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THE INVESTIGATION OF *BEAUVERIA BASSIANA* (ASCOMYCOTA: HYPOCREALES) AS A BIOCONTROL AGENT OF ROSE-STEM SAWFLY, *SYRISTA PARREYSSII* (SPINOLA, 1843) (HYMENOPTERA: SYMPHYTA; CEPHIDAE) LARVAE

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ABSTRACT

The *Rosa* spp. (*Rosa canina* and *Rosa domescana*) used since the earliest times in rituals, cosmetics, perfumes, medicines, aromatherapy, landscape design and repair work are the important economic crop. The rose stem sawfly (*Syrista parreyssii* (Spinola, 1843)) is one of the most important pests of *R. canina*, *R. damascena*. The larvae of *S. parreyssii* bore galleries in and feed on the core sections of the shoots so these shoots completely dry out after a certain time. Because of living in shoot, biological control of this pest is very difficult and important. Besides, recently, biological control of pests are getting more prevalent due to the effects of chemical residues. The present study was carried out to determine insectisidal effect of the entomopathogen fungi *Beauveria bassiana* (Bal.) Vuillemin against *S. parreyssii.*

The insectisidal effect tests were carried out different conidial suspensions (10^6, 10^7 and 10^8 conidia/ml) of *B. bassiana* (ET 10) on laboratory conditions. The effect of these concentrations was observed larvae of *S. parreyssii.* The results of this study showed that all of the concentrations showed remarkable insectisidal effect against this insect larvae. Consequently, these fungal suspensions can be used as new biocontrol agents in controlling *S. parreyssii*.

KEYWORDS:
Beauveria bassiana, biological control, rosehip, *Syrista parreyssii.*

INTRODUCTION

The genus *Rosa* contains approximately 100 species that are widely distributed in Europe, Asia, the Middle East and North America [1]. *Rosa* spp. (*Rosa canina* L. and *Rosa domescana* Mill.) used since the earliest times in rituals, cosmetics, perfumes, medicines, aromatherapy, landscape design and repair work are important economic crop. These deciduous shrubs are widely grown in gardens for their flowers and fruits in Turkey [2].

Rosehip fruit and plant can be damaged by many important insects. One of the most important insect damaged rosehip is *Syrista parreyssii* (Spinola, 1843) (Hymenoptera: Cephidae). *S. parreysii* known as "Gül Filizarısı" in Turkey, has been reported to cause harm to *Rosa* species [3-11]. In addition, *S. parreyssii* is spread in Albania, Azerbaijan, Bulgaria, Armenia, Crete, Spain, Israel, Iran, Italy, Cyprus, Macedonia, Poland, Russia, Serbia, Jordan, Turkey, Turkmenistan and Greece [8, 12-17]. Previous studies on infestation level of this species reported 1-18% for oil-bearing roses [3], and a maximum damage ratio (number of infested shoots) of 14% and 16% for oil-bearing roses [18] and 5-17% for rosehip plants [11].

In this pest control, cultural control methods in terms of reducing the population are very important. Due to the fact that, the insect it lived in shoot, chemical control of this pest may not be successful.

Both this cause and the negative aspects of conventional pest control [19] have led to the investigation of alternative methods such as biological control [20-23].

Entomopathogenic fungi have been mass-produced as biopesticides since the 1970s. [24] and in the recent years, biological pest control, including the use of entomopathogenic fungi, has been attracting much attention. Entomopathogenic fungi are important natural control agents of many insects, including several pests [21, 25].

Most efforts have focused on more production-friendly species, primarily Ascomycetous species of the anamorphic genera *Beauveria*, *Metarhizium*, *Isaria* and *Lecanicillium* (Sordariomycetes: Hypocreales) [26]. The entomopathogenic fungus *Beauveria bassiana* (Bal.) Vuillemin (Ascomycota: Hypocreales) is also widely regarded as one of the most promising species known for potential developments into practical insect biocontrol agents [27-32]. The fungus *B. bassiana* has a great potential as a mycoinsecticide. The microbial insect control agent, *B. bassiana*, has been commercialized for use

as an environmentally friendly bio-pesticide and displays a broad host range capable of infecting and killing a wide range of insect [33].

