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Determination of effects of some fungicides used in hazelnut growing areas against *Trichoderma* species

Fındık üretim alanlarında kullanılan bazı fungisitlerin *Trichoderma* türlerine karşı etkilerinin belirlenmesi

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ABSTRACT

In this study, the effects of some fungicides (boscalid+kresoxim methyl, fluopyram+tebuconazole, sulphur ve tetraconazole) used for control of powdery mildew disease in hazelnuts on *Trichoderma harzianum* (11-TTR-2), *T. hamatum* (F4), *T. atroviride* (T-4-5) and *T. asperellum* (T-11-25) were evaluated under *in vitro* conditions. The study showed that all concentrations (0.25×, 0.5×, 1.0× and 2.0×) of the fungicides significantly reduced the mycelial growth, spore germination and germ-tube elongation of *Trichoderma* isolates, when comparing to the control (P<0.05). Especially, fluopyram+tebuconazole was found to have the higher inhibitory effect to mycelial growth, spore germination and germ-tube elongation of all the isolates. Even at the lowest concentration (0.0625 mL L^{-1}) used in the study, fluopyram+tebuconazole completely inhibited the mycelial growth of *T. hamatum* and *T. asperellum*, whereas it reduced mycelial growth of *T. harzianum* and *T. atroviride* by 93.97% and 89.48%, respectively. On the other hand, tetraconazole at a much higher concentration (1.0 mL L^{-1}) were able to decrease the mycelial growth of *T. harzianum* and *T. atroviride* by 82.16% and 95.61%, respectively. Boscalid+kresoxim methyl and sulphur inhibited the mycelial growth of all four isolates at rates between 26.64-63.59% and 6.75-30.81%, respectively. The EC_{50} and the minimum inhibitory concentration (MIC) values indicated that fluopyram+tebuconazole was more toxic to all the isolates than tetraconazole. As a result, this study showed that boscalid+kresoxim methyl and sulphur can be recommended in hazelnut orchards, where *Trichoderma* spp. should be used against *Xylosandrus germanus*.

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ÖZ

Bu çalışmada, fındıkta külleme hastalığının kontrolü için kullanılan bazı fungisit (boscalid+kresoxim methyl, fluopyram+tebuconazole, kükürt ve tetraconazole)'lerin *Trichoderma harzianum* (11-TTR-2), *T. hamatum* (F4), *T. atroviride* (T-4-5) ve *T. asperellum* (T-11-25) üzerine etkileri *in vitro* koşullarda değerlendirilmiştir. Çalışma, fungisitlerin bütün konsantrasyonları (0.25×, 0.5×, 1.0× ve 2.0×)'nın kontrol ile karşılaştırıldığında, *Trichoderma* izolatlarının misel gelişimini, spor çimlenmesini ve çim tüpü uzamasını önemli derecede azalttığını göstermiştir (P<0.05). Özellikle, fluopyram+tebuconazole'un dört izolatın misel gelişimi, spor çimlenmesi ve çim tüpü uzaması için daha yüksek engelleyici etkiye sahip olduğunu göstermiştir. Çalışmada kullanılan en düşük konsantrasyon (0.0625 mL L^{-1})'da bile fluopyram+tebuconazole, *T. hamatum* ve *T. asperellum*'un misel gelişimi tamamen engellemiştir, halbuki *T. harzianum* ve *T. atroviride*'nin misel gelişimini sırasıyla %93.97 ve %89.48'e kadar azaltmıştır. Diğer taraftan, tetraconazole çok daha yüksek bir konsantrasyonda (1.0 mL L^{-1}) *T. harzianum* ve *T. atroviride*'nin misel gelişimini sırasıyla %82.16 ve %95.61'e kadar azaltabilmiştir. Boscalid+kresoxim methyl ve kükürt, dört *Trichoderma* izolatının misel gelişimini sırasıyla %26.64-63.59 ve %6.75-30.8 arasında değişen oranlarda engellemiştir. Fluopyram+tebuconazole'ün EC_{50} ve minimum engelleyici konsantrasyon (MIC) değerlerinin tüm izolatlar için tetraconazole'den çok daha toksik olduğunu göstermiştir. Sonuç olarak, bu çalışma boscalid+kresoxim methyl ve kükürtün *Trichoderma* spp.'nin *Xylosandrus germanus*'a karşı kullanılması gereken fındık bahçelerinde önerilebileceğini göstermiştir.

