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The Effects of Boron-Containing Compounds against *Monilinia fructigena* Mycelium Growth

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ABSTRACT

Monilinia fructigena is the causative agent of brown rot in pome fruits, contributing to substantial economic losses, especially in storage facilities. The effects of boron-containing compounds have been considered as an environmentally friendly alternative to fungicides. The objective of the study was to determine suitable boron-containing compounds for inhibiting the mycelium growth of *M. fructigena*. Eight different compounds with pH adjusted to neutral (pH 7) and non-neutral were tested with concentrations of 0 (untreated control), 5, 10, 20, 40, 60, 80, and 100 mM *in vitro* conditions. The mycelium growth of the pathogen was totally inhibited with the application of 20 mM of potassium tetrafluoroborate and 10 mM of sodium tetrafluoroborate. The tested concentrations of ammonium pentaborate tetrahydrate, antidot-67, sodium metaborate, and sodium tetraborate decahydrate were not sufficiently effective in inhibiting the mycelium growth of *M. fructigena*, but the experiment of higher concentrations of them could be utility against the pathogen. The pH of boron-containing compounds was crucial in improving the efficacy of compounds, and the non-neutral compounds showed better results against to inhibition of *M. fructigena* mycelium growth. The results showed that boron-containing compounds may be pathogen-specific and that the activity of these compounds is related to pH.

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Bor İçeren Bileşiklerin *Monilinia fructigena* Misel Gelişimine Etkileri

ÖZET

Monilinia fructigena yumuşak çekirdekli meyvelerde kahverengi çürüklük etmenidir ve özellikle depolama tesislerinde önemli ekonomik kayıplara neden olmaktadır. Bor içeren bileşiklerin patojenlere karşı etkileri ise fungisitlere alternatif çevre dostu bir uygulama olarak değerlendirilmektedir. Bu çalışmanın amacı *M. fructigena*'nın misel gelişimini inhibe etmek için uygun bor içeren bileşiklerin belirlenmesidir. Bu bağlamda, nötr (pH 7) ve nötr olmayan pH ile ayarlanmış sekiz farklı bor bileşiğinin, sekiz farklı konsantrasyonun (0; işlenmemiş kontrol, 5, 10, 20, 40, 60, 80 ve 100 mM) patojene etkililikleri *in vitro* koşullarda araştırılmıştır. Uygulamalar arasında 20 mM potasyum tetrafloroborat ve 10 mM sodyum tetrafloroboratın fungusun miselyum gelişimini tamamen engellediği tespit edilmiştir. Amonyum pentaborat tetrahidrat, etidot-67, sodyum metaborat ve sodyum tetraborat dekahidratın test edilen konsantrasyonları etmenin miselyum gelişimini yeterince inhibe etmemiş, ancak daha yüksek konsantrasyonlarının patojene karşı daha yüksek etki gösterebilecekleri değerlendirilmiştir. Bor içeren bileşiklerin pH'sı, bileşiklerin etkinliğinin arttırılmasında önemli bir kriter olmuş ve nötr olmayan bileşiklerin *M. fructigena*'nın miselyum gelişiminin inhibisyonunda daha iyi sonuçlar verdiği gözlenmiştir. Elde edilen sonuçlar, bor içeren bileşiklerin etmene spesifik olabileceğini ve bu bileşiklerin etkinliğinin pH ile ilişkili olduğunu göstermiştir.

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INTRODUCTION

Monilinia fructigena (Aderhold and Ruhland) Honey is one of the most important fungal pathogens which cause brown rot on pome fruits. The pathogen generally infects the fruits before and during storage, while blossom and branch infections in plants are rarely observed (Hrustić et al., 2012). Wounds or growth cracks inflicted by insects and birds cause increased disease occurrence and are known to cause economic losses of more than 1.5 million euros (Batra, 1991; Bautista-Baños, 2014). Brown rot pathogens primarily infect the fruits, and the disease can occur during storage, so reducing fruit injuries during harvest is of great importance for managing the disease (Van Leeuwen et al., 2000). However, fungicides are mostly used to control brown rot in orchards, but the usage of fungicides is not allowed by the European Union as well as Türkiye during the post-harvest and in storage facilities (Karabulut & Baykal, 2004; Hrustić et al., 2012; 2018). Alternative control methods are needed due to the formation of resistance to fungicides such as thiophanate-methyl and benzimidazoles, which are commonly used against the pathogen (Ivić et al., 2021). Eco-friendly disease control methods with UV-C and heat treatment, biocontrol using some yeasts, heated water immersion, thyme essential oils and boron-containing compounds are used by many researchers (Marquenie et al., 2002; Larena et al., 2005; Karabulut et al., 2010; Thomidis & Exadaktylou, 2010; Mari et al., 2012; Tanovic et al., 2015; Thomidis et al., 2017; Yildiz & Coskuntuna, 2019; Zhang et al., 2020; Ardıç et al., 2021).

