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IN VITRO COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI *Beauveria bassiana* (BALS.) VUILL. WITH DIFFERENT FUNGICIDES

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Abstract: *Beauveria bassiana* (Balsamo) Vuillemin is one of the entomopathogenic fungi used against broad host insects and plant pathogens. Fungicides have side effects on entomopathogenic fungi. *In vitro* assay was performed to examine the compatibility of *B. bassiana* with eight commonly used fungicides (Azoxystrobin 75 g/l+Metalaxyl-m 37.5 g/l+ Fludioxonil 12.5 g/l FS, Boscalid 25%+ Pyraclostrobin 12% WG, Copper Hydroxide 361.1 g/l SC, Azoxystrobin 250 g/l SC, Triticonazole 80 g/l + Pyraclostrobin 40 g/l FS, Fludioxonil 12.5 g/l + Metalaxyl 10 g/l SC, Captan 50% WP, Tebuconazole 250 g/l EC) using contact application technique. Fungicides at various concentrations (Recommend Dose-RD, half of the Recommend Dose-0.5 x RD and twice the Recommend Dose-2 x RD) were mixed in Potato Dextrose Agar (PDA) media post-autoclaving. Approximately 25 ml of the mixture was poured into a Petri dish (90 mm) and allowed to cool. Mycelium disc (5 mm in diameter) was taken from 14-days-old *B. bassiana* grown on PDA using a sterile cork borer and placed in the center of each Petri dish containing fungicide + PDA. PDA plates without fungicide were used as a control. Petri dishes were incubated in the dark at 25±1 °C for 14 days. *In vitro* experiments were carried out with three replicates depending on a completely randomized plots design. In the study, the compatible fungicide with *B. bassiana* ET 10 isolate was found to be Copper Hydroxide at 0.5 x RD concentration. Azoxystrobin and Copper Hydroxide were compatible with Bb 18 isolate. Only two (Azoxystrobin + Metalaxyl-m + Fludioxonil and Tebuconazole) of the eight fungicides completely inhibited the mycelial growth of ET 10 isolate and were found harmful. Azoxystrobin + Metalaxyl-m + Fludioxonil completely inhibited the growth of Bb 18 isolate and was not found compatible. This study clearly shows that fungicides have the potential to inhibit the mycelial growth of entomopathogenic fungi under *in vitro* conditions. However, these results need to be further verified *in vitro* under both greenhouse and open-field conditions.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, Fungicides, Compatibility, Inhibition, Harmful

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1. Introduction

Biological control preparations containing entomopathogenic fungi were used in conventional farming systems. However, pesticides are also still used in this production system. Pesticides have effects such as leaving residues, inducing pathogen resistance and harming non-target beneficial organisms. Today, the importance of biological control methods and low-residue chemicals has increased even more. Entomopathogenic fungi (EPF) have been used as a biological control agent for more than 100 years (Roberts, 1989).

Beauveria spp. are entomopathogenic fungi (EPF) that can be easily isolated and produced from almost all ecosystems (Rehner et al., 2011). More than 700 species of entomopathogenic fungi have been described in the kingdom of Fungi, belonging to at least 90 genera (Goetteal et al., 2010). Some of these defined EPFs, such as *B. bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) and *Lecanicillium lecanii*, are commercially produced and used in many countries to control against many pests (Rath, 2000). Nowadays, *B. bassiana* has 707 different hosts. These hosts are contained in 521 genera, 149 families and 15 orders (Zimmermann, 2007). EPF show antagonistic effects against pathogens in the phyllosphere and rhizosphere or plant tissues as endophytes, with their mechanisms of action of parasitism, competition, and antibiosis (Ownley et al., 2010). EPF has many advantages such as being non-toxic to mammals, does not develop resistance to pests, long-term control in nature, being effective at all stages of insect development, can be used with most insecticides, inexpensive and easy to use (Sevim et al., 2015).

