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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

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Abstract

Control potential of 10 *Beauveria bassiana* isolates, isolated from *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) and *Gonioctena fornicata* (Brüggemann) (Coleoptera: Chrysomelidae), collected from alfalfa fields in Tokat province, Turkey, was evaluated against *H. postica* larvae under laboratory conditions. Concentration-response tests were carried out using the concentrations (1×10^3, 1×10^5, 1×10^7, and 1×10^9 conidia/ml) of isolates GN-23, GN-4, HP-30, and HP-6, which performed more than 95% efficacy in screening tests (1×10^7 conidia/ml) 5 days post treatments. LT_{50} and LT_{90} values at 1×10^9 conidia/ml were determined. According to the obtained results, *H. postica* larvae were susceptible to all the tested *B. bassiana* isolates.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, Efficacy, Alfalfa weevil, Larvae, *Hypera postica*

Background

Alfalfa (*Medicago sativa* L.) is one of the well-known and widely used forage crops in the world. Its high yield and quality allows it to be used in feeding programs of different types of livestock (Bates 1998). Alfalfa is attacked by a numerous number of insect species that cause considerable damage and reduce forage yield. Alfalfa weevil *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) is one of the most important pests that attack this crop. Both adults and larvae feed on alfalfa foliage, but the larvae cause the majority of the damage. Larvae feed initially on the inside of terminal leaves and later move to the lower portions of the plant, while adults generally feed on the leaf margins. Injured leaves dry very quickly giving the field a grayish to whitish cast. This pest can be found in all alfalfa production areas of Turkey and worldwide (Cook et al. 2004 and Atanasova 2012).

Frequent cuttings are the most effective cultural method of the pest in the alfalfa fields. Chemical control can be recommended, if there are 25 adults in 1-m^2 area before the first mowing (Erdoğan 2008). However, the chemical insecticides used to manage this pest are extremely hazardous to bees and other beneficial insects (Reddy et al. 2016). Some efficient biological control agents, particularly entomopathogenic fungi (EPF), and some hymenopteran parasitoids have been recorded (Atay et al. 2015; Reddy et al. 2016; Efil 2018 and Yücel et al. 2018).

The application of EPF in bio-control of insects is of immense significance because of their environmental and food safety concerns (Reddy et al. 2016). Species of the genera *Beauveria*, *Metarhizium*, *Lecanicillium* and *Isaria* are commercially produced (Vega et al. 2009). *B. bassiana* was reported to infect 707 species of insect hosts, including 521 genera and 149 families of 15 orders (Imoulan et al. 2017). Some studies have been conducted to determine the potential of *B. bassiana* as a bio-agent against various insect pests in Turkey. These studies

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have mostly focused on lepidopteran and coleopteran pests (Güven et al. 2015; Yanar et al. 2017).

There are several studies concerning biological control of *H. postica* by EPF in Turkey (Atay et al. 2015 and Yücel et al. 2018) and in the world (Hedlund and Pass 1968; Roberts et al. 1994; Mustafa et al. 2014 and Reddy et al. 2016).

The objective of this study was to determine the efficacy of 10 local *B. bassiana* isolates, isolated from *H. postica* and *Gonioctena fornicata,* collected from alfalfa fields in Tokat province, Turkey, against larvae of the alfalfa weevil under laboratory conditions.