Boron products such as boric acid and borax can be used as food preservatives against some types of bacteria and fungi (Estevez-Fregoso et al., 2021). It has been stated that boron application in fruits reduces cuticular microcracks and membrane permeability, and is protective against brown rot factors, especially in storage facilities (Xuan et al., 2000; Thomidis & Exadaktylou, 2010). Various boron-containing compounds, such as Borax, Power B, and the commercial product Nutrel B, have been reported to inhibit *Monilinia laxa* mycelium growth (Thomidis & Exadaktylou, 2010; Thomidis et al., 2017). Similarly, different researchers have reported the inhibitory effects of boric acid and potassium tetraborate on *Botrytis cinerea* (Omaima & Karima, 2007; Qin et al., 2010). The number of identified boron-containing compounds is increasing because of improved natural identification and innovative synthesis procedures, and there is a need to determine the species-specific effects of these compounds on fungi, to find environmentally friendly preservatives, and to select suitable boron-containing compounds and their appropriate concentrations as preservatives against specific fungal pathogens (Estevez-Fregoso et al., 2021).

The effectiveness of boron depends on concentration and pH (Woods 1994). Therefore, determining the optimum pH values of the compounds is very important in terms of increasing their effectiveness. Qin et al. (2010) stated that alkaline potassium tetraborate gave better results than the neutral solution in the control of *B. cinerea*. The pH of the boron compounds tested against *M. laxa* was determined as 7 by Thomidis & Exadaktylou (2010) and Thomidis et al. (2017). The aims of this study were (i) to determine the effects of several boron-containing compounds on *M. fructigena* mycelium growth, (ii) to find suitable concentrations of these compounds, and (iii) to evaluate the interactions between boron-containing compounds and pH with *M. fructigena* mycelium growth.

MATERIAL and METHODS

Isolation of *Monilinia fructigena*

Monilinia fructigena was isolated from an infected Granny Smith apple tree. The infected parts of the tree including fruits and blossoms were sampled and transferred to the laboratory. The surface of the collected samples was sterilized superficially to avoid the development of other microorganisms that might exist on the samples. The samples were washed with tap water and then sterilized with 0.5% sodium hypochlorite for 5 minutes. The sterilized samples were cleaned by rinsing sterile distilled water three times and dried on sterile filter paper. The infected tissues were cut into 2–5 mm pieces with sterile scalpel and inoculated into media of 2% w/w potato dextrose agar (PDA-Merck Millipore) which includes 0.5 g/L streptomycin sulfate powder (Sigma Aldrich) to avoid bacterial contamination. The Petri dishes were incubated at 25 °C for 12 hours for consecutive dark and light periods in a controlled climate chamber. After seven days, the cultures were sub-cultured to a fresh nutrient medium to obtain pure *M. fructigena* isolate. The morphological characters such as color, aerial mycelium, margin shape, etc. were determined under a light microscope for characterization of the isolate (Batra, 1991). The obtained culture of *M. fructigena* has been confirmed by Lane (2002) and Van Leeuwen et al. (2002). The morphological identification was also confirmed with PCR assay as described by Côté et al. (2004). The *M. fructigena* culture was stocked at 4 °C until the experiments.

