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Orijinal araştırma (Original article)

Susceptibility of lucerne beetle [*Gonioctena fornicata* (Brüggemann) (Coleoptera, Chrysomelidae)] larvae to some local enthomopathogenic fungal isolates under laboratory conditions[1]

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Yonca yaprak böceğinin [*Gonioctena fornicata* (Brüggemann) (Coleoptera: Chrysomelidae)] bazı yerel entomopatojen fungus izolatlarına karşı laboratuvar şartlarında duyarlılık düzeyleri

Öz: Tokat ili yonca alanlarından toplanan *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) ve *Gonioctena fornicata* (Brüggemann) (Coleoptera, Chrysomelidae) erginlerinden elde edilen *Beauveria bassiana* (Balsamo) Vuillemin izolatlarının *G. fornicata*'nın larvasına karşı laboratuvar şartlarında etkinliği araştırılmıştır. On *B. bassiana* izolatı tek dozda ($1x10^7$ konidia/ml) *G. fornicata* larvalarına karşı test edilmiştir. 7.günde %90'nın üzerinde etkiye sahip olduğu belirtlenen 4 izolat ile [GN-8-2, GN-4, GN-8-1(2) GN-12-3] $1x10^5$, $1x10^8$ ve $1x10^9$ konidia/ml dozları kulanarak doz ölüm çalışmaları yürütülmüştür. Doz ölüm çalışmalarında, 1×10^9 konidi/ml dozunda 7. günde hemen hemen tüm izolatlar %100 etki göştermiştir. 1×10^8 konidi/ml dozunda LT_{50} ve LT_{90} değerleri belirlenmiştir. Elde edilen sonuçlara göre *G. fornicata* larvalarının doz-ölüm çalışmalarında kullanılan tüm *B. bassiana* izolatlarına karşı hassas olduğu belirlenmiştir.

Anahtar kelimeler: Entomopatojenik fungus, *Beauveria bassiana*, etkinlik, *Gonioctena fornicata*

Abstract: The infectivity of *Beauveria bassiana* (Balsamo) Vuillemin isolates from *Hypera postica* (Gyllenhal) (Coleoptera, Curculionidae) and *Gonioctena fornicata* (Brüggemann) (Coleoptera, Chrysomelidae) collected from alfalfa fields in Tokat Province, Turkey for *G. fornicata* larvae were evaluated under laboratory conditions. Ten *B. bassiana* isolates were used in single screening test to determine their efficacy against *G. fornicata* larvae at a concentration of 1×10^7 conidia/ml. In addition, dose-mortality tests were carried out with the isolates GN-8-2, GN-4, GN-8-1(2) and GN- 12-3. They caused more than 90% mortality at 7 days post-treatment at doses of $1x10^5$, $1x10^8$ and $1x10^9$ conidia/ml. In the dose-mortality tests, almost all isolates caused 100% mortality at $1x10^9$ conidia/ml at 7 days. The LT_{50} and LT_{90} rates at 1×10^8 conidia/ml were also determined. In summary, the *Gonioctena fornicata* larvae were susceptible to all the *B. bassiana* isolates used in the dose- mortality studies.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, effects, *Gonioctena fornicata*

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Introduction

Forage crop cultivation helps sustain animal production (Yolcu & Tan, 2008). Alfalfa (*Medicago sativa* L. Fabaceae) is an important forage crop in many countries throughout the world (Bates, 1998). The lucerne beetle, *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera, Chrysomelidae) causes significant damage to alfalfa and other Fabaceae species. Adults and larvae feed on the leaves, flowers, buds and young shoots (Grigorov, 1976; Çam & Atay, 2006; Atanasova & Semerdjieva, 2009).

Alkan (1946) first recorded the presence and damage of *G. fornicata* in Turkey. Later researchers reported on the presence, biology and damage of *G. fornicata* in different provinces of Turkey (Yıldırım et al, 1996; Coşkuncu & Gençer, 2006). Çam & Atay (2006) stated that larvae and adults cause major damage by feeding on the leaves and stems of alfalfa in Tokat Province, Turkey.

