

**Orijinal araştırma (Original article)**

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

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**Yonca yaprak böceğiinin [*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 ( $1 \times 10^7$  konidia/ml) *G. fornicata* larvalarına karşı test edilmiştir. 7. günde %90'nın üzerinde etkiye sahip olduğu belirtilen 4 izolat ile [GN-8-2, GN-4, GN-8-1(2) GN-12-3]  $1 \times 10^5$ ,  $1 \times 10^8$  ve  $1 \times 10^9$  konidia/ml dozları kulanarak doz ölüm çalışmaları yürütülmüştür. Doz ölüm çalışmalarında,  $1 \times 10^9$  konidi/ml dozunda 7. günde hemen hemen tüm izolatlar %100 etki göstermiştir.  $1 \times 10^8$  konidi/ml dozunda LT<sub>50</sub> ve LT<sub>90</sub> 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  $1 \times 10^7$  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  $1 \times 10^5$ ,  $1 \times 10^8$  and  $1 \times 10^9$  conidia/ml. In the dose-mortality tests, almost all isolates caused 100% mortality at  $1 \times 10^9$  conidia/ml at 7 days. The LT<sub>50</sub> and LT<sub>90</sub> rates at  $1 \times 10^8$  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 entomopathogenic 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 *Plagiодera 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 entomopathogenic 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.

### Susceptibility of *Gonioctena fornicata* larvae to entomopathogenic fungal isolates

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

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

### 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  $1\times10^5$ ,  $1\times10^7$ ,  $1\times10^8$  or  $1\times10^9$  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  $1\times10^7$  conidia/ml concentration. *G. fornicata* larvae were dipped into conidial suspension of  $1\times10^7$  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 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> 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  $1\times10^5$ ,  $1\times10^8$  and  $1\times10^9$  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<sub>50</sub> and LT<sub>90</sub> 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  $1 \times 10^7$  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  $1 \times 10^5$ ,  $1 \times 10^8$  and  $1 \times 10^9$  conidia/ml.

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

Isolates	Mortality $\pm$ SEM*(%)			
	1 DAT**	3 DAT	5 DAT	7 DAT
<b>GN-8-2</b>	31.45 $\pm$ 0.28a***	68.72 $\pm$ 0.48a	95.73 $\pm$ 2.19a	99.71 $\pm$ 0.70a
<b>GN-4</b>	9.15 $\pm$ 4.51abc	44.97 $\pm$ 0.29abc	83.96 $\pm$ 0.47ab	99.71 $\pm$ 0.70a
<b>GN-8-1(2)</b>	26.20 $\pm$ 0.53ab	65.57 $\pm$ 0.88a	69.36 $\pm$ 1.17bcd	96.63 $\pm$ 1.75a
<b>GN-12-3</b>	1.15 $\pm$ 1.12c	22.82 $\pm$ 0.43cd	49.67 $\pm$ 1.08defg	93.30 $\pm$ 1.82ab
<b>HP-30</b>	4.27 $\pm$ 2.19bc	29.50 $\pm$ 0.60cd	58.68 $\pm$ 0.64cdefg	79.14 $\pm$ 0.65bc
<b>GN-23</b>	1.70 $\pm$ 1.74c	24.83 $\pm$ 0.16cd	43.31 $\pm$ 0.11efg	77.34 $\pm$ 0.62bc
<b>GN-5-2</b>	0.00 $\pm$ 0.00c	34.85 $\pm$ 0.30cd	58.49 $\pm$ 0.42cdefg	70.50 $\pm$ 0.60c
<b>GN-20-2</b>	3.36 $\pm$ 1.75bc	29.83 $\pm$ 0.20cd	65.08 $\pm$ 0.14bcde	66.98 $\pm$ 0.49c
<b>HP-6</b>	0.29 $\pm$ 0.70c	4.27 $\pm$ 2.19ef	38.22 $\pm$ 0.25fg	63.48 $\pm$ 0.29c
<b>GN1</b>	3.36 $\pm$ 1.75bc	17.48 $\pm$ 0.66de	34.92 $\pm$ 0.14g	58.57 $\pm$ 0.75c
<b>Control</b>	0.00 $\pm$ 0.00c	1.15 $\pm$ 1.12f	2.57 $\pm$ 1.25h	11.46 $\pm$ 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  $1 \times 10^8$  and  $1 \times 10^9$  conidia/ml three days after inoculation. Effectiveness increased with increase of incubation period. By day 5, all isolates caused over 80% mortality at  $1 \times 10^9$  conidia/ml. At day 7, almost all isolates had 100% efficacy at  $1 \times 10^9$  conidia/ml. At the same time, almost all other doses of the isolates, except  $1 \times 10^5$  conidia/ml of GN-4 and  $1 \times 10^5$  conidia/ml of GN-12-3, had an efficacy of over 70% (Tables 3, 4, 5, 6).