Experimental Design

Eight different boron-containing compounds given in Table 1 were used to determine the effects on *M. fructigena* mycelium growth. The final concentrations of tested boron-containing compounds were provided as 0 (untreated control), 5, 10, 20, 40, 60, 80, and 100 mM. Furthermore, two blocks were created to evaluate how the pH value influenced the efficacy of each tested compound. At the first, the pH of each growth media was measured by using electronic probes (Mettler Toledo S-210) and observed pH values were given in Table 1. In this experiment, the pH of tested media was not intervened and was considered as non-neutral media. In the second, the pH of growth media was adjusted to 7 (± 0.2 unit) by using hydrochloric acid (1N) for alkaline media and potassium hydroxide (1N) or sodium hydroxide (1N) for acidic media and considered as neutral media. The neutral (pH adjusted as 7.0) and non-neutral growth media were used as a factor for each concentration of tested compounds. Agricultural chemicals called Luna® 450 (200 g/L fluopyram + 200 g/L tebuconazole; 25 ml/100 L water) and Signum WG (26.7% boscalid + 6.7% pyraclostrobin; 40 g/100 L water) which are known to control *M. fructigena* mycelium growth were also used as control at the doses recommended by Republic of Türkiye Ministry of Agriculture and Forestry. The solutions of each compound were prepared in sterile distilled water and added to 2% w/w potato dextrose agar (PDA). The media was poured into 9 cm Petri dishes and 8 mm diameter agar disks taken from actively growing *M. fructigena* cultures were inoculated and incubated at 25 °C. Six replicates were used in each experiment and repeated twice.

Table 1. pH values of Boron-containing compounds used in the study
Çizelge 1. Çalışmada kullanılan bor içerikli bileşiklerin pH değerleri

	Tested pH (H_2O)							
Compounds	0	5	10	20	40	60	80	100
Ammonium pentaborate tetrahydrate	5.5	6.2	6.3	6.5	6.6	6.8	6.8	6.8
Boron oxide	5.5	5.8	5.7	5.7	5.5	5.5	5.4	5.3
Etidot-67	5.5	7.1	7.2	7.2	7.3	7.3	7.4	7.5
Potassium metaborate	5.5	7.1	7.6	7.9	8.1	8.3	8.4	8.4
Potassium tetrafluoroborate	5.5	5.4	5.1	4.6	4.2	3.9	3.6	3.6
Sodium metaborate tetrahydrate	5.5	7.5	7.9	8.2	8.4	8.6	8.7	8.7
Sodium tetraborate decahydrate	5.5	7.2	7.3	7.4	7.6	7.7	7.8	7.9
Sodium tetrafluoroborate	5.5	4.9	4.9	4.7	3.9	3.6	3.5	3.5

After seven days of incubation, the colony diameter of each Petri dish was calculated to evaluate the effects of boron-containing compounds on *M. fructigena* mycelium growth. A digital caliper (MarCal 16 EWR IP 67) was used to measure the colony diameter taking two orthogonal measurements and the final colony diameter per Petri dish was calculated as the average of both measurements. The colony growth of treatments was calculated as the diameter of the final colony in Petri dishes minus the diameter of the preliminary agar disk (8 mm). The mycelial development inhibition rates (%) of boron compounds on the 7th day were calculated with the Abbott formula (Karman, 1971).

Statistical Analysis

R software environment (version 3.6.2, R Core Team 2017) was used to carry out the statistical analysis. Before analysis, the normality and homogeneity of data were checked by Shapiro-Wilk (shapiro.test function) and Levene test (leveneTest function in "car" package). The datasets were transformed by Ordered Quantile Normalization (QRQ) to meet the assumptions of Analysis of Variances (ANOVAs) and some noises were added to datasets to break down ties by using the "jitter" function (Peterson & Cavanaugh, 2019). The datasets were back transformed to obtain means of data and to create the illustrations. The effects of concentrations of compounds and the pH (factors) on mycelium growth were analyzed by ANOVA. The posthoc analyses were conducted by Tukey Honest Significant Difference test (TukeyHSD function) when significant interaction between the factors was obtained and the "multcomp" package was used to compare between group means (Hothorn et al., 2008). The effect size statistics (partial eta square - $\eta 2p$) for ANOVAs were calculated by using "etaSquared" function in the "lsr" packages (Navarro, 2013).

