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BIOCONTROL POTENTIAL OF *Beauveria bassiana* AND *Metarhizium anisopliae* ISOLATES FROM TURKEY AGAINST *Hyphantria cunea* (DRURY) (LEPIDOPTERA: ARCTIIDAE) LARVAE UNDER LABORATORY AND FIELD CONDITIONS

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Abstract

Hyphantria cunea is one of the most important pest insects causing significant damage in many plant species. The present study aimed to evaluate the insecticidal activity of Turkey isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against *H. cunea* larvae under laboratory and field conditions. *B. bassiana* isolates YK16, YK23 and YK26, and *M. anisopliae* isolates YK41 and YK45 were sprayed onto the larvae of *H. cunea* at the respective doses of $1x10^5$ and $1x10^6$ conidia mL^{-1} and monitored for seven days. Fungal isolates, bio-insecticide *Bacillus thuringiensis* and chemical insecticide diflubenzuron were used in field studies. Trials were carried out in five replications. All *B. bassiana* isolates caused 100% mortality on first instar larvae in laboratory trials. Mortality ratios ranged from 100 to 96% on second instar larvae. *M. anisopliae* isolates YK45 and YK41 caused 88 and 84%, and 81.33 and 77.11% mortalities for the first and second instar larvae, respectively. The mortality rates fluctuated between 91.78-72.89% for *B. bassiana* on third instar larvae in laboratory conditions. However, *M. anisopliae* isolates YK45 and YK41 caused 77.11 and 60.22% mortality on third instar larvae, respectively. In the field trials, *B. bassiana* YK23 displayed promising insecticidal activity with 80.60% mortality on second instar larvae of *H. cunea*. Other isolates as well caused mortalities ranging from 60.77 to 49.55%. The results revealed that some isolates of *B. bassiana* and *M. anisopliae* have potential to control *H. cunea* larvae. However, additional detailed studies need to be carried out to increase their effectiveness in field conditions.

Keywords: Entomopathogenic Fungi. Fall Webworm. Insecticidal Activity. Microbial Control. Pest Insect.

1. Introduction

The fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae), is one of the important pest insects causing significant damages on the green parts of ornamental trees, shrubs plants, orchards and forests (Schowalter and Ring 2017; Wang et al. 2020). The fall webworm spread to Central Europe and East Asia from the North America in the early 1940s (Ge et al. 2019). Currently, *H. cunea* has been distributed to more than 30 countries in North America, Europe, and Asia and causes serious harms on more than 600 plant species (Lu et al. 2017; Ge et al. 2009). In Turkey, *H. cunea* bring along significant defoliator effect in hazelnut orchards as well as other orchards, parks, gardens and forests for about half a century. In recent years, *H. cunea* population showed a serious increase (Gencer et al. 2020). In Turkey, synthetic chemicals with active substances cypermethrin and diflubenzuron, and a microbial bio-insecticides including *Bacillus thuringiensis kurstaki* (an entomopathogen) are used to control *H. cunea* population. Entomopathogens are successfully used against many pest insects in biological control programs (Karabörklü et al. 2018). Among

the insect pathogens, entomopathogenic fungi have been also produced commercially and successfully used in controlling pest insects for many years (Wakefield 2018). Their infective and killing abilities offer promising control opportunities in different geographic, climatic, and agro-ecological zones (Lacey et al. 2015). That's why entomopathogenic fungi offer environmentally friendly alternatives to conventional synthetic chemicals for pest control.

Entomopathogenic fungi can cling to the cuticle of insects through the producing infective spores and, penetrating into tissues and hemocoel and kill insects in a very short time by their toxins. Apart from the cuticle, entomopathogenic fungi can also penetrate host through tracheal openings, injured areas, digestive system and other openings (Batta and Kavallieratos 2018; Karabörklü et al. 2019). *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikof) Sorokin are among the most important entomopathogenic fungi. About 80% of the commercial bio-insecticides produced using entomopathogenic fungi are based on the genera of *Beauveria* and *Metarhizium* (De Faria and Wraight 2007; Karabörklü et al. 2018; Meyling et al. 2018). Nevertheless, commercial adoption is still hindered by limited field efficacy, sourcing susceptibility to ultraviolet light, low moisture, and difficulties in reaching target pests. However, the endophytic abilities of some genera as *Beauveria* and *Metarhizium* against pest insects add a very important advantage to improve their efficacy (Russo et al. 2021). Although many laboratory studies conducted for evaluating the toxicity of *B. bassiana* and *M. anisopliae* against *H. cunea* (Iskender et al. 2012; Zibaee et al. 2013; Aker and Tuncer 2016; Armas et al. 2020), very few studies were known to be conducted in field conditions (Bi et al. 2018).