1. Introduction

Ambrosia beetles are among the most problematic insects of hazelnut in Turkey, which is the biggest hazelnut producer in the world. Although there are many species of these beetles on hazelnut trees in Turkey, the species including *Xylosandrus germanus* Blandford, *Anisandrus dispar* Fabricius and *Xyleborinus saxesenii* Ratzeburg (Curculionidae: Scolytinae) are widespread and important pests (Tuncer et al. 2017; 2019).

The invasive ambrosia beetle *X. germanus* causes significant product losses on hazelnut due to draining branches or trees, especially in orchards along the Black Sea coast in Turkey where drainage problems occur. In addition to direct damage, *X. germanus* can harm the trees due to tunnelling in the sapwood of host trees and farming symbiotic fungi there. Like other ambrosia beetles, both adults and their larvae feed on the symbiotic fungi growing in the tunnels (Weber and McPherson 1983). Control of *X. germanus* living in the wood tissue of host trees, that protect beetle against insecticides, is very limited. Recently studies showed that entomopathogenic fungi [*Metarhizium anisopliae* (Metch) Sorok, *Beauveria bassiana* (Bals.) Vuill., and *Isaria fumosorosea* Wize)] and endophytic fungi could be an eco-friendly alternative control strategy against *X. germanus* and its symbiotic fungi (Castrillo et al. 2011; 2016; Kushiyev et al. 2018). The entomopathogenic fungi could be used to target adults and their brood, or mycoparasitic fungi, e.g., *Trichoderma* spp., could be used to target their associated symbiotic fungi (*Ambrosiella* spp.) (Castrillo et al. 2016). *Trichoderma* is a natural fungal genus that may be saprophytic or mycoparasitic. The members of the genus produce antifungal metabolites, which may compete, inhibit, or cause lysis of several structures of fungal pathogens (Benítez et al. 2004).

Castrillo et al. (2016) showed that *X. germanus* galleries in *T. harzianum*-treated beech stems had sparse symbiont growth, many with no or only a small number of eggs present. Similarly, in our study (unpublished) demonstrated that 4 *Trichoderma* spp. (*T. harzianum*, *T. hamatum*, *T. atroviride* and *T. asperellum*) to be antagonistic effect against symbiotic fungus (*Ambrosiella grosmanniae*) in the galleries of *X. germanus*. Also, majority of the galleries of the treated females did not have eggs and in some of them, decreased considerably compared to control. As a result, suppressing the growth of the symbiotic fungi will deny the developing brood nutrition for survival and limit beetle population increase (Castrillo et al. 2016).

For the last 6 years, powdery mildew by *Erysiphe corylacearum*, causing highly destructive symptoms and significant economic losses, is the most important disease in almost whole hazelnut producing areas in Turkey (Türkkan et al. 2018). In addition some cultural treatments (removal of infected leaves from orchards) against the powdery mildew, application of fungicides including sulphur, carboxamides, strobilurin (Q_oI) and DeMethylation Inhibitors [(DMI) - Triazoles)] is treated intensely in all hazelnut areas where the disease occurs (GKGM 2020).

This study evaluated the effects of boscalid+kresoxim methyl, fluopyram+tebuconazole, sulphur and tetraconazole on *T. harzianum*, *T. hamatum*, *T. atroviride* and *T. asperellum* isolates.