RESULTS

The effects of several boron-containing compounds on the mycelial development of *M. fructigena* isolate, whose morphological and molecular identification was completed, were tested in the study. The results showed that the boron-containing compounds had statistically different effects on mycelium development (Table 2, Figure 1). The most effective applications in preventing pathogen development were sodium tetrafluoroborate (STFB) and potassium tetrafluoroborate (PTFB), respectively. The different concentrations of STFB significantly affected *M. fructigena* mycelial growth ($F_{7, 181} = 36.46$, $p<0.01$, $\eta^2_p = 0.60$), and the development was not observed when the concentration was higher than 10 mM (Figure 1h). Similar to STFB, mycelium development was greatly inhibited in applications of 20 mM ($F_{7, 184} = 68.80$, $p<0.01$, $\eta^2_p = 0.73$) and above among different concentrations of PTFB. Although pH adjustment had no effects on the growth media ($F_{1, 184} = 0.98$, $p=0.32$, $\eta 2p = 0.005$), the interactions of these two parameters had significant effects ($F_{7, 184} = 3.57$; $p<0.01$, $\eta 2p = 0.12$). The non-neutral growth media showed better inhibitory effects on *M. fructigena* development compared to neutral growth media.

Table 2. Average values of *Monilinia fructigena* growth after seven days of incubation (mm)
Çizelge 2. Yedi gün inkübasyondan sonra Monilinia fructigena gelişiminin ortalama değerleri (mm)

Compounds		Concentrations (mM) 0	5	10	20	40	60	80	100
Ammonium pentaborate tetrahydrate (APBT)	Non-neutral	51.26±5.40	66.23±1.11	66.44±1.53	69.75±0.53	74.03±1.45	28.68±1.45	17.70±2.65	10.93±2.63
	Neutral	67.57±4.96	67.89±1.41	68.38±1.47	72.69±1.00	66.09±1.13	27.06±1.41	15.51±2.02	10.06±1.60
Boron oxide (BO)	Non-neutral	51.26±5.40	39.18±0.48	40.09±0.64	38.85±1.87	39.47±2.70	47.28±0.93	51.49±0.73	51.74±1.01
	Neutral	67.57±4.96	67.55±3.66	58.53±1.28	57.46±2.12	59.54±1.89	64.94±1.27	63.45±1.46	62.12±1.74
Etidot-67 (ETI)	Non-neutral	51.88±5.19	57.30±2.01	58.12±1.39	65.24±2.80	42.05±3.04	26.18±1.40	15.10±2.85	7.03±1.25
	Neutral	67.57±4.96	52.58±2.36	58.03±1.63	65.95±1.35	49.49±1.58	39.27±1.25	34.47±0.61	28.95±1.16
Potassium metaborate (PMB)	Non-neutral	51.26±5.40	57.40±2.14	57.38±3.70	56.05±3.45	57.24±1.60	63.64±2.23	65.66±2.96	65.92±1.40
	Neutral	67.57±4.96	64.11±2.97	61.17±3.65	60.72±4.31	58.09±2.56	58.88±3.05	59.81±4.07	60.43±2.89
Potassium tetrafluoroborate (PTFB)	Non-neutral	51.26±5.40	33.62±1.93	6.65±1.19	ng*	ng	ng	ng	ng
	Neutral	67.57±4.96	50.25±1.65	26.24±0.63	ng	ng	ng	ng	ng
Sodium metaborate tetrahydrate (SMT)	Non-neutral	51.26±5.40	51.22±1.97	46.16±3.31	46.86±5.67	40.93±3.62	35.94±1.72	23.43±3.28	15.86±1.83
	Neutral	67.57±4.96	49.77±3.29	45.97±2.82	45.44±2.39	38.31±3.13	36.69±4.43	35.14±2.09	34.30±4.03
Sodium tetraborate decahydrate (STBD)	Non-neutral	51.88±4.95	54.43±4.82	56.04±2.79	62.34±5.46	50.55±0.93	25.93±2.76	18.53±2.41	2.35±1.50
	Neutral	67.57±4.96	43.67±1.32	43.98±1.67	42.77±0.73	41.24±0.79	33.99±1.22	26.20±0.48	23.10±1.04
Sodium tetrafluoroborate (STFB)	Non-neutral	51.26±5.40	7.10±0.72	ng	ng	ng	ng	ng	ng
	Neutral	67.58±4.96	23.63±0.71	ng	ng	ng	ng	ng	ng