The current study was carried out to determine the insecticidal activity of Turkey native isolates of *B. bassiana* and *M. anisopliae* against the fall webworm *H. cunea* larvae under laboratory and field conditions.

2. Material and Methods

Collection of larvae

The adult emergence of *Hyphantria cunea* was observed for first generation and the larval development was also followed in gardens of hazelnut (*Corylus avellana* L.) in Duzce-Turkey. Just before the experiments, the first, second and third instar larvae were collected together with the small branches of hazelnut trees in June 2020.

Spore suspensions

Previously described five fungal isolates of *B. bassiana* (YK16, YK23 and YK26) and *M. anisopliae* (YK41 and YK45) from soils of Duzce-Turkey were used for preparing spore suspensions (Karabörklü et al. 2019). All isolates were taken from stock cultures, planted in Potato Dextrose Agar (PDA) medium, and kept in a climate chamber at 25°C, 60 ± 5% relative humidity and 14:10 h photoperiod for 10-15 days. Following the developmental period, the conidia were collected from rearing medium and transferred into distilled water including 0.03% Tween 80. Spore concentrations ($1x10^5$ and $1x10^6$ conidia mL^{-1}) were adjusted using a hemocytometer (0.100 mm × 0.0025 mm^2) under light microscope.

Laboratory essays

Ten larvae for each instar were transferred into pet jars (1 L) as five replications. Three fresh hazelnut leaves, approximately 3, 4 and 6 gr weights, were added into each jar for feeding the first, second and third instar larvae for seven days, respectively. Spore suspensions were added to 50 mL spraying bottle and the concentrations were adjusted to $1x10^5$ conidia mL^{-1} for each fungal isolate. Spore suspensions were then applied to larvae using a bottle by spraying method. To the control group distilled water including 0.03% Tween 80 was sprayed. After the spraying process, the plastic jars were covered with thin tulle and transferred to climate chamber. The larvae were kept at 23 ± 2°C, 65 ± 5% humidity and 14: 10 hour (light/dark) photoperiod in the laboratory. At the end of the 7th day of application, the numbers of living and dead larvae were determined. Mortality rates (%) were corrected using Abbott's formula (Abbott 1925) for the mortalities in the controls for each fungal isolate and larval instar.

Field essays

The insecticidal activity of *B. bassiana* YK16, YK23 and YK26, and *M. anisopliae* YK41 and YK45 was tested on the second instar larvae of *H. cunea* from second generation. In the field essay, *B. bassiana*, *M. anisopliae*, a commercial synthetic insecticide diflubenzuron (Dimilin) and commercial bio-insecticide *Bacillus thuringiensis* var. *kurstaki* strain PB-54 (Bio-T Plus) were used in August 2020. A hazelnut garden, infested with high number of larval populations of fall webworm, was chosen for the trials. The numbers of living larvae were recorded for all treatments before the spraying. Spore suspensions of each fungal isolate ($1x10^6$ conidia mL^{-1}) were added to 1 L pet spraying bottles separately. For commercial insecticides, recommended doses by manufacturers were used. Only distilled water including 0.03% Tween 80 was applied for the control groups. Trials were set up in five replicates in accordance with the randomized blocks trial pattern. After the spray of treatments, the hazelnut branches including larval clusters were covered with thin white tulles. At the end of the 7th day, the tulles were opened, and the numbers of living larvae were counted again. The dead larvae were brought to the laboratory to confirm the mortality caused by the isolates. Lethal efficacy of each isolate and commercial product was determined using formula developed by Henderson and Tilton (1955).

Statistical analysis

In comparison of data, variance analysis (one-factor ANOVA) was applied using the SPSS (SPSS 17.0 commercial software, SPSS, Inc., Chicago, IL) program. The averages were compared using a Tukey-Kramer HSD post-test at a 5% significance level.

3. Results

At the end of 7^{th} day, all entomopathogenic fungal isolates exhibited high insecticidal activities on first, second and third instars larvae of *H. cunea* at a concentration of $1x10^5$ conidia ml^{-1} under laboratory condition. When compared with the control group, it was determined that *B. bassiana* and *M. anisopliae* isolates had a significant lethal effect on first instar larvae of *H. cunea* ($F = 30.400$; $df = 4$; $p < 0.0001$). When the efficacies of fungal isolates were evaluated, it was seen that their mortality effects ranged from 100% to 84% (Figure 1). All isolates of *B. bassiana* caused 100% mortality on first instar larvae of *H. cunea* and were significantly different from the two *M. anisopliae* isolates. *M. anisopliae* YK45 isolate caused 88% mortality on the same larval instar (Figure 1).