Methods

Isolation of fungi

Overwintered adults of *H. postica* and *G. fornicata* emerged from the soil were collected from alfalfa fields of Tokat, Turkey, during the period, April–May 2015 (Table 1). The collections were brought to the laboratory with fresh alfalfa plants, transferred to separate cages and checked daily. The dead adults were subjected to surface sterilization with 1% sodium hypochlorite solution for 1 min, washed twice with distilled water, placed in a sterile Petri-dish containing moistened filter paper and kept at 25 ± 2 °C and 16L: 8D h photoperiod (Ali-Shtayeh et al. 2002). By the end of incubation period, the fungus was isolated from the adults, with external fungal growth, with special care (Sevim 2010). PDAY (PDA + 1% yeast extract) media were used for isolation. To prevent bacterial contamination, 50 μg/ml ampicillin, 20 μg/ml tetracycline, and 200 μg/ml streptomycin were added to the medium (Ihara et al. 2001). Single-spore isolates of all the isolates were obtained by serial dilution (Dhingra and Sinclair 1995) and were identified as *B. bassiana*. Totally, ten *B. bassiana* isolates were isolated from the field-collections of *H. postica* and *G. fornicata* adults (Table 1), and they were deposited in the fungal culture collection of the Mycology Laboratory at the Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection in Tokat, Turkey. In order to obtain sufficient amounts of spore suspensions, the fungi were sub-cultured in PDA (Potato Dextrose Agar) medium. The fungi cultures were incubated at 25 ± 2 °C for 17 days. Ten milliliters of sterilized water with 0.02% Tween 80 was added to each plate, and spore harvesting was done by gently rubbing the culture surface, using a sterilized glass hokey. Spore suspension from each isolate was adjusted to 1×10^3, 1×10^5, 1×10^7, and 1×10^9 conidia/ml (Şahin 2006).

Table 1 Hosts and locations of the tested enthomopathogenic *Beauveria bassiana* isolates

Isolates	Information of Locations				Hosts
	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*
GN5–2	Güryıldız, Tokat, Turkey	40° 19′ 49″	36° 22′ 04″	525	*G. fornicata*
GN8–2	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*

Bioassays

Primarily, screening tests were conducted to determine the efficacy of the isolates against *H. postica* larvae at 1×10^7 conidia/ml. To test the effect of each isolate, *H. postica*

Table 2 Mortality of *Hypera postica* exposed to the ten isolates at $1x10^7$conidia/ml

Mortality±SEM*(%)				
ISOLATES	1 DAT**	3 DAT	5 DAT	7 DAT
GN-23	0.29 ± 0.70a***	24.83 ± 0.16c	98.85 ± 1.12abc	100.00 ± 0.00a
GN-20-2	0.29 ± 0.70a	60.22 ± 0.52ab	94.43 ± 1.49abcd	99.71 ± 0.70a
GN-12-3	0.00 ± 0.00a	51.82 ± 0.92ab	85.66 ± 0.49d	100.00 ± 0.00a
GN-1	0.60 ± 1.45a	39.79 ± 0.68bc	86.99 ± 0.22 cd	99.71 ± 0.70a
GN-4	2.57 ± 1.25a	56.84 ± 0.62ab	97.43 ± 1.25abcd	99.71 ± 0.70a
GN-5-2	0.29 ± 0.70a	53.50 ± 0.79ab	83.64 ± 0.22d	100.00 ± 0.00a
GN-8-2	2.57 ± 1.25a	60.22 ± 0.52ab	88.54 ± 0.14bcd	99.71 ± 0.70a
GN-8-1(2)	2.57 ± 1.25a	65.50 ± 0.72a	85.36 ± 0.25d	100.00 ± 0.00a
HP-30	1.70 ± 1.74a	60.22 ± 0.52ab	99.40 ± 1.45ab	100.00 ± 0.00a
HP-6	4.53 ± 1.12a	68.72 ± 0.48a	99.71 ± 0.70a	100.00 ± 0.00a
CONTROL	0.00 ± 0.00a	0.00 ± 0.00d	1.15 ± 1.12e	13.01 ± 0.22b