*ng: no grow

The different concentrations of ammonium pentaborate tetrahydrate (APBT) have controlled *M. fructigena* mycelial growth by varying degrees ($F_{7, 175}= 183.58$, $p<0.001$, $\eta 2p = 0.88$). Although pH adjustment of media did not show a significant impact ($F_{1, 175} = 0.09$, $p=0.75$), the interaction of these two factors had significant effects on *M. fructigena* development ($F_{7, 175} = 27.54$, $p<0.01$, $\eta 2p = 0.54$). APBT with higher levels than 40 mM had a better controlling tendency and 100 mM concentration of APBT showed the best results to control with 10.06±1.60 mm and 10.93±2.63 mm mycelial growth for neutral and non-neutral growth media, respectively (Table 2, Figure 1a). Unfortunately, none of the concentrations of APBT tested in this study were able to fully inhibit *M. fructigena* development. The concentrations of boron oxide (BO) ($F_{7, 165} = 35.11$, $p<0.01$, $\eta 2p = 0.59$) and the pH adjustment ($F_{1, 165} = 735.66$, $p<0.01$, $\eta 2p = 0.82$) had statistically significant effects, but the applications did not able to control *M. fructigena* mycelium growth (Figure 1b). The neutral growth media supported mycelium growth compared to non-neutral growth media and the pH factor had higher effects than the concentration of BO. The interactions of these two factors had significant effects on mycelium growth ($F_{7, 165} = 11.63$, $p<0.01$, $\eta 2p = 0.34$). *M. fructigena* development decreased significantly with increasing concentrations of epidote-67 application (disodium octaborate tetrahydrate – ETI) and the best result was observed when the concentration was 100 mM non-neutral medium with 7.03±1.25 mm mycelium growth (Table 2, Figure 1c). Although potassium metaborate (PMB) concentrations and pH changes have statistically different effects, it was observed that they could not prevent fungal growth ($F_{7, 184} = 8.30$, $p<0.01$, $\eta^2_p = 0.25$ for concentration, $F_{1, 184} = 17.20$, $p<0.01$, $\eta^2_p = 0.09$ for pH adjustment) (Figure 1d).