2. Materials and methods

2.1. *Fungal isolates*

The isolates of *T. harzianum* (11-TTR-2), *T. hamatum* (F4), *T. atroviride* (T-4-5) and *T. asperellum* (T-11-25) were obtained from the culture collection of the Ondokuz Mayis University, Faculty of Agriculture, Department of Plant Protection to use in this study. The isolates were maintained on potato dextrose agar (PDA; Oxoid Ltd., Basingstoke, UK) slants stored at 4°C for further studies.

2.2 *Chemical fungicides*

Collis SC, Luna Experience SC 400, Domark 10 EC and Saupolo 80 WG fungicides registered to control powdery mildew of hazelnut were purchased from BASF (Germany), BAYER (Germany), HEKTAŞ (Turkey) and ASTRANOVA (Turkey), respectively (Table 1). These fungicides were used at four concentrations (0.25×, 0.5×, 1.0× and 2.0×; where x is the field rate recommended by the manufacturer).

2.3. *Effect of the fungicides on mycelial growth, conidial germination and germ-tube elongation*

The antifungal effect of four fungicides on mycelial growth of *Trichoderma* spp. was evaluated according to Erper et al. (2018). Four concentrations of the fungicides were added to autoclaved PDA media, and then the ameliorated PDA media were dispensed aseptically into 9-cm-dia. Petri dishes (20 mL per Petri). Same amount of unamended PDA media were dispensed into the dishes for control. Mycelial discs (5-mm-dia.) cut from 7-day-old cultures of *T. harzianum* (11-TTR-2), *T. hamatum* (F4), *T. atroviride* (T-4-5) and *T. asperellum* (T-11-25) were placed on the centre of each medium, and the dishes incubated at 25°C in the dark. When the control fungal colonies had grown to the point of nearly covering the dishes, all dishes were measured at two perpendicular points. Mycelial growth values were converted into the percentage of mycelial growth inhibition (MGI), in relation to the control treatment by using the formula MGI (%)= [(dc – dt)/dc] × 100, where dc and dt represented mycelial growth diameter in control and amended Petri dishes, respectively. Each treatment has five replications, and the experiment was conducted once.

Table 1. The fungicides selected for *in vitro* tests.

Chemical group	Active ingredient	Trade name	Manufacturer	Registered concentrations in Turkey
Mixture	Boscalid+kresoxim methyl	Collis SC	Basf	0.30 mL L^{-1}
	Fluopyram+tebuconazole	Luna Experience SC 400	Bayer	0.25 mL L^{-1}
DMI[a]- Triazoles	Tetraconazole	Domark 10 EC	Hektaş	0.50 mL L^{-1}
Sulphur	Sulphur	Saupolo 80 WG	Astranova	4.0 g L^{-1}

[a]DeMethylation Inhibitors (DMI).

The effect of fungicide on conidia germination and germ-tube elongation was carried out using the concentrations mentioned above. The concentrations of the fungicides were added to autoclaved PDA medium, and then the ameliorated PDA medium was dispensed aseptically into 6-cm-dia. Petri dishes (10 mL per Petri). Aliquots of 100 µl of spore suspension (1×10^5 conidia mL^{-1}) prepared from 7-10 day fungal cultures were added to the dishes containing PDA medium with the 4 concentrations of the fungicides added. The media without the fungicide were used as a control. The dishes were incubated at 24±1°C for 24 h under dark conditions. The percentage of inhibition of spore germination and germ-tube elongation were determined by measuring the germinated conidia in 4 different microscopic fields for each dish, using a CX31 model compound microscope (Olympus, Tokyo, Japan) at ×200 magnification. A total of 200 spores were observed for each dish. Conidia were regarded as germinated when germ-tube length was equal or greater than conidial length. The inhibition was expressed as percentage: {[control (number of conidia or germ-tube length)–fungicide amended (number of conidia or germ-tube length)]} control (number of conidia or germ-tube length)} × 100. Three replicates were used for each fungicide, and each of the experiments was conducted once.