B. bassiana kills the pest by infection as a result of the insect coming into contact with fungal spores. An insect can come into contact with the fungal spores in several ways: by having the spray droplets land on its body, by moving on a treated surface, or by consuming plant tissue treated with the fungus (the latter is not a major method of uptake). Once the fungal spores attach to the insect's skin (cuticle), they germinate sending out structures (hyphae) that penetrate the insect's body and proliferate. It may take 3-5 days for insects to die, but infected cadavers may serve as a source of spores for secondary spread of the fungus. Insects can also spread the fungus through mating [34]. High humidity and free water enhance activity of the conidia and the subsequent infection of the insect [35-36].

The aims of this study were to assess the insecticidal effect of different conidial suspensions (10^6, 10^7 and 10^8 conidia/ml) of *B. bassiana* (ET10) isolated from *Sphenoptera antiqua* Illiger (Coleoptera: Buprestidae) larvae with respect to the control of *S. parreyssii* larvae under controlled condition.

MATERIALS AND METHODS

Host Plant and Harmful Insect. Naturally infested (included larvae of *S. parreyssii*) rosehip twigs (*R. canina*) and *S. parreyssii* larvae were used as host plant and harmful insect in this study, respectively.

Fungal Isolate. *B. bassiana* (ET10) isolated from *S. antiqua* larvae damaged on *Onobrychis sativa* L. (Fabacea) in Erzurum province were used as biological control agent in this study. *S. antiqua* dead larvae were washed in a solution of 2% sodium hypochlorite for 1 min. Then they were dried on filter paper. After drying, they were transferred to Petri dishes containing 20 ml of Potato Dextrose Agar (PDA, Difco), and incubated at 25 ºC for 1 week with high humidity (80±10% rh). These colonies were obtained from each dead larva and morphological characters of colonies were identified according to Kulu et al. [37].

Entomopathogenic fungal isolate was grown on PDA and maintained on Sabourd Dextrose Agar (SDA, Merck, Darmstadt, Germany) slant cultures were maintained at 4°C in the refrigerator until used in fungal collection of Department of Plant Protection, Faculty of Agriculture, Ataturk University.

Molecular Characterization of *Beauveria bassiana* Isolate. DNAs of *B. bassiana* (ET10) isolate was obtained from mycelia growth on petri dish culture. Around 200 mg mycelia were ground with liquid nitrogen and 1 ml of extraction buffer (0.2 M Tris 8.5, 0.25 M NaCl, 25 mM EDTA, 0.5% SDS) was added. Following that, phenol/chloroform clarification and ethanol precipitation were applied. DNA's were diluted in 50 µl TE (10 mM Tris 7.5- 1 mM EDTA) buffer. ITS region from DNA samples were amplified using primers ITS1 (TCCGTAGGTGAACCTGCGG) located on 18S rRNA [38] and ITS4 (TCCTCCGCTTATTGATATGC) located on 28S rRNA [39]. PCR amplifications were performed in 50 µl reaction mix containing 1,5 mM MgCl, 0,2 mM dNTP mix, 0,3 pmol each primer, 1,5U Taq polymerase, 1X polymerase buffer (100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl, 0.8% (v/v) Nonidet P40) (Fermentas, Germany) and 1 µl of template DNA (20–50 ng). The analyze was done in 94 ºC for 1 second, 30 a in 94 ºC 45s + 50C 45s + 72 ºC 45s. PCR products were separated in 1.5 % agarose gels, stained with ethidium bromide, and visualized under UV light. Sequence analysis was done by RefGen (Ankara, Turkey). The result of this analyze was run ABI 3100 Genetic Analyzer.

