.007	10.327 (9.331-11.229)	22.808 (21.219-24.875)	10.29
HP15	0.043±0.007	18,615 (16.437-21.966)	48,581 (39.975-64.900)	99.394

When the LT₅₀ values of the isolates applied in the study were analysed, the most effective isolate was GN22-1 (10.327 days) and this followed by HP3-1 (17.186), HP-15 (18.615) and HP5-2 (28.813), respectively. LT₉₀ rates were ranged like those of the LT₅₀ (Table 2).

Main effects of different EPF isolates and exposure intervals on percent mortality of *T. castaneum* were highly significant. The isolates used in this study did not cause mortality more than 39% on the 7th day. Akmal et al. (2020) observed a low effect (32.5%) against *T. castaneum* adults 7th day after the application with 5×10⁸ spores/ml concentration of *B. bassiana*. GN22-1 has started to show a significant effect from the 13th day and this effect increased to over 80% on the 17th day. The effect of all other isolates did not exceed 55% at the end of the 23rd day. Similarly, Moore et al. (2000) stated that the virulence of fungal strains differed significantly against stored grain insect pests. Padin et al. (2002) reported that *B. bassiana* had no significant insecticidal effect on *T. castaneum* by exposing pest-infested wheat and bean seeds to conidia of *B. bassiana*. Rice and Cogburn (1999), recorded an efficiency more than 80% *B. bassiana* isolate against *T. castaneum* adult at 21 days after treatment. Similarly, in this study we revealed 82% mortality with isolates GN22-1 at the end of 21 days. On the other hand, Rizwan et al. (2019) reported that *B. bassiana*-treated wheat gave mortality rate with 31.67% against *T. castaneum* at the highest concentration (1×10⁸ conidia kg⁻¹ of wheat) after 21-day exposure time. Also, that study, revealed an additive effect of *B. bassiana*, when used with diatomaceous earth against adults of this pest. In our study all isolates used showed that more than effect 32.5% effect at the end of 21 days. This differences between two studies might be related to the variation in virulence of the isolates tested in both studies.

We arrive to the conclusion that especially GN22-1 isolate can has a potential in the control of this insect and may serve an alternative to chemical insecticides.

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