2.4. *EC_{50} and MIC values of the fungicides*

Effective concentrations of fungicides causing a 50% reduction (EC_{50}) in mycelial growth of *Trichoderma* spp. were calculated using SPSS Probit Analysis (Erper et al. 2018). Mycelial growth was assessed, as described above, in PDA containing 0.25×, 0.5×, 1.0× and 2.0× concentrations of the fungicides. Minimum inhibitory concentration (MIC) values required to completely inhibit mycelial growth were also identified in parallel experiments.

2.5. *Statistical analysis*

The results of this study were separately subjected to analysis of variance (One-Way ANOVA) using the SPSS Statistics Program, and significant differences between the means were determined by using Tukey's HSD test (P<0.05).

3. Results and Discussion

In this study, the effect of different concentrations of four fungicides (boscalid+kresoxim methyl, fluopyram+tebuconazole, sulphur and tetraconazole) on *Trichoderma* spp. was evaluated under laboratory conditions. The fungicides significantly reduced mycelial growth of *T. harzianum* (11-TTR-2), *T. hamatum* (F4), *T. atroviride* (T-4-5) and *T. asperellum* (T-11-25) compared to control (P<0.05) (Table 2). Even at the lowest concentration (0.0625 mL L^{-1}), fluopyram+tebuconazole completely inhibited mycelial growth of *T. hamatum* and *T. asperellum*, whereas it reduced mycelial growth of *T. atroviride* and *T. harzianum* by 89.48% and 93.97%, respectively. On the other hand, boscalid+kresoxim-methyl and sulphur reduced the mycelial growth of *T. harzianum*, *T. hamatum*, *T. atroviride* and *T. asperellum* by 55.53% and 30.81%, 58.78% and 29.97%, 63.59% and 28.40%, and 60.97% and 29.68% respectively, at their highest concentrations. With a few exceptions, tetraconazole at 1.0% had similar inhibitory effects as fluopyram+tebuconazole. Moreover, the inhibitory effects of these two fungicides were significantly different from those of boscalid+kresoxim-methyl and sulphur (P<0.05). These results are compatible with those of Sonavane and Venkataravanappa (2017), who showed 2000 ppm concentrations of sulphur to reduced the mycelial growth of *T. harzianum* by 16.02%. On the other hand, Suneeta et al. (2017) found that 250 ppm concentrations of tebuconazole, propioconazole, difenoconazole, propineb, and tebuconazole+trifloxystrobin totally inhibited the mycelial growth of *T. harzianum*. The same researchers observed that azoxystrobin, kresoxim-methyl, carbendazim and fosetyl aluminium reduced mycelial growth by 41.11%, 32.22%, 8.99% and 54.44%, respectively, at the highest concentration (2000 ppm). Similarly, Singh et al. (2016) reported that the thiophanate methyl and tebuconazole at 500 ppm concentration completely inhibited the mycelial growth of *T. harzianum*, while mancozeb+metalaxyl-M and carbendazin, even at the

Table 2. The inhibitory effects of different concentrations of four fungicides on mycelial growth of *Trichoderma* spp.

Fungicides	Concentrations (g mL L^{-1})	Inhibition of mycelial growth (%)			
		T. harzianum	*T. hamatum*	*T. atroviride*	*T. asperellum*
Boscalid+kresoxim methyl	0.075	26.64[a] ±2.49[b] hi[c]	30.03±1.13 e	31.13±0.72 h	32.68±0.65 d
	0.15	40.14±0.96 ef	4296±1.05 d	49.12±0.71 g	42.821±.28 c
	0.3	44.76±2.35 e	50.25±0.86 c	56.31±0.48 f	45.92±4.12 c
	0.6	55.53±0.46 d	58.78±0.62 b	63.59±0.54 e	60.97±0.28 b
Fluopyram+tebuconazole	0.0625	93.97±0.46 a	100.00±0.00 a	89.48±0.53 c	100.00±0.00 a
	0.125	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
	0.25	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
	0.5	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
Sulphur	1.0	6.75±0.58 jk	7.23±0.53 h	7.28±0.52 k	6.81±0.29 f
	2.0	11.34±1.53 j	17.44±1.86 g	13.83±1.23 j	11.92±0.41 f
	4.0	21.92±1.10 i	21.44±1.62 f	20.60±2.98 i	20.67±0.46 e
	8.0	30.81±0.78 gh	29.97±0.76 e	28.40±0.44 h	29.68±0.49 d
Tetraconazole	0.125	35.43±0.23 fg	100.00±0.00 a	72.59±0.47 d	100.00±0.00 a
	0.25	41.98±4.15 ef	100.00±0.00 a	75.24±0.35 d	100.00±0.00 a
	0.5	68.54±1.77 c	100.00±0.00 a	92.72±0.37 bc	100.00±0.00 a
	1.0	82.16±0.93 b	100.00±0.00 a	95.61±0.17 ab	100.00±0.00 a
Control	0	0.00±0.00 k	0.00±0.00 i	0.00±0.00 l	0.00±0.00 g