Preparation of Conidial Suspensions. *B. bassiana* ET 10 isolate was cultured on SDA with 1% yeast extract (SDAY) plates in several Petri dishes (9 cm in dia-meter), and were grown for 2-3 weeks at 25 ± 1°C under a 16 h/8 h (light/dark) photoperiod and 60 ± 5% RH for fungal growth and conidial production. Conidial suspensions were prepared by scraping conidia from the cultures into an aqueous solution of 0.02% Tween 80 [40]. Surface of a 14-day-old culture was gently scratched with inoculation needle and transferred to vials containing 5 ml sterile Tween-80 solution (0.1% v/v). The concentration of conidia in stock suspensions were determined by direct count using hemocytometer. Three different conidial suspensions (10^6, 10^7 and 10^8 conidia/ml) were prepared in sterile distilled water containing Tween 80 and vortexed for 3 min to produce a homogenous suspension for the bioassay.

Bioassay. Infested rosehip twigs with *S. parreyssii* larvae were cut from rosehip in Ataturk University Campus (1854 m), Erzurum, Turkey in late June 2015 and were brang to laboratory. Twigs were divided into 8 cm in length including one larvae by lancing. Three conidial suspensions (10^6, 10^7 and 10^8 conidia/ml) were sprayed to *S. parreyssii* larvae in rosehip twigs opened with lancet. Rosehip twigs applicated by different fungal conidial suspensions were closed with parafilm. They placed in petri dishes (9 cm x 1.5 cm deep), and humidity of twigs was provided by wetted cotton. Experiment was planned to randomized block design with three replications and each replicate contained twigs with 9 larvae. Totally 81 larvae were sprayed by different conidial suspensions of *B. bassiana* ET 10 and 27 positive control were sprayed by sterile distilled water, too. All Petri dishes were incubated at 23±2 ºC. The number of dead larvae and mortality rates (%)

of *S. parreyssii* were recorded after 48, 96 and 120 h. Mortality rate calculated follow formula;

$$\text{Mortality rate (\%)} = \frac{100 \text{ x the number of dead larvae in treatment}}{\text{Total larvae in treatment}}$$

Dead larvae were transferred to Petri dishes lined with PDA to encourage fungal growth and sporulation in order to confirm that death was due to infection by *B. bassiana*. The insecticidal effect of different conidial suspensions of fungal isolate was expressed as death insects.

Statistical Analyses. All data in the present study were processed by JUMP 5.0 and the means were separated by LS Means Students tests. The statistical analyses of percentage values were performed by using transformed values.

RESULTS

Macroscopic characters of *B. bassiana* colony that observed were growth pattern, color, shape, surface texture, colony elevation and diameter/day on Petri dish. Macroscopic and microscopic characters of *B. bassiana* isolate and appearance on PDA were given Table 1 and Fig. 1. Macroscopic morphology character shown that *B. bassiana* colony color was white, texture was smooth. Colony growth pattern was disperse, without pattern and not concentrical (Fig. 1).

Genome of pure isolate was identified using PCR method with primer specific for identification *B. bassiana*. The molecular identification results of tested fungal strain (ET 10) are shown Table 2, and sequence of this fungus is shown Table 3. According to the molecular identification test results, ET 10 fungal isolate was identified as *Beauveria bassiana* at 0.99 similarity index.

TABLE 1
Macroscopic characters of *B. bassiana* isolate on PDA

Isolate Code	Colony Observation					
	Growth pattern	Color	Shape	Texture	Elevation	Diameter/ days
ET 10	Disperse	White	Round	Smooth	Raised	4.5 cm/10 days