**Susceptibility of *Gonioctena fornicata* larvae to entomopathogenic fungal isolates**

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

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

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

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

<b>Doses</b>	<b>Mortality<math>\pm</math>SEM* (%)</b>			
	<b>1 DAT**</b>	<b>3 DAT</b>	<b>5 DAT</b>	<b>7 DAT</b>
<b>1x10<sup>5</sup></b>	0.60 $\pm$ 1.45ab***	15.26 $\pm$ 1.92c	48.32 $\pm$ 0.40bc	59.68 $\pm$ 2.92b
<b>1x10<sup>8</sup></b>	0.29 $\pm$ 0.70ab	41.58 $\pm$ 0.24ab	60.14 $\pm$ 0.34b	95.47 $\pm$ 1.12a
<b>1x10<sup>9</sup></b>	7.02 $\pm$ 0.70a	53.43 $\pm$ 0.45a	82.52 $\pm$ 0.66a	98.86 $\pm$ 1.12a
<b>Control</b>	0.00 $\pm$ 0.00b	1.15 $\pm$ 1.12d	2.57 $\pm$ 1.25d	11.46 $\pm$ 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<sub>50</sub> 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<sub>90</sub> 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<sub>50</sub> and LT<sub>90</sub>) values (days) of *Gonioctena fornicata* larvae treated with isolates of the entomopathogenic fungus *Beauveria bassiana*

Isolates	Slope $\pm$ SE	LT <sub>50</sub> (95% fiducial limit)	LT <sub>90</sub> (95% fiducial limit)	$\chi^2$
<b>GN-4</b>	0.563 $\pm$ 0.066	3.101 ( 2.689-3.512)	5.377 (4.819-6.184)	0.92
<b>GN-8-1(2)</b>	0.315 $\pm$ 0.045	2.406 (1.632-2.997)	6.475 (5.637-7.829)	0.87
<b>GN-8-2</b>	0.463 $\pm$ 0.062	1.904 (1.313-2.371)	4.673 (4.092-5.544)	0.67
<b>GN-12-3</b>	0.498 $\pm$ 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 ( $1\times 10^3$ ,  $1\times 10^5$ ,  $1\times 10^7$ ,  $1\times 10^8$  and  $1\times 10^9$  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  $1\times 10^9$  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  $1\times 10^9$  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  $1\times 10^3$ ,  $1\times 10^5$   $1\times 10^7$  and  $1\times 10^9$  conidia/ml and reported that on day 7 the mortality rate reached 100% for almost all isolates at  $1\times 10^7$  and  $1\times 10^9$  conidia/ml. Similarly, Atay & Yanar (2016) reported that conidial suspensions of  $1\times 10^8$  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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## References

Alkan B., 1946. Tarım Entomolojisi. T.C. Tarım Bakanlığı Ankara, Yüksek Ziraat Enstitüsü Ders Kitabı. 232 s.

Atanasova D.Y. & I.B. Semerdjieva, 2009. Population density of *Phytonomus variabilis* Hrbst. and *Phytodecta fornicata* Brugg. on multifoliolate and trifoliolate alfalfa in relation to anatomical characteristics on their leaves. *Journal of Central European Agriculture*, 10(4): 321-326

Atay T. & D. Yanar, 2016. Effects of entomopathogenic fungus isolates against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) under laboratory conditions. Turkey 6. Plant Protection Congress. 5-8 September 2016, Konya-Turkey, 72.

Atay T., D. Yanar, E. Baysal & Y. Yanar, 2017a. Pathogenicity of entomopathogenic fungus *Beauveria bassiana* Bals. (Vuill) isolates against *Gonioctena fornicata* (Brüggeman) (Coleoptera: Chrysomelidae). Uluslararası Katılımlı İç Anadolu Bölgesi 3. Tarım ve Gıda Kongresi, 26-28 Ekim 2017, Sivas, 106.

Atay T., D. Yanar & Y. Yanar, 2017b. Effects of entomopathogenic fungus isolates against *Gonioctena fornicata* (Brüggeman) (Coleoptera: Chrysomelidae) under laboratory conditions. Ecology 2017, International Symposium, 11-13 May 2017, Kayseri-Turkey, 141.