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

Table 3 Mortality of *Hypera postica* exposed to GN-23 isolate

Mortality±SEM* (%)				
Doses (conidia/ml)	1 DAT**	3 DAT	5 DAT	7 DAT
1×10^3	1.65 ± 1.25a***	13.66 ± 0.25b	70.95 ± 1.10b	86.70 ± 0.54b
1×10^5	0.29 ± 0.70a	20.86 ± 0.65b	82.19 ± 0.40b	99.71 ± 0.70a
1×10^7	0.29 ± 0.70a	24.83 ± 0.16b	98.85 ± 1.12a	100.00 ± 0.00a
1×10^9	0.60 ± 1.45a	48.58 ± 1.49a	98.85 ± 1.12a	99.71 ± 0.70a
Control	0.00 ± 0.00a	0.00 ± 0.00c	1.15 ± 1.12c	13.01 ± 0.22c

**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 5 Mortality of *Hypera postica* exposed to Hp-30 isolate

Mortality±SEM* (%)				
Doses(conidia/ml)	1 DAT**	3 DAT	5 DAT	7 DAT
1×10^3	0.41 ± 0.84a***	62.22 ± 0.52a	78.11 ± 0.11b	94.13 ± 2.44a
1×10^5	0.00 ± 0.00a	56.03 ± 0.12a	80.82 ± 0.68b	95.18 ± 1.78a
1×10^7	1.70 ± 1.74a	60.22 ± 0.52a	99.40 ± 1.45a	100.00 ± 0.00a
1×10^9	0.00 ± 0.00a	66.92 ± 0.33a	100.00 ± 0.00a	100.00 ± 0.00a
Control	0.00 ± 0.00a	0.00 ± 0.00b	1.15 ± 1.12c	13.01 ± 0.22c

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

larvae were dipped into conidial suspension of 1×10^7 conidia/ml of each isolate for 4–5 s and placed in a Petri-dish (10 larvae per dish) containing fresh alfalfa leaves. Mortality rates were recorded on the 1st, 3rd, 5th, and 7th days post treatment. In addition, concentration-response tests were carried out with isolates proved to have a high effect, using such tested concentrations. The experiments were carried out in completely randomized block design, with three replications and replicated two times.

Statistical analysis

Test results were converted into percentages and arcsine transformed. The transformed data was analyzed by analysis of variance (ANOVA) and the means compared by Tukey's multiple comparison tests. All statistical analyses were carried out using the MINITAB Release 16 packet program. LT_{50} and LT_{90} values of the concentration causing the fastest effect were determined, using the probit analysis.

Results and discussion

The ten EPF isolates tested against *H. postica* at 1×10^7conidia/ml caused 100% mortality 7 days post treatment in almost all isolates (Table 2). Concentration-response tests, carried out using concentrations of 1×10^3, 1×10^5, 1×10^7, and 1×10^9 conidia/ml of the isolates (GN-23, GN-4, HP-30, HP-6), caused more than 95% mortality rate on the 5th day.

The mortality rates of *H. postica* larvae varied from 13.66 to 72.08% 3 days post treatment. The highest mortality rate (72.08%) was recorded for HP-6 at 1×10^9 conidia/ml (Table 6). HP-30 was the most effective isolate with mortality rate of 62.22% at 1×10^3 conidia/ml (Table 5). On the 5th day, all isolates and concentrations caused more than 70% mortality, while on the 7th day the mortality rate reached 100% by almost all isolates at 1×10^7 and 1×10^9 conidia/ml (Tables 3, 4, 5, and 6).

Table 4 Mortality of *Hypera postica* exposed to GN-4 isolate

Mortality±SEM* (%)				
Doses(conidia/ml)	1 DAT**	3 DAT	5 DAT	7 DAT
1×10^3	1.15 ± 1.12a***	28.24 ± 0.09b	77.18 ± 0.43b	96.63 ± 1.75a
1×10^5	1.15 ± 1.12a	41.43 ± 0.75ab	80.69 ± 0.54b	99.71 ± 0.70a
1×10^7	2.57 ± 1.25a	56.84 ± 0.62a	97.43 ± 1.25a	99.71 ± 0.70a
1×10^9	0.00 ± 0.00a	50.00 ± 0.21a	98.35 ± 1.25a	100.00 ± 0.00a
Control	0.00 ± 0.00a	0.00 ± 0.00c	1.15 ± 1.12c	13.01 ± 0.22b