[a]Values represent the mean of five replications of fungicides concentrations used for *Trichoderma* spp. [b]Mean values followed by standard error of the mean. [c]Means followed by the same letter within same column are not significant different according to the Tukey's HSD (P<0.05).

highest concentration (2000 ppm) used in the study, could reduce the growth of the fungus by up to 94.5%, but the captan did not. Khan and Shahzad (2007) determined that carbendazim and thiophanate methyl suppressed the mycelial growth of *T. harzianum*, *T. pseudokoningii*, *T. longibrachiatum* and *T. viride* even at very low concentrations, and the latter fungicide completely inhibited the growth of *T. harzianum* at 10 ppm.

Of all four fungicides used in the present study, the most toxic for isolates of *T. harzianum*, *T. hamatum*, *T. atroviride* and *T. asperellum* was fluopyram+tebuconazole (Table 3). While the effectiveness of boscalid+kresoxim methyl and tetraconazole varied according to the species of *Trichoderma*, sulfur was non-toxic to none. The previous studies reported that systemic fungicides (hexaconazole, tridemorph propiconazole, triflumizole, triflumizole, bitertanol and azoxystrobin) are more toxic to *T. harzianum* than contact fungicides that have no inhibitory effects at low concentrations such as copper oxychloride and copper hydroxide (Sarkar et al. 2010). Similarly, Ranganathaswamy et al. (2012) found that benzimidazoles showed higher toxicity to *T. harzianum* and *T. virens* compared to chlorothalonil and triazoles, but the toxicities of sulphur, Bordeaux mixture, azoxystrobin and mancozeb were found to be much lower than that of the latter group. Bagwan (2010) found that *T. harzianum* and *T. viride* were most sensitive to captan, tebuconazole, carboxin+thiram, propiconazole and chlorothalonil, but were not susceptible to thiram, copper oxychloride and mancozeb. Roberti et al. (2006) determined that all of *Clonostachys rosea*, *T. atroviride*, *T. harzianum*, *T. longibrachiatum* and *T. viride* had low sensitivity to carboxin and thiram, but they had a high sensitivity to prochloraz. They also showed that guazatine, prochloraz and triticonazole were highly toxic for the mycelial growth of *T. viride*, and carboxin, guazatine and thiram were moderately insensitive for the mycelial growth of *T. harzianum*.

In the previous studies, several fungicides including prochloraz, guazatine, cyprodinil, fludioxonil, azoxystrobin, metalaxyl+mancozeb, metalaxyl+copper oxide, copper hydroxide, copper sulphate and copper oxide were found to be more effective to conidial germination of *Trichoderma* spp. (Roberti et al. 2006; Marcellin et al. 2018; Silva et al. 2018). The results of the present study showed that fluopyram+tebuconazole reduced the conidial germination of *T. harzianum* and *T. atroviride* by 84.13% and 81.58%, although it completely inhibited the conidial germination of *T. hamatum* and *T. asperellum* at the lowest concentration (0.0625 ml L^{-1}) (Table 4). In addition, this inhibitory effect was statistically different from the effects of other fungicides, with a few exceptions ($P<0.05$).