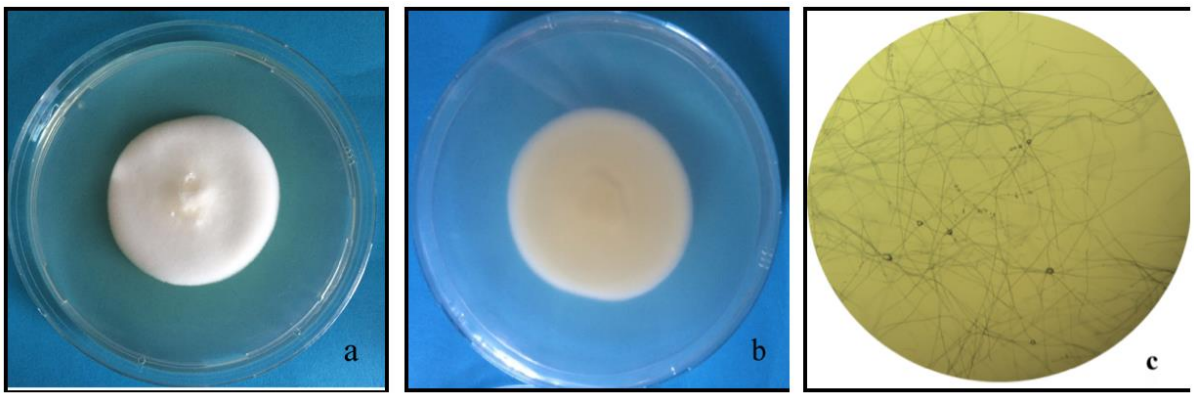


FIGURE 1
Apperance of *B. bassiana* isolate ET 10 on PDA in ten days: a) top view; b) bottom view; c) hypha

TABLE 2
The molecular identification result of fungal isolate ET 10, similarity index and ITS sequences analyzed

Isolate	ITS identification results	Similarity index	ITS 1 sequences
ET 10	*Beauveria bassiana*	0.99	GB |KY806126|

TABLE 3
ITS1+5.8S rDNA+ITS2 sequence of ET 10

1	ATTCCCGGGGGAGGTCTACCTGATTCGAGGTCACGTTCAG
41	AAGTTGGGTGTTTTACGGCGTGGCCACGTCGGGGTTCCGG
81	TGCGAGTTGGTTTACTACGCAGAGGTCGCCGCGGACGGGC
121	CGCCACTCCATTTCAGGGCCGGCGGTGTGCTGCCGGTCCC
161	CAACGCCGACTTCCCCAAAGGGAGGTCGAGGGTTGAAATG
201	ACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGC
241	AATGTGCGTTCAAAGATTCGATGATTCACTGGATTCTGCA
281	ATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGA
321	TGCCAGAGCCAAGAGATCCGTTGTTGAAAGTTTTAATTTA
361	TTTGTTTTGCCTTGCGGCGTATTCAGAAGATGCTGATAAT
401	ACAAGAGTTTGATGGTCCCCGGCGGCCGCTGGTCCAGTCC
441	GCGTCCGGCTGGGGCGAGTCCGCCGAAGCAACGATAGGTA
481	GGTTCACATAAGGGTTTGGGAGTTGAAAACTCGGTAATGA
521	TCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTT
561	TTACTTCTTCTAAGGGGACCAAGGAG

TABLE 4
The insecticidal effect of three different conidial suspensions of *B. bassiana* ET 10 against larvaes of *S. parreyssii* under laboratory condition

Applications	The number of dead larvae			Average	Mortality rate (%)			Average
	48 h	96 h	120 h		48 h	96 h	120 h	
10^6 conidia/ml	6.0	7.33	9.0	**7.44±1.50 A**	66.67	81.48	100	**82.72±16.70 A**
10^7 conidia/ml	6.0	7.67	9.0	**7.56±1.50 A**	66.67	85.19	100	**83.95±16.70 A**
10^8 conidia/ml	7.0	8.33	9.0	**8.11±1.02 A**	77.78	92.59	100	**90.12±11.31 A**
Control	0.0	0.0	0.33	**0.11±0.19 B**	0.0	0.0	3.7	**1.23±2.14 B**
CV			**29.61**				**29.61**	
LSD			**1.65**				**18.38**	