Atay T., Y. Yanar, E. Baysal & I. Kepenekci, 2015. Occurrence of entomopathogenic fungus *Beauveria* sp. on overwintered adults of *Hypera postica* (Gyllenhal) (Coleoptera, Curculionidae) and *Gonioctena fornicata* (Brüggemann) (Coleoptera, Chrysomelidae) in soil. 5th Entomopathogens and Microbial Control Congress. 09-11 September 2015, Ankara, 82.

Atay T., 2018. Tachinid (Diptera: Tachinidae) parasitoids of the lucerne beetle, *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae), with a new parasitoid record and their parasitism rates. *Türkiye Entomoloji Dergisi*, 42 (2): 141-147.

Bates G.E., 1998. Alfalfa: High-quality forage production. SP434-C. The University of Tennessee Extension, Knoxville. <https://extension.tennessee.edu/publications/Documents/sp434c.pdf>. Web Accessed 30 December 2018.

Baysal E., T. Atay & Y. Yanar, 2018. Efficacy of some local isolates of the fungus *Beauveria bassiana* (Balsamo) Vuillemin on the alfalfa weevil *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) larvae, under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 28 (65): 1-5.

Coşkuncu K.S. & N.S. Gençer, 2006. *Gonioctena fornicata* (Brüggeman) (Coleoptera: Chrysomelidae)'nın Bursa ili yonca ekiliş alanlarında biyolojisi, yayılışı ve popülasyon dalgalanması. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, 21(2): 15-19.

Çam H. & T. Atay, 2006. Tokat ili Chrysomelinae ve Cryptocephalinae (Coleoptera: Chrysomelidae) türleri üzerinde faunistik araştırmalar. *Türkiye Entomoloji Dergisi*, 30(4): 285-302.

Çam H., D. Yanar, Y. Yanar, M. Oztürk & M. Kılınç, 2017. Potential of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). 6th Entomopathogens and Microbial Control Congress.14-16 September 2017, Tokat, 31.

Demir I., E.S. Seçil, Z. Demirbağ & A. Sevim, 2012. Molecular characterization and virulence of the entomopathogenic fungus *Beauveria bassiana* from *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae). *Türkiye Entomoloji Bülteni*, 2 (1): 23-30.

Demir I., S. Kocaçevik, E. Dönmez, Z. Demirbağ & A. Sevim, 2013. Virulence of entomopathogenic fungi against *Plagiodera versicolora* (Laicharting, 1781) (Coleoptera: Chrysomelidae). *African Journal of Agricultural Research*, 8 (18): 2016-2021. .

Dhingra O.D. & J.B. Sinclair, 1995. Basic Plant Pathology Methods (2<sup>nd</sup> ed.). Boca Raton: CRC Press, 448 pp.

Grigorov S., 1976. Population dynamics of the most important useful and destructive insects on alfalfa in the Sofia area. *Rastitelnozashchitna Nauka*, 3: 50-63.

Güven O., D. Çayır, R. Baydar & I. Karaca, 2015. The effects of entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill isolates on Colorado potato beetle, [Leptinotarsa decemlineata Say. (Coleoptera: Chrysomelidae)]. *Turkish Journal of Biological Control* 6 (2): 105-114.

Imoulan A., M Hussain, P.M. Kirk, A. El Meziane & Y.J. Yao, 2017. Entomopathogenic fungus Beauveria: Host specificity, ecology and significance of morpho-molecular characterization in accurate taxonomic classification. *Journal of Asia-Pacific Entomology*, 20(4): 1204-1212.

Inanlı C., Z. Yoldaş & A.K. Birgucü, 2012. Effects of entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarrhizium anisopliae* (Metsch.) on larvae and egg stages of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 49 (39): 239-242.

Komaki A., Ş. Kordali, A. Usanmaz Bozhüyüük, H.H. Altınok, M. Kesdek, D. Şimşek & M.A. Altınok, 2017. Laboratory assessment for biological control of *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) by entomopathogenic fungi. *Türkiye Entomoloji Dergisi*, 41(1): 95-103.

Öztop A., M. Keçeci, I. Tekşam & A. Unlü, 2016. Investigations on control of Leopard Moth Borer (*Zeuzera pyrina* L. (Lepidoptera: Cossidae) in pomegranate orchards of Antalya province. *Derim*, 33(1):57-68.