So far, no chemical insecticide has been registered for the control of *G. fornicata* in Turkey. Early and frequent harvesting of alfalfa crops may cause mortality of the pest. There have also been some biological control studies. In particular, entomopathogenic fungi and some parasitoids contribute to the regulation of *G. fornicata* populations. Atay et al (2015) stated that 36% of overwintered *G. fornicata* adults in alfalfa growing areas of Tokat Province in Turkey were naturally infested with *Beauveria* spp. In addition, Atay (2018), reported that larvae of *G. fornicata* were parasitized by *Meigenia mutabilis* (Fallén, 1810) and *Macquartia tenebricosa* (Meigen, 1824) (Diptera: Tachinidae) in Tokat Province in Turkey.

Having an average lifespan of approximately 5 years, alfalfa cropping can provide a relatively stable environment. Using chemicals against alfalfa pests have undesirable effects on non-target organisms including pollinators and natural enemies. Therefore, environmentally friendly pest management practices are required. The application of entomopathogenic fungi in the biocontrol of insects has come to prominence because of environmental and food safety concerns (Sinha et al, 2016; Reddy et al, 2016).

There are approximately 90 genera and 700 species of entomopathogenic fungi (Roberts & Humber, 1981). Among them, species of *Beauveria*, *Metarhizium*, *Lecanicillium* and *Isaria* have been produced commercially (Vega et al, 2009). *Beauveria bassiana*, which attacks its host by causing acute mycoses, has been isolated and tested against different pests in various cropping systems (Rahman et al, 2010). This enthomopathogenic fungus has been reported from 707 insect host species, including 521 genera from 149 families of 15 orders (Imoulan et al, 2017).

Several studies have been conducted to determine the potential of *B. bassiana* as a bioinsecticide against various insect pests in Turkey. These studies have mostly focused on lepidopteran pests, including *Tuta absoluta* (Gelechiidae) (Inanlı et al, 2012; Yüksel et al, 2017), *Ostrinia nubilalis* (Hubner) (Pyralidae) (Demir et al, 2012), *Sesamia cretica* (Noctuidae) (Yanar et al, 2016), *Zeuzera pyrina* L.

(Cossidae) (Öztop et al, 2016), *Hyphantria cunea* Durry (Arctidae) (Saruhan et al, 2017) and coleopteran pests, including *Plagiodera versicolora* (Laicharting, 1781)] (Demir et al, 2013), *Leptinotarsa decemlineata* Say. (Güven et al, 2015; Yanar et al, 2017), *Hypera postica* (Curculionidae) (Atay et al, 2015; Yücel et al, 2015; 2018; Baysal et al, 2018), *Rhynchites bacchus* L. (Rhynchitidae) (Sevim et al, 2014), *Tribolium confusum* Duv. (Tenebrionidae) (Komaki et al, 2017) and *Sitophilus granarius* (L.) (Curculionidae) (Atay & Yanar, 2016; Çam et al, 2017).

There have been several studies on the microbial control of *G. fornicata* with enthomopathogenic fungi in Turkey (Atay et al, 2015; 2017a; 2017b; Yanar et al, 2018). The aim of this study was to determine the infectivity of 10 local *B. bassiana* isolates, obtained from *H. postica* and *G. fornicata* collected from alfalfa fields in Tokat Province, against the larvae of the lucerne beetle (*G. fornicata*) under laboratory conditions.

Materials and Methods

Isolation of fungi

Hibernating adults of *Hypera postica* and *Gonioctena fornicata* were collected from alfalfa fields in Tokat Province during April and May, 2015 (Table 1). They were brought to the laboratory with fresh alfalfa plants and transferred to separate cages 19.5 cm in diameter. The insects were checked daily and the dead adults were transported to a moist chamber for 7 days to induce fungal sporulation. Naturally infected adults were also transferred from the alfalfa fields to the laboratory. Fungi were isolated from mycosed adults. Single-spore isolates of all the isolates were obtained by serial dilution (Dhingra & Sinclair, 1995) and were identified as *B. bassiana*. A total of ten *B. bassiana* isolates were obtained from the field collections of *H. postica* and *G. fornicata* adults (Table 1). They were deposited in the fungal culture collection of the Mycology Laboratory at the Tokat Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection in Tokat, Turkey.