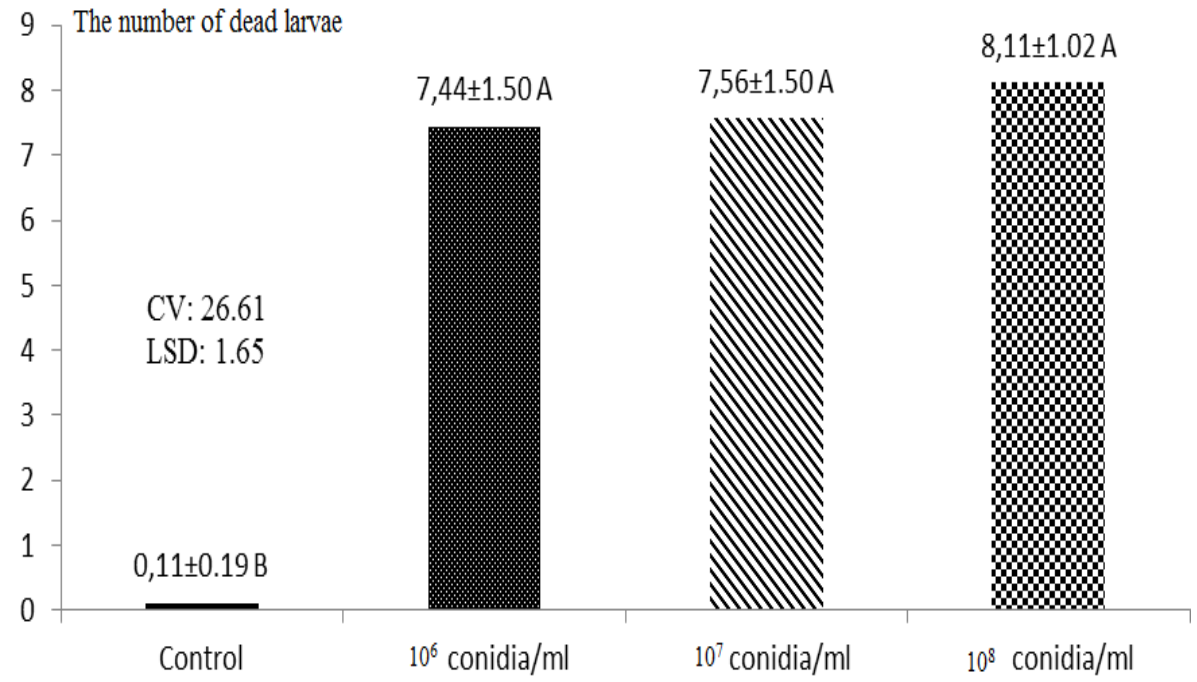
*Mean values (Mean ± Standart deviation) in the same column by the same letter are not significantly different to the test of LS Means Differences Student's ($p<0.01$)

The number of dead larvae and mortality rates (%) of *S. parreyssii* recorded after 48, 96 and 120 h are summarized in Table 4. The results show that all of the different conidial suspensions of *B. bassiana* ET 10 isolate have insecticidal effect on the larvae of *S. parreyssii*. Insecticidal activities of the tested three different conidial suspensions were statistically different from the control at three different times ($p< 0.001$).