Generally, all four fungicides strongly decreased the germ-tube elongation of the *Trichoderma* species compared to control (Table 5). The effectiveness of the lowest fluopyram+tebuconazole concentration on germ tube elongation, with the exception of sulfur, was also similar to the highest level of the other two fungicides ($P<0.05$).

Table 3. The EC_{50} and MIC values of the fungicides inhibiting mycelial growth of *Trichoderma* spp.

Fungicides	*Trichoderma* spp.							
	T. harzianum		*T. hamatum*		*T. atroviride*		*T. asperellum*	
	EC_{50}[a]	MIC[b]	EC_{50}	MIC	EC_{50}	MIC	EC_{50}	MIC
Boscalid+kresoxim methyl	0.396	>0.6	0.297	>0.6	0.212	>0.6	0.301	>0.6
Fluopyram+tebuconazole	0.025	0.125	<0.0625	0.0625	0.032	0.125	<0.0625	0.0625
Sulphur	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
Tetraconazole	0.26	>1.0	<0.125	0.125	0.052	>1.0	<0.125	0.125

[a]The concentration that caused 50% reduction. [b]Minimum inhibitory concentration.

Table 4. The inhibition effects of different concentrations of four fungicides on conidial germination of *Trichoderma* spp.

Fungicides	Concentrations (g mL L^{-1})	Inhibition of conidial germination (%)			
		T. harzianum	*T. hamatum*	*T. atroviride*	*T. asperellum*
Boscalid+kresoxim methyl	0.075	62.00[a]±0.50[b] f[c]	54.66±0.66 de	56.44±1.44 ef	51.06±1.06 e
	0.15	66.13±0.53 e	57.28±2.28 d	60.50±0.50 e	60.85±0.85 d
	0.3	71.69±0.19 d	72.70±0.70 c	72.84±0.84 d	70.60±0.39 c
	0.6	73.32±0.64 d	76.07±0.52 bc	77.97±0.97 cd	76.86±1.86 b
Fluopyram+tebuconazole	0.0625	84.13±0.25 c	100.00±0.00 a	81.58±1.58 c	100.00±0.00 a
	0.125	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
	0.25	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
	0.5	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
Sulphur	1.0	27.58±1.58 ı	27.36±1.36 h	25.66±0.66 h	21.70±1.70 h
	2.0	35.21±0.21 h	34.08±2.08 g	30.76±0.23 h	30.95±0.95 g
	4.0	37.77±0.77 h	39.71±0.71 f	43.10±1.10 g	41.74±0.25 f
	8.0	44.91±0.91 g	50.50±0.50 e	51.32±1.31 f	51.16±1.16 e
Tetraconazole	0.125	66.16±0.50 e	80.42±0.42 b	77.94±0.05 cd	100.00±0.00 a
	0.25	74.77±0.22 d	100.00±0.00 a	80.52±0.52 c	100.00±0.00 a
	0.5	83.52±0.85 c	100.00±0.00 a	88.76±1.76 b	100.00±0.00 a
	1.0	90.83±0.83 b	100.00±0.00 a	92.34±1.34 b	100.00±0.00 a
Control	0	0.00±0.00 j	0.00±0.00 ı	0.00±0.00 ı	0.00±0.00 ı

[a]Values represent the mean of three replications of fungicides concentrations used against *Trichoderma* spp. [b]Mean values followed by standard error of the mean. [c]Means followed by the same letter within same column are not significant different according to the Tukey's HSD ($P<0.05$).

Table 5. The inhibition effects of different concentrations of four fungicides on germ-tube elongation of *Trichoderma* spp.