Table 1. Hosts and locations of collection of the tested entomopathogenic *Beauveria bassiana* isolates

Isolates	Locations				Host
	Location	Coordinates		Altitude (m)	
		N	E		
GN-23	Gümenek, Tokat, Turkey	40° 21 ' 56"	36° 38 ' 39"	637	*G.fornicata*
GN-20-2	Yağmurlu, Tokat, Turkey	40° 30 ' 51"	36° 49 ' 17"	829	*G.fornicata*
GN-12-3	Emirseyit, Tokat, Turkey	40° 20 ' 16"	36 ° 24 ' 21"	572	*G.fornicata*
GN-1	Ulaş, Tokat, Turkey	40° 19 ' 18"	36° 26 ' 12"	600	*G.fornicata*
GN-4	Güryıldız, Tokat, Turkey	40° 19 ' 58"	36 ° 22 ' 35"	582	*G.fornicata*
GN-5-2	Güryıldız, Tokat, Turkey	40° 19 ' 49"	36 ° 22 ' 04"	525	*G.fornicata*
GN-8-2	Büyükyıldız, Tokat, Turkey	40° 20 ' 12"	36 ° 23 ' 37"	567	*G.fornicata*
GN-8-1(2)	Büyükyıldız, Tokat, Turkey	40° 20 ' 12"	36 ° 23 ' 37"	567	*G.fornicata*
HP-30	Bedirkale, Tokat, Turkey	40° 03 ' 56"	36° 26 ' 48"	1133	*H.postica*
HP-6	Güryıldız, Tokat, Turkey	40° 19 ' 45"	36 ° 21 ' 40"	585	*H.postica*

Insect culture

The lucerne beetle larvae used in tests were collected from alfalfa fields in Ballıdere and Ulaş villages of Tokat Province in May, 2016.

Inoculum preparation from entomopathogenic fungal isolates

Fungal isolates were subcultured on PDA (Potato Dextrose Agar) medium at 25±2°C for 17 days. Spores were harvested with 10 ml of sterilized water containing 0.02% Tween 80. Conidia suspensions were filtered through 3 layers of sterile cheesecloth to remove particles. The number of spores in suspensions was determined with a hemocytometer under a light microscope. The spore concentration of each isolate were then adjusted to 1×10^5, 1×10^7, 1×10^8 or 1×10^9 conidia/ml by dilution from the stock suspensions (Saruhan et al, 2017).

Application of Fungal Inoculum and Larval Mortality Experiments

Screening tests were conducted with ten isolates against *G. fornicata* larvae at $1x10^7$ conidia/ml concentration. *G. fornicata* larvae were dipped into conidial suspension of 1×10^7 conidia/ml of each isolate for 4-5 sec and placed in a Petri dish (10 larvae per dish) containing fresh alfalfa leaves. Death rates were recorded on the 1st, 3rd, 5th and 7th days post-treatment. Dose-mortality tests were carried out with four isolates, namely GN-8-2, GN-4, GN-8-1(2) and GN-12-3, which had been determined to be highly effective, using doses of $1x10^5$, $1x10^8$ and $1x10^9$ conidia/ml. The experiments were carried out with a completely randomized design, with 3 replications, and they were repeated twice.

Statistical analysis

Data was analyzed with analysis of variance (ANOVA) and the means were compared with Tukey's multiple comparison test. All statistical analyses were carried out by using the MINITAB Release 16 packet program. LT_{50} and LT_{90} values were calculated by using the Probit analysis.