In laboratory and field studies, fungicides affect natural infections with entomopathogenic fungi, reduce infection

rates, and delay disease in animals (Sosa-Gomez et al., 2003). Therefore, EPF isolates selected as mycopesticides need to be tested for compatibility with chemical pesticides for their application in IPM programs (Shah et al., 2009). Studies have shown that fungicides affect radial growth and sporulation, but not germination (Li and Holdom, 1994). Kouassi et al. (2003) observed that delayed application of fungicides increases the effectiveness of *B. bassiana*. Faion (2004) reported that sulphur is not compatible with *B. bassiana* and *M. anisopliae*. In some laboratory studies, researchers have reported that fungicides do not effect on EPFs. For example, *B. bassiana*, *P. lilacinus*, *Metarhizium* spp., *M. anisopliae*, *Evlacovaea* sp. and *Tolypocladium cylindrosporum* were reported to grow better on media modified with Guanidine, Dodine, Benomyl, Thiabendazole or Copper Sulphate (Beilhartz et al., 1982; Luz et al., 2007). Some researchers have reported that Mancozeb and Copper Oxychloride are highly toxic to many EPF isolates (Rachappa et al., 2007).

This study aims to determine the effect of eight fungicides commonly used by conventional farmers in Türkiye, on the mycelial growth of native isolates of *B. bassiana* under laboratory conditions.

2. Materials and Methods

2.1. Fungal Cultures

B. bassiana ET 10 isolate was isolated from *Sphenoptera antiqua* in Erzurum province, Türkiye (Tozlu et al., 2017) and Bb 18 isolate was isolated from field soil in Düzce province, Türkiye (Erdoğan and Sağlan, 2023). EPF isolates were grown in the dark at 25±1°C for 14 days and after that subcultured on Potato Dextrose Agar (PDA-Difco) medium.

2.2. Fungicide Treatments

The commercial fungicides selected for *in vitro* studies were the most frequently used by farmers and described in Table 1. For compatibility tests, the formulations of fungicides were tested at three different doses viz., field recommended dose (RD), half of the recommended dose (0.5 × RD) and twice the recommended dose (2 × RD) (De Olivera and Neves, 2004).

2.3. Influence of Fungicides on Mycelial Growth of *Beauveria bassiana*

The inhibitory effect of commercial fungicides against *B. bassiana* isolates (ET 10 and Bb 18) was investigated according to the contact application test with Petri dishes under *in vitro* conditions. PDA was prepared and sterilized in autoclave at 121 °C for 15 minutes. Eight fungicides at different concentrations (RD, 0.5 x RD and 2 x RD) were mixed into PDA post-autoclaving, while the media was still liquid (45±5 °C), approximately 25 ml of the mixture was poured into 90 mm Petri dishes and allowed to cool and solidify. Mycelium plug (5 mm in diameter), taken from the leading growth edge of a 14-day-old culture of *B. bassiana* grown on PDA, was placed in the center of a Petri dish containing fungicide + PDA. PDA plates without fungicide were used as a control. Parafilm-sealed Petri dishes were incubated in the dark at 25±1 °C for 14 days. *In vitro* experiments were performed using three replicates in a completely randomized plots design. Inhibition rates for *B. bassiana* were calculated using the formula (Equation 1) described by Wang et al. (2012). Compatibility ratings for commercial fungicides were classified according to Hassan (1989) in evaluation categories of 1-4 scoring index (Table 2).

Mycelial growth inhibition (%): (C-T) x 100 / (C-6) (1)

where; C is the diameter of the mycelial growth in control petri plates, 6: the diameter of pathogen disk, T: the diameter of mycelial growth in treated petri plates.

2.4. Statistical Analysis

The data were analyzed by performing the ANOVA (one-way analysis of variance). Statistically significant differences between mean values were determined using LS Means Differences Student's" multiple comparison test (LSD) (P≤0.01). All statistical analyses were performed using JMP software version 13 (SAS Institute Inc., Cary, NC, USA).