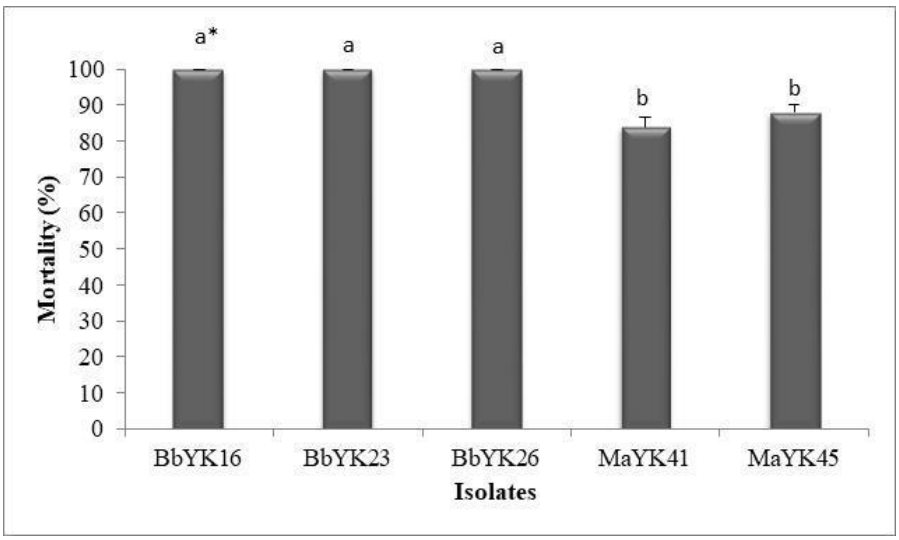

Figure 1. Lethal effect of *Beauveria bassiana* and *Metarhizium anisopliae* isolates on first instar larvae of *H. cunea.* *Among the means indicated by different letters there is a significant difference ($p < 0.05$). Bb: *Beauveria bassiana,* Ma: *Metarhizium anisopliae.* Bars indicate standard errors of mean. Mortality (%) corrected based on Abbott's formula (Abbott 1925).

Beauveria bassiana and *M. anisopliae* isolates also exhibited pronounced mortality rates on second instar larvae of *H. cunea* at the same dose and period (Figure 2). Their mortality effects ranged from 77.5 to 100% on the second instar larvae ($F = 13.189$; $df = 4$; $p < 0.0001$). Complete mortality was obtained with the application of *B. bassiana* YK23 isolate. Other *B. bassiana* isolates caused 96% mortality on the same instar larvae (Figure 2).

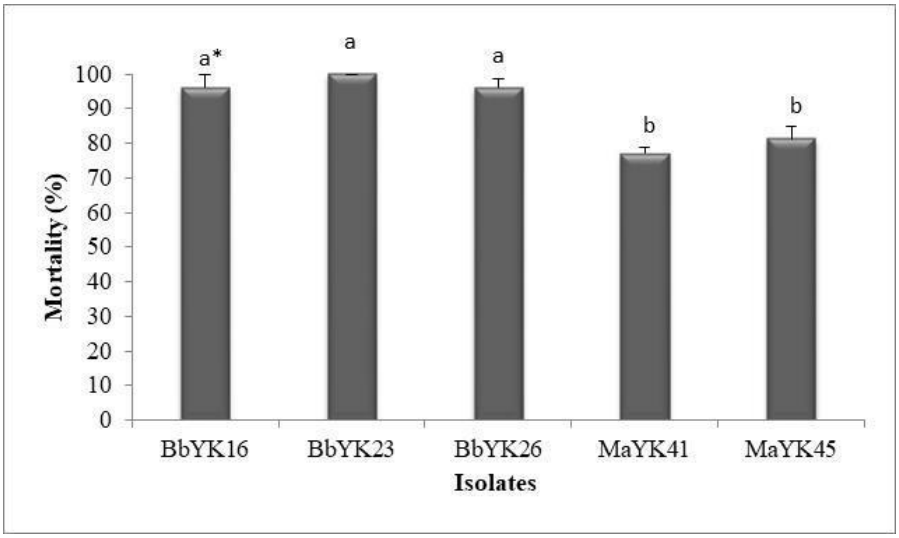

Figure 2. Lethal effect of *Beauveria bassiana* and *Metarhizium anisopliae* isolates on second instar larvae of *H. cunea.* *Among the means indicated by different letters there is a significant difference ($p < 0.05$). Bb: *Beauveria bassiana,* Ma: *Metarhizium anisopliae.* Bars indicate standard errors of mean. Mortality (%) corrected based on Abbott's formula (Abbott 1925).