Rahman K.M.A., M. Barta & L Cagáň, 2010. Effects of combining *Beauveria bassiana* and *Nosema pyrausta* on the mortality of *Ostrinia nubilalis*. *Central European Journal of Biology*, 5 (4): 472-480.

Reddy G.V.P., F.B. Antwi, G. Shrestha & Y. Kuriwada, 2016. Evaluation of toxicity of biorational insecticides against larvae of the alfalfa weevil. *Toxicology Reports* 3: 473-480.

Roberts D.W. & R.A. Humber, 1981. Entomogenous Fungi, (Editors: G. T. Cole & B. Kendrick, Biology of Conidial Fungi), Academic Press, New York, 201-236 pp.

Saruhan İ., S. Toksoz & İ. Erper, 2017. Evaluation of some entomopathogenic fungi against the fall webworm (*Hyphantria cunea* Durry, Lepidoptera: Arctidae). *Selcuk Journal of Agricultural and Food Sciences*, 31(2): 76-81.

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Sevim A., E. Sevim, I. Demir & Z. Demirbağ, 2014. Molecular characterization and pathogenicity of *Beauveria bassiana* Isolated from *Rhynchites bacchus* L. (Coleoptera: Rhynchitidae). *Nevşehir Journal of Science and Technology*, 3(2): 33-47.

Sinha K.K., A.K. Choudhary & P. Kumari, 2016. Entomopathogenic fungi, (Editor: Omkar, Ecofriendly Pest Management for Food Security), Academic Press, Newyork, 475-505 pp.

Vega F.E., M.S. Goettel, M. Blackwell, D. Chandler, M.A. Jackson, S. Keller, M. Koike, N.K. Maniania, A. Monzo'n, B.H. Ownley, J.K. Pell, D.E.N. Rangel, H.E. Roy, 2009. Fungal entomopathogens: new insights on their ecology. *Fungalecology*, 2:149-159.

Yanar Y., D. Yanar, T. Atay, M. Çankaya & E. Baysal, 2017. Effects of entomopathogenic fungi *Beauveria bassiana* isolates on Colorado Potato Beetle [*Leptinotarsa decemlineata* (Say)] (Coleoptera: Chrysomelidae). 6th Entomopathogens and Microbial Control Congress. 14-16 September 2017, Tokat, 39.

Yanar Y., D. Yanar, T. Atay, M. Yalçın & O. Beldek, 2016. Entomopathogen fungi isolates on Pink Corn Borer *Sesamia cretica* Led under laboratory conditions. VII International Scientific Agriculture Symposium, , Jahorina-Bosnia and Herzegovina, 347.

Yanar D., Y. Yanar & B. Solmaz, 2018. Efficacy of Entomopathogenic fungus *Beauveria bassiana* isolate GOPT-228 against *Gonioctena fornicata* (Bruggeman). IX International Agricultural Symposium, , 4-7 Ekim 2018, Jahorina-Bosnia and Herzegovina, 1084-1087.

Yıldırım E., I. Aslan & H. Özbek, 1996. Erzurum ve Erzincan illerinde önemli bir yonca (*Medicago sativa* L.) zararlısı, *Gonioctena fornicata* (Brüggemann) (Coleoptera, Chrysomelidae)'nın tanımı, biyolojisi ve zararı. Türkiye 3. Çayır-Mera ve Yem Bitkileri Kongresi, 17-19 Haziran 1996, Erzurum, 816-822.

Yolcu H. & M. Tan, 2008. Ülkemiz Yem Bitkileri Tarımına Genel Bir Bakış. *Tarım Bilimleri Dergisi*, 14(3): 303-312.

Yücel B., Z. Demirbağ, C. Gözüaçık & I. Demir, 2015. Enthomopathogenic fungi from the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae). 5<sup>th</sup> Enthomopathogens and Microbial Control Congress. 09-11 September 2015, Ankara, 76.

Yücel B., C. Gözüaçık, D. Gencer, I. Demir & Z. Demirbağ, 2018. Determination of fungal pathogens of *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae): Isolation, characterization, and susceptibility. *Egyptian Journal of Biological Pest Control*, (2018): 28-39

Yüksel, E., Ç. Açıkgöz, F. Demirci and M. Muştu, 2017. Effects of the entomopathogenic fungi, *Beauveria bassiana*, *Isaria farinosa* and *Purpureocillium lilacinum*, on eggs of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Turkish Journal of Biological Control*, 8(1): 39-48.