**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 6 Mortality of *Hypera postica* exposed to Hp-6 isolate

Mortality±SEM* (%)				
Doses(conidia/ml)	1 DAT**	3 DAT	5 DAT	7 DAT
1×10^3	0.41 ± 0.84ab***	52.01 ± 0.49b	86.34 ± 0.25b	99.59 ± 0.84a
1×10^5	0.00 ± 0.00b	59.68 ± 0.34ab	86.99 ± 0.22b	97.43 ± 1.25a
1×10^7	4.53 ± 1.12a	68.72 ± 0.48a	99.71 ± 0.70a	100.00 ± 0.00a
1×10^9	2.57 ± 1.25ab	72.08 ± 0.48a	100.00 ± 0.00a	100.00 ± 0.00a
Control	0.00 ± 0.00b	0.00 ± 0.00c	1.15 ± 1.12c	13.01 ± 0.22b

**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 7 Lethal time (LT_{50} and LT_{90}) values of the entomopathogenic fungi, *Beauveria bassiana* isolates (day)

Isolates	Slope ± SE	LT_{50} (95% fiducial limit)	LT_{90} (95% fiducial limit)	χ^2
GN-4	1.040 ± 0.148	3.110 (2.808–3.404)	4.343 (3.977–4.907)	7.4
GN-23	0.984 ± 0.122	3.074 (2.796–3.350)	4.376 (4.024–4.890)	15.7
HP-6	1.132 ± 0.151	2.476 (2.215–2.735)	3.608 (3.283–4.097)	5.9
HP-30	0.174 ± 0.167	2.401 (1.348–2.885)	4.196 (3.768–5.014)	5.8

LT_{50} for HP-30 was 2.401 days, followed by HP-6 (2.476 days), GN-23 (3.074 days), and GN-4 (3.110 days). LT_{90} values for the isolates of HP-6, HP-30, GN-4, and GN-23 were 3.608, 4.196, 4.343, and 4.376 days, respectively (Table 7).

Yücel et al. (2018) tested 7 isolates of *B. bassiana* and one isolate of *B. pseudobassiana,* isolated from *H. postica*, on larvae and adults of *H. postica* at 1×10^5, 1×10^6 1×10^7, and 1×10^8 conidia/ml. The results showed that the highest mortality rate in larvae was obtained by the isolate HpA-5 (*B. bassiana*) (100%) and HpI-4 (*B. pseudobassiana*) (97%), within 14 days at 1×10^8 conidia/ml. The highest mortality rate of adults were obtained by the isolates HpA-5 (*B. bassiana*) and HpI-4 (*B. pseudobassiana*) with 98 and 95% mortality rates within 14 days at 1×10^8 concentration, respectively.

Mustafa et al. (2014) reported that conidial suspension with 1×10^7 conidia/ml of two isolates of *B. bassiana* caused 100% mortality on adults of *H. postica,* 6 days post treatments. Also, Reddy et al. (2016) investigated the efficiency of six biorational-insecticides against *H. postica* larvae under laboratory conditions and found that Mycotrol® ESO (*B. bassiana* GHA) lasted 5–9 days to kill 100% of *H. postica* larvae in all the tested concentrations (0.072, 0.36, 0.72, and 1.44 ml/l). In addition, Atay et al. (2015) stated that 23.25% of *H. postica* adults, overwintered in alfalfa growing areas of Tokat province in Turkey, were found naturally infected with *Beauveria* spp. Harcourt et al. (1977) reported that larvae of this pest were found infected with *Entomophthora phytonomi* Arthur, which considerably reduced the weevil population in Canada.

Conclusions

The present study showed that the local isolates of *B. bassiana* could be suggested as bio-control agent against *H. postica* larvae; however, further studies should be conducted under field conditions.

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Availability of data and materials

The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s)).

Authors' contributions

The whole team jointly planned the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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