Fungicides	Concentrations (g mL L^{-1})	Inhibition of germ-tube elongation (%)			
		T. harzianum	*T. hamatum*	*T. atroviride*	*T. asperellum*
Boscalid+kresoxim methyl	0.075	23.32[a]±2.41[b] i[c]	30.68±0.83 f	38.63±2.32 g	41.24±1.89 f
	0.15	67.79±1.26 f	75.33±0.45 c	66.32±1.67 f	49.47±1.50 e
	0.3	78.18±1.20 e	76.18±0.48 c	84.19±0.77 cde	79.44±1.27 c
	0.6	87.76±0.61 bc	89.54±0.59 b	89.65±0.47 bc	91.37±0.70 b
Fluopyram+tebuconazole	0.0625	92.61±0.58 b	100.00±0.00 a	91.34±0.64 b	100.00±0.00 a
	0.125	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
	0.25	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
	0.5	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
Sulphur	1.0	17.64±1.62 i	21.55±1.30 g	20.09±2.20 h	20.49±1.10 h
	2.0	30.04±1.52 h	37.15±1.47 e	42.65±1.60 g	29.46±2.04 g
	4.0	56.69±1.94 g	67.85±1.29 d	67.21±1.07 f	63.85±1.39 d
	8.0	78.82±1.47 de	76.64±0.41 c	79.59±0.99 e	84.05±0.49 c
Tetraconazole	0.125	29.19±2.17 hı	99.02±0.07 a	69.52±1.23 f	100.00±0.00 a
	0.25	61.04±1.31 g	100.00±0.00 a	82.28±0.81 de	100.00±0.00 a
	0.5	84.42±0.25 cd	100.00±0.00 a	85.89±0.66 bcd	100.00±0.00 a
	1.0	89.11±0.59 bc	100.00±0.00 a	98.55±0.10 a	100.00±0.00 a
Control	0	0.00±0.00 j	0.00±0.00 h	0.00±0.00 ı	0.00±0.00 ı

[a]Values represent the mean of three replications of fungicides concentrations used against *Trichoderma* spp. [b]Mean values followed by standard error of the mean. [c]Means followed by the same letter within same column are not significant different according to the Tukey's HSD ($P<0.05$).

4. Conclusion

Invasive ambrosia beetle, *X. germanus* is one of the most economically important pests in hazelnut orchards of Turkey. Adults and larvae of the beetle feed only on symbiotic fungi cultivated by females in the galleries, so controlling the fungi means depriving the beetles from a food source. Mycoparasitic fungi, *Trichoderma* spp. could be used to target the symbiotic fungi. The fungicides applied for the control of *E. corylacearum* in hazelnut growing areas adversely affect the use of *Trichoderma* species. Consequently, the present study shown boscalid+kresoxim methyl and sulphur used hazelnut orchards to be less harmful against biocontrol fungi *Trichoderma* spp. than fluopyram+tebuconazole and tetraconazole. Therefore, boscalid+kresoxim methyl and sulphur, especially at low concentrations may be recommended in hazelnut orchards where *Trichoderma* spp. should be used against *X. germanus*. However, these fungicides should not be used at the same time with *Trichoderma* spp.

References

Bagwan NB (2010) Evaluation of *Trichoderma* compatibility with fungicides, pesticides, organic cakes and botanicals for integerated management of soil borne diseases of soybean [*Glycine max* (L.) Merril]. International Journal of Plant Protection 3: 206-209.

Benítez T, Rincón AM, Limón MC, Codon AC (2004) Biocontrol mechanisms of *Trichoderma* strains. International Microbiology 7(4): 249-260.

Castrillo LA, Griggs MH, Ranger CM, Reding ME, Vandenberg JD (2011) Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycota: Hypocreales) against adult *Xylosandrus germanus* (Coleoptera: Curculionidae) and impact on brood. Biological Control 58(2): 121-126.

Castrillo LA, Griggs MH, Vandenberg JD (2016) Competition between biological control fungi and fungal symbionts of ambrosia beetles *Xylosandrus crassiusculus* and *X. germanus* (Coleoptera: Curculionidae): mycelial interactions and impact on beetle brood production. Biological Control 103: 138-146.