Results and Discussion

The ten entomopathogenic fungal isolates were tested against *G. fornicata* larvae at a concentration of $1x10^7$conidia/ml. At 7 days, GN-8-2, GN-4, GN-8-1(2) and GN-12-3 had more than than 90% efficacy (Table 2). Therefore, dose-mortality tests were carried out with these isolates, using doses of $1x10^5$, $1x10^8$ and $1x10^9$ conidia/ml.

Table 2. Mortality of *Gonioctena fornicata* exposed to Turkish isolates of the entomopathogen *Beauveria bassiana*

	Mortality±SEM*(%)			
Isolates	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
GN-8-2	31.45±0.28a***	68.72±0.48a	95.73±2.19a	99.71±0.70a
GN-4	9.15±4.51abc	44.97±0.29abc	83.96±0.47ab	99.71±0.70a
GN-8-1(2)	26.20±0.53ab	65.57±0.88a	69.36±1.17bcd	96.63±1.75a
GN-12-3	1.15±1.12c	22.82±0.43cd	49.67±1.08defg	93.30±1.82ab
HP-30	4.27±2.19bc	29.50±0.60cd	58.68±0.64cdefg	79.14±0.65bc
GN-23	1.70±1.74c	24.83±0.16cd	43.31±0.11efg	77.34±0.62bc
GN-5-2	0.00±0.00c	34.85±0.30cd	58.49±0.42cdefg	70.50±0.60c
GN-20-2	3.36±1.75bc	29.83±0.20cd	65.08±0.14bcde	66.98±0.49c
HP-6	0.29±0.70c	4.27±2.19ef	38.22±0.25fg	63.48±0.29c
GN1	3.36±1.75bc	17.48±0.66de	34.92±0.14g	58.57±0.75c
Control	0.00±0.00c	1.15±1.12f	2.57±1.25h	11.46±0.14d

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

According to the results of the dose-mortality tests, all isolates had a significant effect at $1x10^8$ and $1x10^9$ conidia/ml three days after inoculation. Effectiveness increased with increase of incubation period. By day 5, all isolates caused over 80% mortality at $1x10^9$ conidia/ml. At day 7, almost all isolates had 100% efficacy at $1x10^9$ conidia/ml. At the same time, almost all other doses of the isolates, except $1x10^5$ conidia/ml of GN-4 and $1x10^5$ conidia/ml of GN-12-3, had an efficacy of over 70% (Tables 3, 4, 5, 6).

Table 3. Mortality of *Gonioctena fornicata* exposed to four concentrations of the isolate GN-8-2 and control over 7 days from treatment

	Mortality±SEM* (%)			
Doses	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
1x10^5	21.52±2.01ab***	39.79±0.68c	75.86±0.83b	86.10±1.68b
1x10^8	33.02±0.49a	68.79±0.64ab	94.72±2.58ab	99.40±1.45a
1x10^9	16.36±0.22ab	84.87±2.08a	99.40±1.45a	99.71±0.70a
Control	0.00±0.00c	1.15±1.12d	2.57±1.25c	11.46±0.14c

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

Table 4. Mortality of *Gonioctena fornicata* exposed to four concentrations of the GN-4 isolate and control over 7 days from treatment

	Mortality±SEM* (%)			
Doses	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
1x10^5	2.57±1.26bc***	31.28±0.48bc	51.75±0.41c	67.39±1.06b
1x10^8	5.28±2.58bc	55.17±0.64b	93.52±2.00a	98.85±1.12a
1x10^9	53.35±0.28a	83.32±2.12a	98.30±1.74a	100±0.00a
Control	0.00±0.00c	1.15±1.12d	2.57±1.25d	11.46±0.14c

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

Table 5. Mortality of *Gonioctena fornicata* exposed to four concentrations of the GN-8-1(2) isolate and control over 7 days from treatment

	Mortality±SEM* (%)			
Doses	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
1x10^5	3.37±1.75b***	21.21±0.37c	50.00±0.33c	75.54±0.46b
1x10^8	21.21±0.37a	73.48±0.15a	82.19±0.40b	83.96±0.47b
1x10^9	27.92±0.48a	69.12±0.80a	97.43±1.25a	100.00±0.00a
Control	0.00±0.00c	1.15±1.12d	2.57±1.25d	11.46±0.14c