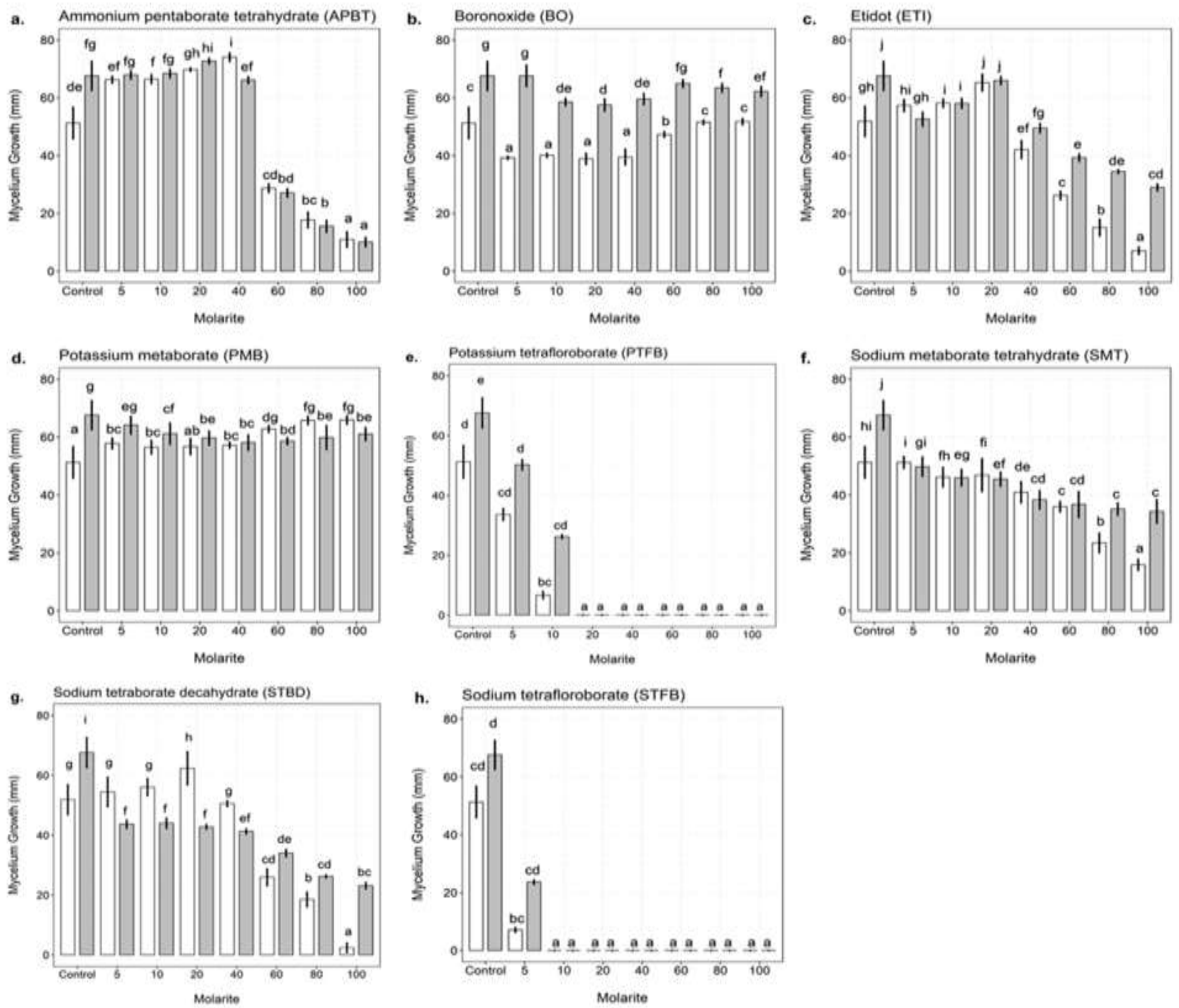

Figure 1. Effects of boron-containing compounds on the growth of *Monilinia fructigena* after seven days of incubation (a. Ammonium pentaborate tetrahydrate, b. Boron oxide, c. Etidot-67, d. Potassium metaborate, e. Potassium tetrafloroborate, f. Sodium metaborate tetrahydrate, g. Sodium tetraborate decahydrate, h. Sodium tetrafloroborate. *The white bars indicate non-neutral growth media while grey bars are neutral growth media)

*Şekil 1. Yedi gün inkübasyondan sonra bor içerikli bileşiklerin Monilinia fructigena gelişimine etkileri (a. Ammonium pentaborate tetrahydrate, b. Boron oxide, c. Etidot-67, d. Potassium metaborate, e. Potassium tetrafloroborate, f. Sodium metaborate tetrahydrate, g. Sodium tetraborate decahydrate, h. Sodium tetrafloroborate. * Beyaz barlar nötr olmayan gelişme ortamını gösterirken gri barlar nötr gelişme ortamını göstermektedir)*

The increasing concentration of sodium metaborate tetrahydrate (SMT) and sodium tetraborate decahydrate (STBD) substantially reduced *M. fructigena* mycelium growth by 15.86±1.83 mm and 2.35±1.50 mm, respectively, at 100 mM concentrations. Although pH adjustment of the growing medium in the SMT application had significant, albeit low, effects on mycelium growth ($F_{1, 184} = 31.66$, $p<0.01$, $\eta^2_p = 0.15$), pH adjustment in the STBD application did not have a significant effect on mycelium growth ($F_{1, 176} = 1$, $p=0.31$, $\eta^2_p = 0.006$). However, the interaction between these factors significantly affected mycelium growth for both treatments ($F_{7, 184} = 19.74$ $p<0.01$, $\eta^2_p = 0.44$ for SMT; $F_{7, 176} = 89.19$, $p<0.01$, $\eta^2_p = 0.79$ for STBD). The results showed that 100 mM SMT and STBD with non-neutral growth media can control *M. fructigena* development (Figure 1f, 1g). Additionally, Luna and Signum used as controls completely inhibited *M. fructigena* mycelium growth.