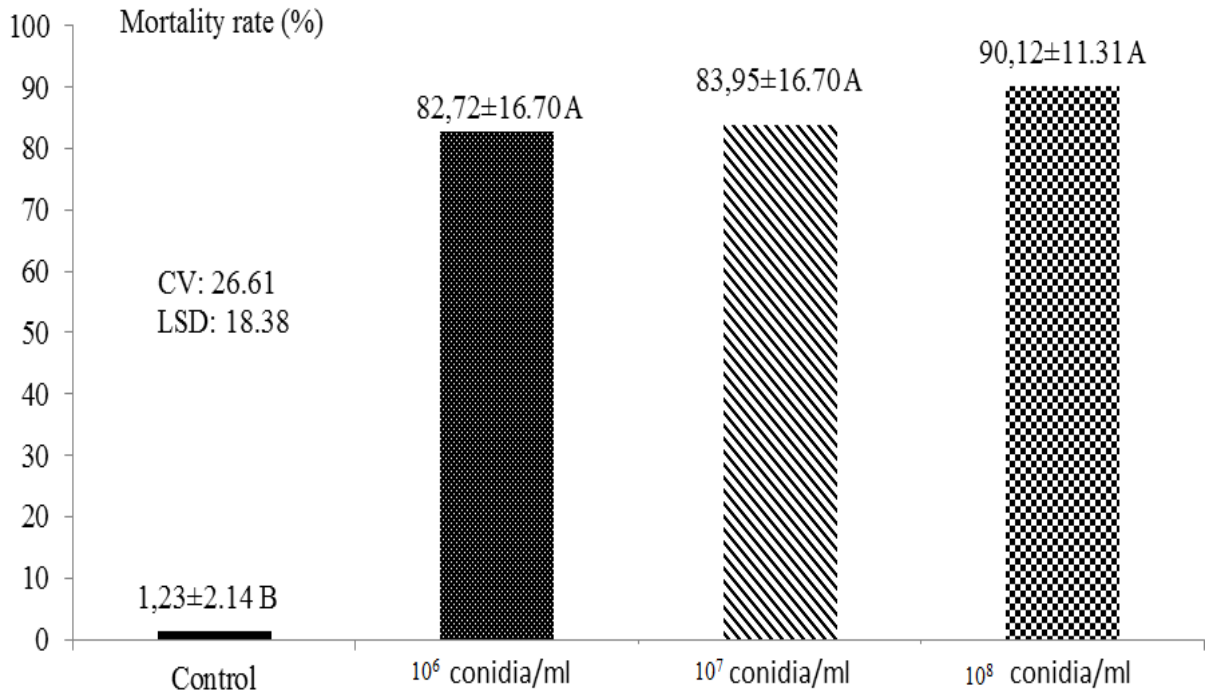
At 48 h, the lowest the number of dead larvae and mortality rate (0%) were observed from control application. All of the fungal suspensions used in this study caused mortality but the number of dead larvae and mortality rates were changed from 6 and 66.67 to 7 and 77.78 at 48 h respectively. All of the tested conidial suspensions were statistically different from the control. At 96 h, the lowest number of dead larvae and mortality rates were observed from control application, too. The number of dead larvae and mortality rate of the suspensions were different from the control. The dead larvae and mortality rates number of conidial suspensions including 10^6, 10^7 and 10^8 conidia/ml was 7.33, 7.67, 8.33 and 81.48, 85.19, 92.59 (%), respectively. At 96 h the number of dead larvae and mortality rate of 10^8 conidial suspension of *B. bassiana* isolate (ET 10) were found to be the highest. At 120 h, all of the applications, except control, showed insecticidal activity 100%. The lowest the number of dead larvae and mortality rate observed from control (0.33 and 3.7%) (Table 4).

The average of the number of dead larvae and mortality rate (%) of treatments are given in Fig. 2a, 2b. Result of LSD multiple comparison test to explore the difference among the suspensions, there were statistical difference for insectisidal effect of treatments. The results show that all suspensions of this isolate have a significant insecticidal effect on *S. parreysii* ($P< 0.01$) (Fig. 2a, 2b).

According to conidial suspensions, the highest number of dead larvae (8.11) and mortality rate (90.12%) were observed at 10^8 and the lowest number of dead larvae (7.44) and mortality rate (82.72%) at 10^6 conidial suspension of the fungi. The means of dead larvae and mortality rate (%) of 10^6, 10^7 and 10^8 conidial suspensions of the fungi were 7.44, 7.56, 8.11 and 82.72%, 83.95%, 90.12%, respectively (Fig, 2a, 2b).



(a)



(b)

FIGURE 2

Insecticidal effect of *B. bassiana* ET 10 against larvae of *S. parreyssii* under laboratory condition the average of a) the number of dead larvae at all treatments, b) mortality rate (%) of all conidial suspensions at different times

Insecticidal effect of different conidial suspensions of the fungi (10^6, 10^7 and 10^8) were different from the control (Fig. 2a, 2b). The fungus was re-isolated from dead larvae. The photographs of larvae of *S. parreyssii* with *B. bassiana* (ET 10) were given from 48 h to 120 h in Fig. 3.