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

Table 6. Mortality of *Gonioctena fornicata* exposed to four concentrations of GN-12-3 isolate and controls over 7 days from treatment

	Mortality±SEM* (%)			
Doses	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
1x10^5	0.60±1.45ab***	15.26±1.92c	48.32±0.40bc	59.68±2.92b
1x10^8	0.29±0.70ab	41.58±0.24ab	60.14±0.34b	95.47±1.12a
1x10^9	7.02±0.70a	53.43±0.45a	82.52±0.66a	98.86±1.12a
Control	0.00±0.00b	1.15±1.12d	2.57±1.25d	11.46±0.14c

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

When the LT_{50} rates of the isolates used in the study were compared, the most effective was GN8-2 (1.904 days). This was followed by GN8-1(2) (2.406 days),

GN-4 (3.101 days) and GN-12-3 (4.089 days). LT_{90} values for the isolates of GN8-2, GN-4, GN8-1(2) and GN-12-3 were 4.673, 5.377, 6.475 and 6.662 days, respectively (Table 7).

Table 7. Lethal time (LT_{50} and LT_{90}) values (days) of *Gonioctena fornicata* larvae treated with isolates of the entomopathogenic fungus *Beauveria bassiana*

Isolates	**Slope±SE**	**LT_{50} (95% fiducial limit)**	**LT_{90} (95% fiducial limit)**	χ^2
GN-4	0.563±0.066	3.101 (2.689-3.512)	5.377 (4.819-6.184)	0.92
GN-8-1(2)	0.315±0.045	2.406 (1.632-2.997)	6.475 (5.637-7.829)	0.87
GN-8-2	0.463±0.062	1.904 (1.313-2.371)	4.673 (4.092-5.544)	0.67
GN-12-3	0.498±0.054	4.089 (3.692-4.486)	6.662 (6.089-7.474)	0.67

Previous studies conducted in Turkey with *Beauveria bassiana* isolates have also showed a high efficacy against *G. fornicata*. Atay et al (2017a) tested 4 isolates (GN-23, GN-4, GN5-2, GN8-1) of *B. bassiana* against adults of *G. fornicata* at five different concentrations (1×10^3, 1×10^5, 1×10^7, 1×10^8 and 1×10^9 conidia/ml) and reported that all isolates showed an efficacy of more than 85% at day 13. In addition, Atay et al (2017b) evaluated the effectiveness of 16 *B. bassiana* isolates against *G. fornicata* adults at 1×10^9 conidia/ml and reported that 8 isolates had an efficacy of more than 90% at day 13. In another study, Yanar et al (2018) stated that a conidial suspension of 1×10^9 conidia/ml of the GOPT-228 isolate of *B. bassiana* caused 90% mortality of *G. fornicata* adults at 7 days after treatment.

Also, some isolates of *B. bassiana* have been effective against some other pests. Baysal et al (2018) tested 4 isolates (GN-23, GN-4, HP-30, HP-6) of *B. bassiana* on larvae of *H. postica* at 1×10^3, 1×10^5 1×10^7 and 1×10^9 conidia/ml and reported that on day 7 the mortality rate reached 100% for almost all isolates at 1×10^7 and 1×10^9 conidia/ml. Similarly, Atay & Yanar (2016) reported that conidial suspensions of 1×10^8 conidia/ml of 3 isolates of *B. bassiana* had caused the mortality of more than 70% of adults of *Sitophilus granarius* at 14 days post treatment.

In this study, the entomopathogenic potential of ten *B. bassiana* isolates against *G. fornicata* larvae was determined under laboratory conditions. In general, the local isolates of *B. bassiana* applied to *G. fornicata* larvae in dose-mortality tests significantly reduced their numbers but the isolates need to be tested under field conditions.

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