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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of antifungal effects of boron products against fungal disease agents causing rots in carrots

Havuçlarda çürümelere sebep olan bazı fungal hastalık etmenlerine karşı bor ürünlerinin antifungal etkilerinin belirlenmesi

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ABSTRACT

In this study, antifungal effects of boron products were investigated against *Geotrichum candidum, Alternaria alternata, Fusarium solani, Fusarium avenaceum* which cause postharvest rots in carrots. For boric acid, 0.05, 0.1, 0.2, 0.2, 0.3, 0.4, 0.5 %; for borax, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 %; for borax 0,1, 0.2, 0.4, 0.6, 0.8, 1.0%; for borik acid + borax mixture 0.05+0.1, 0.1+0.2, 0.2+04, 0.3+0.6, 0.4+0.8, 0.5+1.0% concentrations were used. Mycelial and conidia germination decreased linearly with increasing concentrations of the chemicals used. Boric acid and borax at different doses completely inhibited the mycelial growth of *G. candidum* and *F. solani*, while *A. alternata* and *F. avenaceum* were not completely inhibited. When both components were used as a mixture, they showed activity against *G. candidum* and *F. solani* at the relatively lower doses, but activity against *F. avenaceum* and *A. alternata* were lowest. Boric acid and borax at different concentrations completely inhibited spore germination of fungal agents apart from *A. alternata*. Boron products and mixtures significantly reduced the mycelial weights developed in liquid culture. In semi *in vivo* conditions, boron products were found to be more effective against *F. solani*, either singly or in mixtures, than their therapeutic properties. The results obtained showed that boron products are promising applications against soil-borne diseases that are problematic in carrots.

ÖZET

Bu çalışmada, havuçlarda hasat sonrası çürümelere sebep olan *Geotrichum candidum, Alternaria alternata, Fusarium solani, Fusarium avenaceum* hastalık etmenlerine karşı bor ürünlerinin antifungal etkileri araştırılmıştır. Borik asit uygulamasında %0.05, 0.1, 0.2, 0.3, 0.4, 0.5; boraks için %0,1, 0.2, 0.4, 0.6, 0.8, 1.0 ve borik asit + boraks karışımı için ise %0.05+0.1, 0.1+0.2, 0.2+04, 0.3+0.6, 0.4+0.8, 0.5+1.0 konsantrasyonlarında misel gelişim ve konidi çimlenmesinin engellenmesindeki antifungal etkileri incelenmiştir. Kullanılan kimyasalların konsantrasyonlardaki artış oranlarına paralel olarak misel ve konidi çimlenmesinde doğrusal olarak azalmalar kaydedilmiştir. Borik asit ve boraks kullanılan farklı dozlarda *G. candidum* ve *F. solani*'nin misel gelişimini tamamen engellerken, *A. alternata* ve *F. avenaceum* misel gelişimini tamamen engellenememiştir. Her iki bileşen karışım halinde kullanıldığında *G. candidum* ve *F. solani*'ye karşı nispeten daha düşük dozda etkinlik gösterirken, *F. avenaceum* ve *A. alternata*'ya karşı etkinlikleri düşük düzeyde gerçekleşmiştir. Borik asit ve boraks farklı konsantrasyonlarında *A. alternata* dışındaki fungal etmenlerin spor çimlenmesini tamamen engellemiştir. Bor ürünleri ve karışımların sıvı kültürde gelişen misel ağırlıklarını önemli düzeyde düşürmüştür. Yarı *in vivo* koşullarda bor ürünlerinin teksel veya karışım halinde *F. solani* etmenine karşı koruyucu özelliğinin tedavi edici özelliğine kıyasla daha etkili olduğu belirlenmiştir. Elde edilen sonuçlar bor ürünlerinin havuçta sorun olan toprak kökenli hastalıklara karşı umut verici uygulama olduğunu göstermiştir.

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INTRODUCTION

Carrot (*Daucus carota* var. *sativus*), a member of the Umbelliferae-Apiaceae family, is native to Central Asia and the Middle East (Kasap, 2010). According to the Food and Agriculture Organization (FAO), the total world production of carrots is 41,666,714 tonnes grown on 1,096,000 ha (FAO, 2021). Turkey ranks 12th among the carrot producing countries in the world with a production of 788,578 tonnes on an area of 10,159 ha (TÜİK, 2022).