The highest inhibition values of boron-compounds in *M. fructigena* mycelial development were observed in STFB and PTFB applications similar to the effect of mycelial diameter values (Figure 2). Mycelial development was prevented 100% at concentrations of ≥10 mM of STFB and ≥ 20 mM of PTFB. Doses of 60 mM ≥ APBT and STBD applications, gradually increased the percentage of inhibition of mycelial development of the pathogen, regardless of pH. However, 100 mM concentration of STBD inhibited mycelial growth at a higher rate than neutral, with a 95.47% inhibition rate in non-neutral media. BO application inhibited mycelial growth by up to 24.20% and showed that inhibition rates varied between neutral and non-neutral media. While an increasing inhibition from 18.95% to 86.45% was recorded with the ETI application, this range varied from 9.95 to 69.06% with the SMT application. Pathogen development could not be significantly prevented in PMP application, but it was revealed that there was an adverse effect between media.

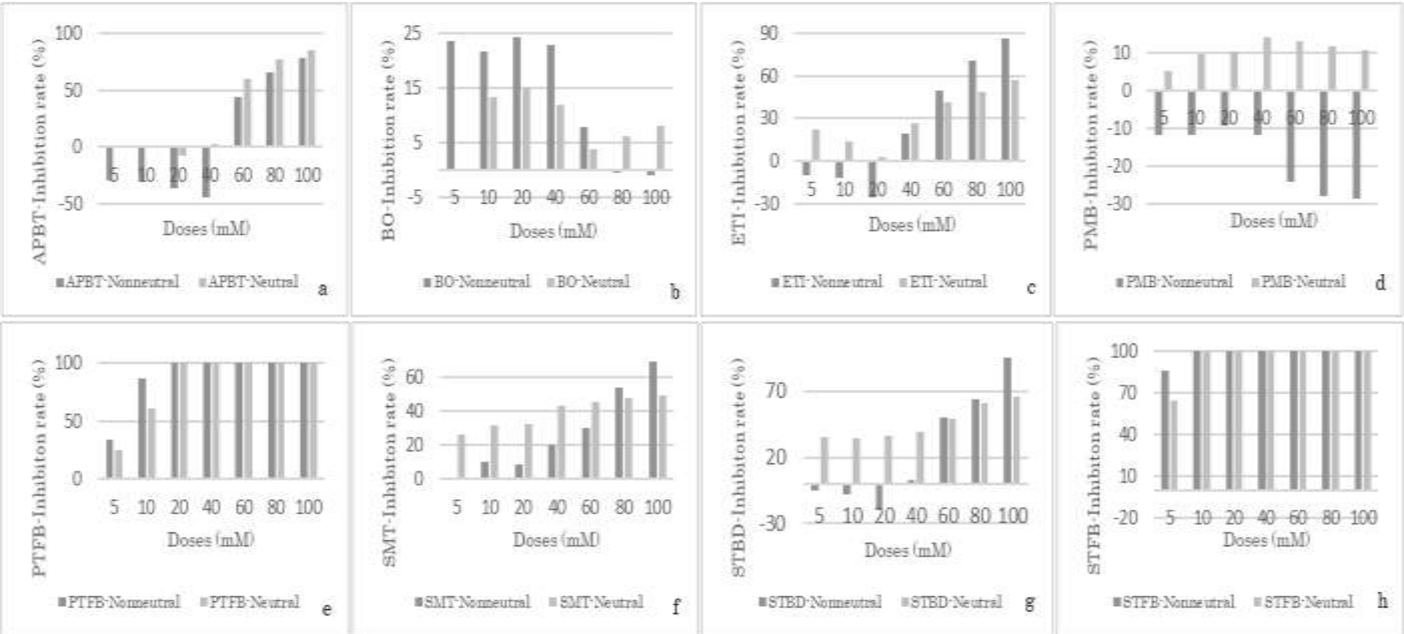

Figure 2. Inhibition rates (%) of boron-compounds on *Monilinia fructigena* mycelial growth (a; APBT, B; BO, c; ETI; d; PMB, e; PTFB, f; SMT, g; STBD, h; STFB).