Mortality rates caused by fungal isolates ranged from 91.78 to 60.22% on third instar larvae ($F = 7.901$; $df = 4$; $p < 0.001$) (Figure 3). The most effective isolate was found to be *B. bassiana* YK23 with 91.78% mortality rate, followed by *M. anisopliae* YK45 isolate with 77.11% mortality rate (Figure 3).

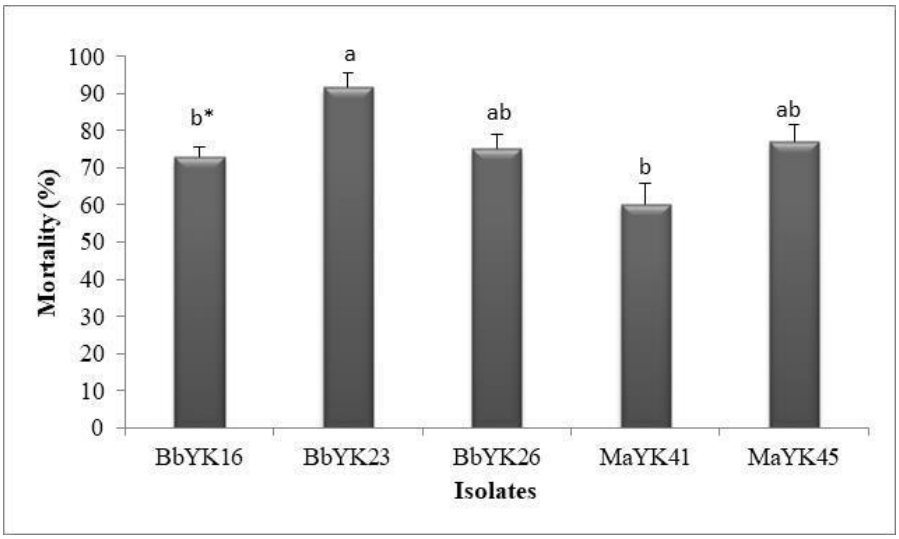

Figure 3. Lethal effect of *Beauveria bassiana* and *Metarhizium anisopliae* isolates on third instar larvae of *H. cunea.* *Among the means indicated by different letters there is a significant difference ($p < 0.05$). Bb: *Beauveria bassiana,* Ma: *Metarhizium anisopliae.* Bars indicate standard errors of mean. Mortality (%) corrected based on Abbott's formula (Abbott 1925).

Beauveria bassiana and *M. anisopliae* isolates also revealed significant insecticidal activity against second instar of *H. cunea* in field conditions ($F = 40.585$; $df = 6$; $p < 0.0001$) (Table 1). In the field essay, at the end of the 7th day *B. bassiana* YK23 displayed promising insecticidal activity (80.60%) on second instar larvae at $1x10^6$ conidia ml^{-1} dose, and it did not differ from the efficacy of diflubenzuron and *Bacillus*

thuringiensis var. *kurstaki*. Other fungal isolates caused mortality varying between 49.55% and 60.77% at the same dose and period.

Table 1. Lethal effect of *Beauveria bassiana* and *Metarhizium anisopliae* isolates against second larvae instar of *Hyphantria cunea* in field condition.

Treatment	Number of alive larvae (Mean± SD)		Efficacy (%)
	Before application	After application	
Control	311.4±28.15	309.00±28.19	-
Diflubenzuron	305.2±17.95	12.80±04.97	95.82±01.47[a]
Btk	305.2±24.10	36.60±13.28	87.83±04.70[ab]
BbYK16	304.6±29.03	118.20±21.19	60.77±06.87[c]
BbYK23	312.6±23.39	60.20±13.44	80.60±04.21[b]
BbYK26	300.4±15.06	121.80±19.82	59.24±05.27[c]
MaYK41	302.2±12.87	150.40±27.69	49.55±11.08[c]
MaYK45	299.4±09.63	120.00±14.87	59.63±04.70[c]

[a]Means within each column followed by the different letter are significantly different ($p \leq 0.05$). Control: distilled water with 0.03% Tween 80, Btk: *Bacillus thuringiensis* var. *kurstaki,* Bb: *Beauveria bassiana*, Ma: *Metarhizium anisopliae*, SD: Standard deviation. Efficacy (%) calculated based on Henderson and Tilton's formula (Henderson and Tilton 1955).