Table 1. Basic data about fungicides used *in vitro* assay

Brand name	Active ingredient	Recommended dosage g or ml/100 liter water	Manufacturer
ASTRADYN	Azoxystrobin 75 g/l+Metalaxyl-m 37.5 g/l+ Fludioxonil 12.5 g/l FS	250 ml	ASTRANOVA
Bellis®	Boscalid 25% + Pyraclostrobin 12% WG	40 g	BASF
CASNOX H2 F	Copper Hydroxide 361.1 g/l SC	250 ml	SAFA
FUNGIROL	Azoxystrobin, 250 g/l SC	75 ml	CANSA
Insure®	Triticonazole 80 g/l + Pyraclostrobin 40 g/l FS	250 ml	BASF
MALVIN	Fludioxonil 12.5 g/l + Metalaxyl 10 g/l SC	250 ml	SAFA
massCAPTAN	Captan 50% WP	250 g	ERTAR
SOLIZOL	Tebuconazole 250 g/l EC	50 ml	DOĞAL

Table 2. 1-4 Scoring index

Score index	The average reduction in growth over an untreated control	Compatibility status
1	<20 % reduction in growth	Harmless
2	20-35 % reduction in growth	Slightly harmful
3	36-50 % reduction in growth	Moderately harmful
4	>50 % reduction in growth	Harmful

3. Results

Mycelial growth of *B. bassiana* ET 10 and Bb 18 isolates was significantly different between fungicides (P≤0.01). All the fungicides inhibited the mycelial development of *B. bassiana* isolates (ET 10 and Bb 18) in PDA medium either partially or completely at all three concentrations (Table 3 and 4). All the concentrations of Azoxystrobin + Metalaxyl-m + Fludioxonil and Tebuconazole completely inhibited mycelial growth of *B. bassiana* ET 10 isolate and showed 100% effect. At 0.5 x RD concentration, the best mycelial growth (22.40 mm) and the lowest percentage of inhibition (26.80%) were detected in Copper Hydroxide compared to the control. Mycelial growth of other fungicides was determined between 3.25 mm-12.23 mm and % inhibition rate between 60.13%-89.38%. The best mycelial development (15.13 mm, 14.97 mm) and the lowest effect (50.65%, 50.98%) were also observed with Copper Hydroxide at RD and 2 x RD concentrations, respectively. Other fungicides showed inhibition of 60% or higher at RD and 2 x RD concentrations. At 0.5 x RD concentration, only Copper Hydroxide showed slightly harmful (2 on the scale), while other fungicides were found to be harmful (4 on the scale). The RD and 2 x RD concentrations of Azoxystrobin + Metalaxyl-m + Fludioxonil completely suppressed mycelial growth of *B. bassiana* Bb 18 isolate and showed 100% inhibition. The best mycelial growth (30.57 mm, 30.00 mm) and the lowest effect (21.74%, 23.27%) were determined with Azoxystrobin at 0.5 x RD and RD concentrations, respectively. At 0.5 x RD concentration, Captan moderately inhibited *B. bassiana* Bb 18 isolate (53.96%), while at RD concentration, Copper Hydroxide (56.52%) and Captan (58.06%) also exhibited moderate inhibition. The best mycelial development (21.00 mm) and the lowest percentage of inhibition (46.29%) were observed in Copper Hydroxide at 2 x RD concentration. Boscalid (47.31%) and Azoxystrobin (53.71%) moderately inhibited Bb 18 isolate. At 0.5 x RD concentration, only Azoxystrobin was found to be slightly harmful, while other fungicides were found to be harmful. ET 10 isolate was found to be susceptible to all fungicides except Copper Hydroxide at all three concentrations. Bb 18 isolate was found to be susceptible to all fungicides except Azoxystrobin at 0.5 x RD and RD concentrations, and Copper Hydroxide at 2 x RD concentration (Table 3 and 4).

Table 3. Effect of different fungicides on mycelial growth of *B. bassiana* (I)