Erper İ, Kushiyev R, Türkkan M, Tuncer C (2018). Evaluation of some fungicides against symbiotic fungus *Ambrosiella hartigii* associated with *Anisandrus dispar* Fabricius and *Xylosandrus germanus* Blandford (Coleoptera: Curculionidae: Scolytinae). Selçuk Tarım ve Gıda Bilimleri Dergisi 32(1): 60-66.

GKGM (2020) Bitki Koruma Ürünleri Veri Tabanı. https://bku.tarim.gov.tr. Erişim 10 Şubat 2020.

Khan MO, Shahzad S (2007) Screening of *Trichoderma* species for tolerance to fungicides. Pakistan Journal of Botany 39(3): 945-951.

Kushiyev R, Tuncer C, Erper İ, Özdemir İO, Saruhan İ (2018) Efficacy of native entomopathogenic fungus, *Isaria fumosorosea*, against bark and ambrosia beetles, *Anisandrus dispar* Fabricius and *Xylosandrus germanus* Blandford (Coleoptera: Curculionidae: Scolytinae). Egyptian Journal of Biological Pest Control 28(1): 55.

Marcellin ML, François ME, Valteri VA, Endali EMJ, Begoude BAD (2018) In vitro study of the compatibility of six fungicides with two strains of *Trichoderma asperellum*, biocontrol agents used against cacao black pod disease in Cameroon. International Journal of Innovation and Applied Studies 24(4): 1834-1848.

Ranganathaswamy M, Patibanda AK, Rao GN (2012) Evaluation of toxicity of agrochemicals on *Trichoderma* isolates in vitro. Journal of Biological Control 26(4): 391-395.

Roberti R, Badiali F, Pisi A, Veronesi A, Pancaldi D, Cesari A (2006) Sensitivity of *Clonostachys rosea* and *Trichoderma* spp. as potential biocontrol agents to pesticides. Journal of Phytopathology 154(2): 100-109.

Sarkar S, Narayanan P, Divakaran A, Balamurugan A, Premkumar R (2010) The in vitro effect of certain fungicides, insecticides, and biopesticides on mycelial growth in the biocontrol fungus *Trichoderma harzianum*. Turkish Journal of Biology 34(4): 399-403.

Silva MAFD, Moura KED, Moura KED, Salomão D, Patricio FRA (2018) Compatibility of *Trichoderma* isolates with pesticides used in lettuce crop. Summa Phytopathologica 44(2): 137-142.

Singh C, Sharma N, Singh BR (2016) *Trichoderma harzianum*: mass multiplication and its interaction with different fundicides. Journal of Biotechnology and Biosafety 4(1): 332-338.

Sonavane P, Venkataravanappa V (2017) Compatibility Studies of *Trichoderma harzianum* isolate with fungicides used against soil borne disease in Coorg Mandarin-Pepper-Coffee Plantations. International Journal of Current Microbiology and Applied Sciences 6(8): 346-354.

Suneeta P, Kumar SV, Aiyanathan KEA, Nakkeeran S (2017) Promissory Action of *Trichoderma* spp. and fungicides in the management of *Fusarium* wilt of gerbera. Journal of Pure and Applied Microbiology 11(1): 241-247.

Tuncer C, Knizek M, Hulcr J (2017) Scolytinae in hazelnut orchards of Turkey: clarification of species and identification key (Coleoptera, Curculionidae). ZooKeys 710: 65.

Tuncer C, Kushiyev R, Erper İ, Özdemir İO, Saruhan İ (2019). Efficacy of native isolates of *Metarhizium anisopliae* and *Beauveria bassiana* against the invasive ambrosia beetle, *Xylosandrus germanus* Blandford (Coleoptera: Curculionidae: Scolytinae). Egyptian Journal of Biological Pest Control 29(1): 28.

Türkkan M, Erper İ, Eser Ü, Baltacı A (2018) Evaluation of inhibitory effect of some bicarbonate salts and fungicides against hazelnut powdery mildew. Gesunde Pflanze 70: 39-44.

Weber BC, McPherson JE (1983) Life history of the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). Annals of the Entomological Society of America 76(3): 455-462.