DISCUSSION

It is believed that environmental safety and ecosystem stability considerations lead to the conclusion that the use of native isolates in a microbial control program is more convenient [41]. *B. bassiana* is one of the most ubiquitous and extensively studied of the entomopathogenic fungal species and

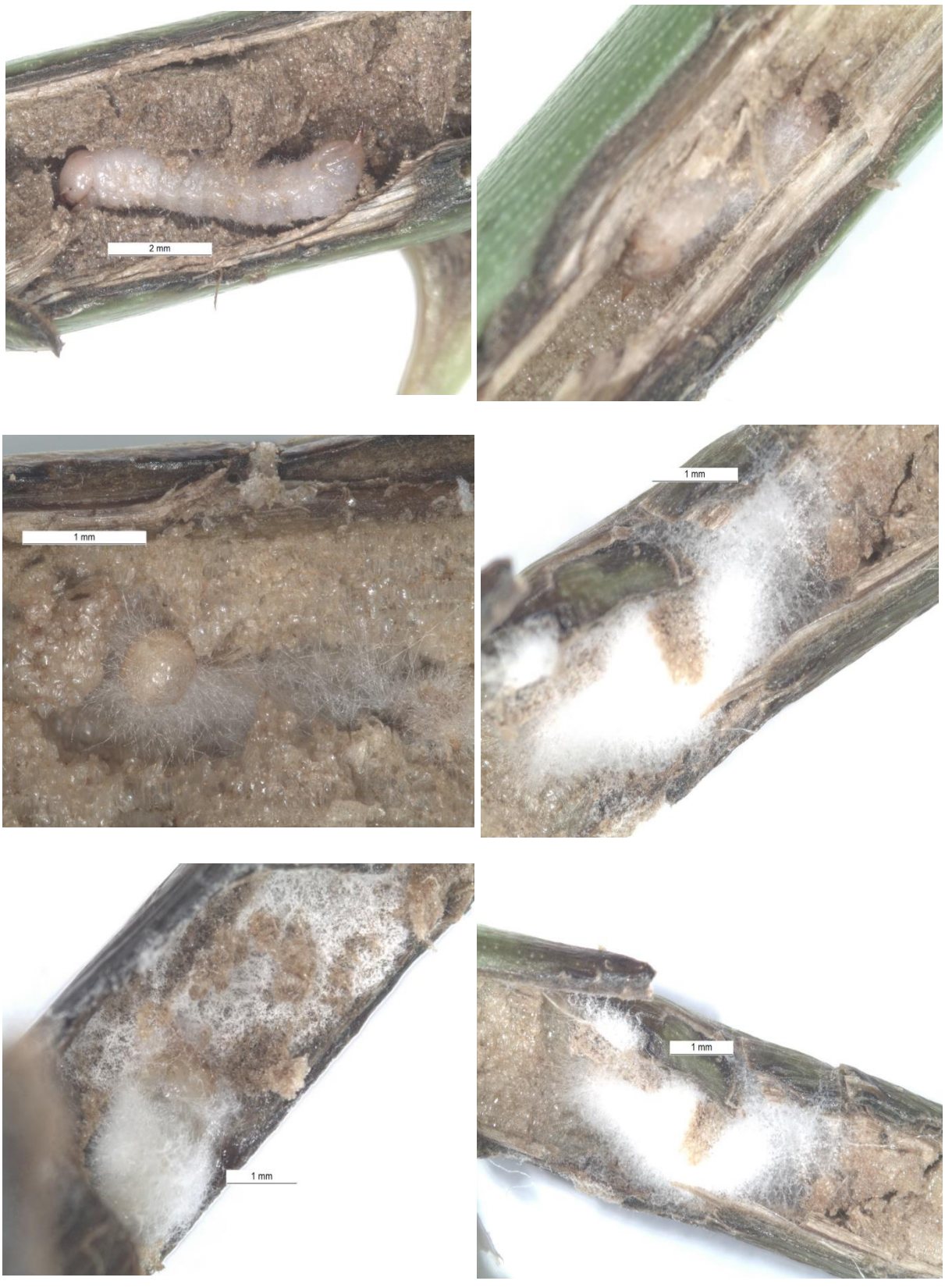


FIGURE 3
The dead larvae of *S. parreyssii* applicated by *B. bassiana* ET 10

is the active agent in many products currently in use and under development worldwide [21, 42-48].