Active ingredient	*Beauveria bassiana* ET 10 isolate						
	0.5 x RD		RD		2 x RD		Scoring at
	MG[1]	PI	MG[1]	PI	MG[1]	PI	0.5 x RD
Azoxystrobin 75 g/l+Metalaxyl-m 3.5g/l+ Fludioxonil 12.5 g/l FS	0.00^{e*}	100.00	0.00^{e*}	100.0	0.00^{e*}	100.00	4
Boscalid 25%+ Pyraclostrobin 12% WG	3.25^{d}	89.38	4.47^{de}	85.29	4.73^{de}	84.64	4
Copper Hydroxide 361.1 g/l SC	22.40^{b}	26.80	15.13^{b}	50.65	14.97^{b}	50.98	2
Azoxystrobin, 250 g/l SC	11.58^{c}	62.09	11.63^{bc}	62.09	9.47^{bcd}	76.80	4
Triticonazole 80g/l + Pyraclostrobin 40 g/l FS	4.40^{d}	85.62	6.57^{cd}	78.43	5.23^{de}	83.01	4
Fludioxonil 12.5 g/l + Metalaxyl 10 g/l SC	10.07^{c}	66.99	7.13^{cd}	76.80	9.20^{cd}	69.93	4
Captan 50% WP	12.23^{c}	60.13	12.40^{bc}	59.48	12.83^{bc}	58.17	4
Tebuconazole 250 g/l EC	0.00^{e}	100.00	0.00^{e}	100.00	0.00^{e}	100.00	4
Control	30.57^{a}	0.00	30.57^{a}	0.00	30.57^{a}	0.00	-
$CV_{(0.01)}$	3.2	-	3.8	-	3.4	-	
LSD	5.66	-	5.71	-	6.50	-	

[1]Data are means of three replicates; *Means followed by different letters within a column are significantly different according to LSD test (P≤0.01); MG= Mycelial growth (mm); PI= Per cent inhibition; RD= Recommended dosage; 1= Harmless (<20% inhibition); 2= Slightly harmful (20-35% inhibition); 3= Moderately harmful (36-50% inhibition); 4= Harmful (>50% inhibition).

Table 4. Effect of different fungicides on mycelial growth of *B. bassiana* (II)

Active ingredient	*Beauveria bassiana* ET 10 isolate						
	0.5 x RD		RD		2 x RD		Scoring at
	MG[1]	PI	MG[1]	PI	MG[1]	PI	0.5 x RD
Azoxystrobin 75 g/l+Metalaxyl-m 37.5g/l+ Fludioxonil 12.5 g/l FS	2.90^{g*}	92.58	0.00^{e*}	100.00	0.00^{e*}	100.00	4
Boscalid 25%+ Pyraclostrobin 12% WG	6.57^{efg}	83.12	15.57^{c}	60.10	20.57^{bc}	47.31	4
Copper Hydroxide 361.1 g/l SC	15.30^{cd}	60.87	17.00^{c}	56.52	21.00^{b}	46.29	4
Azoxystrobin, 250 g/l SC	30.57^{b}	21.74	30.00^{b}	23.27	18.13^{bc}	53.71	2
Triticonazole 80g/l + Pyraclostrobin 40 g/l FS	10.03^{ef}	74.35	9.90^{d}	74.68	8.13^{e}	79.28	4
Fludioxonil 12.5 g/l + Metalaxyl 10 g/l SC	10.80^{de}	72.38	7.73^{de}	80.31	10.00^{de}	74.42	4
Captan 50% WP	17.97^{c}	53.96	16.40^{c}	58.06	14.57^{cd}	62.66	4
Tebuconazole 250 g/l EC	5.63^{fg}	85.68	4.50^{e}	88.49	5.07^{ef}	86.96	4
Control	39.17^{a}	0.00	39.17^{a}	0.00	39.17^{a}	0.00	-
$CV_{(0.01)}$	1.9	-	1.3		2.4	-	
LSD	5.02	-	3.62		6.28	-	

[1]Data are means of three replicates; *Means followed by different letters within a column are significantly different according to LSD test (P≤0.01); MG= Mycelial growth (mm); PI= Per cent inhibition; RD= Recommended dosage; 1= Harmless (<20% inhibition); 2= Slightly harmful (20-35% inhibition); 3= Moderately harmful (36-50% inhibition); 4= Harmful (>50% inhibition).