4. Discussion

Beauveria bassiana and *M. anisopliae* isolates displayed significant insecticidal activity against the fall webworm *H. cunea* larvae in laboratory conditions. Fungal isolates were very effective especially on first and second instar larvae. All isolates of *B. bassiana* caused complete mortality on first instar larvae at the dose of $1x10^5$ conidia ml^{-1}. Mortality ratios ranged from 100 to 96% on second instar larvae. Iskender et al. (2012) reported that *B. bassiana* isolates (PaF04, PaF09 and PaF76) caused mortalities varying between 90 and 96.66% on the larvae at the dose of $1x10^6$ conidia ml^{-1}. In another study, Zibae et al. (2013) indicated that *B. bassiana* caused the highest mortality (76%) on fourth instar larvae of *H. cunea* at the dose of 10^7 conidia ml^{-1} for insect dipping method. *Metarhizium anisopliae* isolates caused 88 and 84% mortality on first instar and 81.33 and 77.11% on second instar larvae. The results in present study revealed that *B. bassiana* is more effective than *M. anisopliae* on the larval stages of *H. cunea*. Similar results were reported when *B. bassiana* and *M. anisopliae* were applied to fourth instar larvae of *H. cunea* in laboratory conditions (Armas et al. 2020). Fite et al. (2020) also reported that *B. bassiana* was more virulent to the third instar larvae of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) than *M. anisopliae* under laboratory conditions. Efficacy of fungal isolates decreased gradually depending on larval development. Third instar larvae of *H. cunea* appeared to be more resistant to fungal isolates compared with first and second instars at the same dose and period. The mortality values fluctuated between 91.78 and 72.89% for *B. bassiana* and 77.11 and 60.22% for *M. anisopliae* on 3^{th} instar of *H. cunea*. Similarly, Aker and Tuncer (2016) reported that *M. anisopliae* caused 85% mortality on second instar larvae of *H. cunea* but decreased to 68.33% in third instar larvae at $1x10^8$ conidia ml^{-1} dose at the end of 16 days.

The *B. bassiana* and *M. anisopliae* also exhibited significant insecticidal activity on *H. cunea* larvae in field conditions. *B. bassiana* YK23 isolate displayed promising insecticidal activity by reaching 80.60% mortality rate on second instar larvae of *H. cunea,* and it did not differ from the efficacy of the commercial insecticides, diflubenzuron (95.82%) and *Bacillus thuringiensis* var. *kurstaki* (87.83%). Other fungal isolates caused mortalities varying between 49.55 and 60.77% at $1x10^6$ conidia ml^{-1} dose at the end of 7^{th} day. To the best of our knowledge, only one study (Bi et al. 2018) was conducted on the efficacy of *B. bassiana* and *M. anisopliae* against *H. cunea* larvae in field conditions. A trap-strips system was designed for *H. cunea* larvae in field conditions and 10 days later spore suspension of *B. bassiana* sprayed under each trap ($1x10^8$ conidia ml^{-1} dose) by Bi et al. (2018). The trap-strips provided an appropriate microenvironment for *H. cunea* to pupate and an acceptable humidity for *B. bassiana* to infect and reproduce. It was reported that the average infection rates for trap-strips systems were more than 90% against *H. cunea*.

However, there are some reports on their effectiveness on other lepidopteran insect pests. Godonou et al. (2009) reported that *B. bassiana* (Bba5653) and *M. anisopliae* (Ma182) caused 94% and 80% mortality

on third instar larvae of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), respectively, at $1x10^8$ conidia ml^{-1} dose in laboratory conditions. In field conditions, both emulsion and water-based formulations (equivalent to 10^{12} spore ml^{-1}) of *B. bassiana* (Bba5653) were compared with the commercial insecticide bifenthrin by being practically 100% more efficient (Godonou et al. 2009). In addition, Agboyi et al. (2020) indicated that *B. bassiana* (Bb11) was able to reduce the density of *P. xylostella* by 83 and 93% in farm, compared with unsprayed control and deltamethrin application, respectively at the dose of 53 g/ha twice a week. Moreover, Fite et al. (2020) also indicated that *B. bassiana* is also effective against *H. armigera* by reducing larval infestations, decreasing pod damage and increasing chickpea yield in field conditions. Despite these, additional detailed studies need to be carried out to assess the effectiveness of *B. bassiana* and *M. anisopliae* in field conditions.

5. Conclusions

All Turkey isolates of *B. bassiana* and *M. anisopliae* tested were pathogenic to the three first instars of *H. cunea* under laboratory condition, where the isolate YK23 of *B. bassiana* caused highest percent of mortality to the first and second instar of *H. cunea*. In the field condition, this isolate was also the most effective to cause the mortality of the second instar of *H. cunea* among all isolates tested, and it had the same efficacy of the commercial insecticides.

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Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

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