*B. bassiana (*ET10) isolated from *S. antiqua* larvae was used as biological control agent in this study. Sequences of fragments were compared with

data from the GenBank, and the isolates were characterized as *B. bassiana* with high degree of identity in this study. ET 10 isolate developed white mycelium on PDA in this study. This development was related to study of Ahmad [49], Utami and Isnawati [50] and Kulu et al. [37] which shown that *B. bassiana* will grow on PDA medium as white mycelium and form white powder layer.

There are many articles which are insecticidal effect of *B. bassiana*, most of them on Coleoptera orders, such as Adane et al. [51], Eken et al. [52], Marannino et al. [53], Sabour et al., [54]. The different mortality rates were observed on different pest group applicated by *B. bassiana*. The infestation level of *B. bassiana* (10^4 conidia/ml) were recorded about 88% mortality against *Sitophilus zeamais* (Coleoptera: Curculionidae) in eight day [51]. Both *B. bassiana* and *Metarhaizum anisopliae* gave 77% mortality against *Bruchus rufimanus* L. (Coleoptera: Chrysomelidae) [54]. 10^8 conidia/ml and 10^7 conidia/ml of *B. bassiana* caused mortalities 100% within 4.6 and 4.4 days respectively on *S. populnea* larvae under laboratory conditions [52].

Besides, there are some article about *B. bassiana* on Hymenopteran species. Since certain entomopathogenic fungi present a high potential to control *Atta bisphaerica*, pathogenicity tests have been performed with fungi widely used within the microbial control of insects, such as *M. anisopliae* and *B. bassiana*. They have proved to be highly virulent in the laboratory, although field tests do not reproduce the same results [55-56]. The pathogenity of two isolates *B. bassiana*, two isolates *M. anisoplia* and two isolates *Paecilomyces farinosus* against soldier of *Atta sexdens sexsdens* (Hymenoptera: Formicidae) were studied in laboratory conditions in the other study. Three species of fungi were highly pathogenic to *A. sexdens sexdens* soldiers causing high mortality rates, above 80%, killing the ants in the first four days after inoculation [57]. 100% mortality within 2.90 and 2.77 days were obtained against *Caliroa cerasi* (Hymenoptera: Tenthredinidae) larvaea by Aslantaş et al. [58] under laboratory condition. The other study, *B. bassiana* effectively reduced wheat stem damage caused by *Cephus cinctus* (Hymenoptera: Cephidae) larvae compared to untreated plots or water spray only [48].

Additionaly, Swiergiel et al. [59] observed the effect of an isolate of the *B. bassiana* strain GHA biocontrol product (BotaniGard wettable powder [WP], Lindesro, Sweden) against *Hoplocampa testudines* (Hymenoptera: Tenthredinidae) under laboratory conditions. They indicated that their laboratory infection experiment showed a significant effect of *B. bassiana* GHA.

According to the result of our study, the number of dead larvae and mortality rate (%) of 10^6, 10^7 and 10^8 conidial suspensions of *B. bassiana* were 7.44, 7.56, 8.11 and 82.72%, 83.95% and 90.12% on *S. parreyssii*, respectively. To our knowledge, only Yanar et al. [60] the insecticidal effects of 14 isolates of *B. bassiana* on *S. parreyssii* were tested under laboratory conditions. They observed mortality rate of O-80 as 91.7% and indicated that O-80 isolate can be used for microbial and integrated management of this species. The results coincide with the findings of our studies.

The development of entomopathogen fungi which may be effective, selective, and bio-degradable, with little or no resistance by the pest and no toxicity to the environment will help to reduce the negative effects of synthetic insecticides.

CONCLUSIONS

According to the present results, it can be concluded that all tested concentrations (10^6, 10^7, 10^8) of the *B. bassiana* has effected against *S. parreyssii* larvae under laboratory conditions. This study matters to highlight the successful usage of an environment-friendly, natural, and for health of humans and other livings, risk-free product against *S. parreyssii* in substitution for the chemical pesticides that are intensely used and harmful for environment, natural balance and human health.

Additional field research is needed to determine how effective *B. bassiana* is at controlling *S. parreyssii* larvae in the field conditions.

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