4. Discussion

This study clearly showed that fungicides have variable effects on the mycelial growth of *B. bassiana* isolates. Indeed, Shah et al. (2009) and Martins et al. (2012) reported decreased fungal growth when the concentration of the active ingredient of a fungicide increased. The enhanced effects of *B. bassiana* on the processes of radial growth and sporulation vary depending on the fungal isolates and the nature and concentrations of the fungicide (Olmert and Kenneth, 1974). In the study, out of the total eight tested fungicides, only Azoxystrobin + Metalaxyl-m + Fludioxonil and Tebuconazole completely inhibited the mycelial growth of *B. bassiana* ET 10 isolate at all three concentrations. Again, Azoxystrobin + Metalaxyl-m + Fludioxonil completely inhibited the mycelial growth of *B. bassiana* Bb 18 isolate at RD and 2 x RD concentrations and showed a negative effect. Copper Hydroxide against *B. bassiana* ET 10 isolate, Azoxystrobin and Copper Hydroxide against Bb 18 isolate was determined as a slightly harmful fungicide (Table 3 and 4). Similar to our findings, Loureiro et al. (2002) reported that fungicides viz. Thiophanate Methyl, Captan, Tebuconazole, Metalaxyl and Mancozeb inhibited the mycelial growth and sporulation of *B. bassiana*. In triazole fungicides (Tebuconazole), ergosterol biosynthesis is inhibited and consequently fungal cell membrane formation is prevented (Bartlett et al., 2002). Kouassi et al. (2003) found that Copper Oxide, Metalaxyl, and Mancozeb, at the recommended doses, inhibited the radial growth of *B. bassiana* (MK2001 isolate) on solid medium after 8 days of application. Er and Gökçe (2004) showed that Captan and Iprodione suppressed the conidial germination of *Isaria fumosorosea* and also inhibited mycelial development. Gatarayiha et al. (2010) reported that Azoxystrobin showed very little effect on the mycelial growth of *B. bassiana* at a concentration of 10^{-2}. Azoxystrobin was most compatible with *B. bassiana*, while Flutriafol was the most harmful fungicide. Shah et al. (2009) determined that Azoxystrobin inhibited the spore germination of *M. anisopliae* and *Lecanicillium longisporum*, and only Captan exhibited fungistatic effects on *I. fumosorosea.* The tolerance of *B. bassiana* to Cu-based fungicides was investigated and at the recommended application rate, Cu hydroxide did not significantly inhibit mycelial growth (Martins et al., 2012). Khan et al. (2012) found that among the 12 fungicides tested, Chlorothalonil (0.1%), Thiram (0.2%), and Metalaxyl (0.1%) exhibited the lowest spore germination and vegetative growth in both *B. bassiana* and *M. anisopliae.* Fiedler and Sosnowska (2017) reported that Chlorothalonil, Azoxystrobin, and Thiophanate-methyl fungicides inhibited the sporulation and mycelial growth of *B. bassiana*. Reddy et al. (2018) found that Tebuconazole was highly toxic and completely inhibited the mycelial growth of all tested entomopathogenic fungi (*B. bassiana*, *M. anisopliae*, and *L. lecanii*) at concentrations of 1000 and 10,000 ppm. Furthermore, even at a concentration of 100 ppm, Tebuconazole completely inhibited the growth of *L. lecanii*. Celar and Kos (2020) found that only in two cases, Copper Oxide at 15°C and Copper Hydroxide at 25°C, at the lowest concentration of 6.5%, did not significantly inhibit the mycelial growth of *B. bassiana*.

5. Conclusion

As indicated by the results of the study, Azoxystrobin + Metalaxyl-m + Fludioxonil and Tebuconazole fungicides completely inhibited the mycelial growth of *B. bassiana* ET 10 isolate at all three concentrations and were found to be incompatible. Azoxystrobin + Metalaxyl-m + Fludioxonil fungicides completely inhibited the mycelial growth of Bb 18 isolate and were found to be incompatible. The current study demonstrates the potential risks posed by fungicides on the entomopathogenic fungus *B. bassiana*. Only Copper Hydroxide was found to be compatible with *B. bassiana* ET 10 isolate, while Azoxystrobin and Copper Hydroxide were found to be compatible with Bb 18 isolate. These fungicides were determined to be slightly harmful. However, additional field and/or greenhouse studies are being done to confirm the compatibility of Copper Hydroxide and Azoxystrobin fungicides and *B. bassiana* within an Integrated Pest Management strategy.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	O.E.	Z.S.
C	50	50
D	50	50
S	100	
DCP	50	50
DAI	100	
L	50	50
W	100	
CR	50	50
SR	100	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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