Şekil 2. Borlu bileşiklerin Monilinia fructigena miselyal gelişimini engelleme oranları (%) (a; APBT, B; BO, c; ETI; d; PMB, e; PTFB, f; SMT, g; STBD, h; STFB).

DISCUSSION and CONCLUSION

Boron is widely used in many fields such as industry and medicine and is also one of the important micronutrients for plants (Woods, 1994; Bolt et al., 2017; Shireen et al., 2018). The effects of boron-containing compounds in fungi have been investigated in recent years and the attention to the application of boron has been increasing because of its environmental-friendly preservative effects against several plant pathogens (Gedük et al., 2020; Estevez-Fregoso et al., 2021; Gür et al., 2021). Finding pathogen-specific boron-containing compounds and determining their appropriate concentrations are important for the management of plant diseases. The aim of this study was to investigate various boron-containing compounds to control brown rot caused by *Monilinia fructigena*.

Sodium tetrafluoroborate (STFB) and PTFB were the most effective compounds in inhibiting pathogen growth among applications. It was observed that mycelial development was prevented completely after 10 mM STFB and 20 mM PTFB applications (Figure 1). Moreover, although the pH of these compounds did not have a significant effect on mycelium development, non-neutral media with lower concentrations showed better results than neutral media. Qin et al. (2010) showed that alkaline potassium tetraborate (pH 9.2) was more effective in controlling *B. cinera* than neutral compounds. *Monilinia fructigena* can optimally grow at pH 3.5 and it can also tolerate the alkaline growth medium at pH 9 (Holb, 2004; Hrustić et al., 2020). Holb (2004) hypothesized that *M. fructigena* decreased the pH of the substrate, thus the disease grows exponentially after acidity levels of fruits reached the optimal degree. However, the pH of boron-containing compounds applied against brown rot agents generally adjusted at 7 (Thomidis & Exadaktylou, 2010). Even though the results did not show significant effects of the pH of STFB and PTFB on *M. fructigena* mycelium growth, the pH of other tested compounds was significant (Figure 1). The main acidification agent of brown rot caused by *M. fructicola* was gluconic acid and the accumulation of gluconic acid created an unstable cell membrane as well as increased disease susceptibility (De Cal et al., 2013). Boron application could improve the cell membrane integrity in pears and reduce the micro cracking on the fruit surface (Thomidis & Exadaktylou, 2010; Xuan et al., 2002). Tetrafluoroborate anions can be associated with antifungal effects on *M. fructigena* because it is likely that fluorine improves the antifungal effects of these compounds (Kirk 2006).

Tested concentrations of APBT, ETI, SMT, and STBD failed to completely inhibit mycelium growth, but it appears that higher concentrations of these compounds may be useful in controlling *M. fructigena* mycelial growth. In particular, the non-neutral growth media with these compounds (except APBT) showed better results in controlling pathogen development. The higher concentrations of these compounds are slightly alkaline to alkaline and the effectiveness of these compounds against *M. fructigena* mycelium growth is increasing with the high level of pH. Boronoxide could not inhibit mycelial growth, and interestingly, the higher concentrations of BO increased it. Similarly, the pH of PMB was observed as one of the highest levels, but it cannot inhibit mycelium growth. The concentration and pH of boron-containing compounds are crucial for selecting appropriate pathogen-specific preservatives, and it is thought that these compounds may be pathogen-specific. Therefore, the selection of suitable boron-containing compounds against specific pathogens is necessary to find an environmentally friendly alternative to fungicides.

In conclusion, this study showed that the boron-containing compounds with tetrafluoroborate anions could be candidates as an alternative to fungicides against *M. fructigena*. Likewise, APBT, ETI, SMT, and STBD could be useful to control *M. fructigena* mycelium growth with higher concentrations than 100 mM when the pH of these compounds was alkaline. Further studies are needed to investigate how much boron can be absorbed by fruits after application and the toxicological effects of this boron on human health.

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Researchers' Contribution Rate Statement

Boron applications and experiments were carried out by İ. Ş. and F. Y. Ş. K. provided funding and supervised the experiment. Identification of the isolate and first draft of the manuscript was prepared by G. P. All authors read previous versions of the manuscript and approved the final manuscript.

Conflicts of Interest Statement

No potential conflict of interest was reported by the authors.

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