In Turkey, carrots are stored by different methods such as leaving them in the field after harvesting, burying them in pits and storing them in cold stores (Tatlıdil, 2000). Carrot plant and root are affected by many biotic and abiotic factors both during the growth period in field conditions and after harvest in packing house and storage. Among the biotic factors, weeds, fungal, bacterial and viral pathogens are the most important limiting factors (Üremiş et al., 2020). The most important fungal diseases causing yield and quality losses in carrots are Fusarium dry rot (*Fusarium avenaceum*), Phytophthora root rot (*Phytophthora megasperma*), black rot (*Alternaria radicina*, *Pythium violae, Rhizoctonia solani*), white rot (*Sclerotinia sclerotiorum, Sclerotinia minor*), crater rot (*Rhizoctonia carotae*), root collar rot (*Rhizoctonia solani*), purple root rot (*Fusarium* spp., *Helicobasidium brebissonii, Rhizoctonia solani*) Cercospora leaf blight (*Cercospora carotae*), powdery mildew (*Erysiphe heraclei*), Alternaria leaf blight (*Alternaria dauci*), sour rot (*Geotrichum candidum*) (Davis & Raid, 2002; Koike et al, 2007; Horita & Hatta, 2016; Hameed et al., 2019; Favaro et al., 2024; Latvala et al., 2024). It has been determined that there are fungal pathogens that cause significant product loss and reduce product quality in carrots grown and stored in our country as well as worldwide (Yanmaz, 1994; Tülek & Dolar, 2011). *Geotrichum candidum* (sour rot), *Botrytis cinerea* (grey mould), *Chalara elegans* (black root rot) *Rhizoctonia carotae* (crater rot), *Penicillium* spp. (blue mold), *Aspergillus niger* (sooty rot), *Mycocentrospora acerina* (licorice rot), *Candida* spp. (yeast rot), and *Rhizophus oryzae* (Rhizopus soft rot) are common fungal disease agents encountered as post-harvest diseases. Apart from these disease agents; *Alternaria radicina* (black rot), *Fusarium* spp. (Fusarium dry rot), *Sclerotinia sclerotiorum* (white rot), and *Pectobacterium caratovora* subs. *caratovora* (bacterial soft rot) cause economic losses in carrots under storage conditions (Tülek & Dolar, 2011; Soylu et al., 2022a; Pascouau et al., 2023). Crater rot disease caused by *Rhizoctoni carotae* (Kurt et al., 2004) and leaf blight caused by *Alternaria dauci* (Soylu et al., 2005) were reported in carrot cultivation areas in Hatay province.

Although physical and chemical control methods are used in different countries against the disease agents of carrots in warehouses (Lockhart & Delbridge, 1972; Heltoft & Thomsen, 2023), there is no registered fungicides for these diseases in Turkey. However, environmentally friendly control strategies have been implemented against post-harvest fungal disease agents worldwide as an alternative to chemicals (Soylu et al., 2010; Kordowska-Wiater et al., 2012; de Lima et al., 2016; Soylu et al., 2021; Soylu et al., 2022b; Atay & Soylu, 2023; Kara et al., 2024; Oğuz et al., 2024; Uysal et al., 2024). During the washing process of carrots, some synthetic chemicals such as sodium hypochloride and chlorine gas can be mixed with water containing 50-100 ppm of active chlorine (Yıldız & Yıldız, 1999). The use of SOPP (Sodium-Orthophenylphenylphenate) alone or in a mixture with 0.1 M potassium carbonate was found to be an effective method of preventing the mycelial growth of the crater rot disease agent *Rhizoctonia carotae* (Ricker & Punja, 1991). One study compared treatments with potassium carbonate, sodium bicarbonate, ammonium bicarbonate and water were compared for disease control. When carrot slices and roots artificially wounded and inoculated with the fungal disease agent *Chalara elegans* disease agent were immersed in 0.1 or 0.05 M calcium proponate ($Ca(C_2H_5COO)$) and potassium solutions, it was found that disease development was reduced compared to treatments with standard sodium hypochlorite ($NaClO$). Sodium bicarbonate ($NaHCO_3$), potassium carbonate (K_2CO_3) and ammonium bicarbonate (NH_4HCO_3) applications were more found to be more effective in controlling the disease than water applications. However, it was reported that these applications were not economically viable (Punja & Gaye, 1993). Ozone applications in warehouses were found to be effective in reducing the symptoms caused by white rot and lead mould disease agents. Türkkan (2019) evaluated the efficacy of ammonium, calcium, potassium and sodium salts as possible alternatives to synthetic fungicides in the control of *Geotrichum candidum*, which causes sour rot in carrots. Although there are studies on the antifungal activity of boron derivatives against the citrus sour rot disease agent *Geotrichum citri-aurantii* (Kurt et al., 2018; Gedik, 2019), there are no studies on the antifungal activity of boron and its products against fungal disease agents in carrot fruit. In this study, the antifungal activities of environmentally friendly boron derivatives were determined against *Fusarium avanecaeum* (*F. avanecaeum*)*, Geotrichum candidum* (*G. candidum*)*, Fusarium solani (F. solani*) and *Alternaria alternata* (*A. alternata*).

MATERIALS and METHODS

Fungal isolates and chemicals

F. avanecaeum, G. candidum, F. solani and *A. alternata* isolates, obtained from diseased carrot plants grown in the region, identified and maintained in the fungal culture collection of Hatay Mustafa Kemal University Plant Health Application and Research Center, were used in the study. Boric acid and borax, which were used as boron products in the experiment, were obtained from the National Boron Research Institute of Turkey (Boren, 2014). The technical specifications of the chemicals used are given in Table 1

Table 1 Technical characteristics of the chemicals used in the trials

Çizelge 1. Denemelerde kullanılan kimyasalların teknik özellikleri

Chemicals	Formulations	Content	Value (min)	pH
Borik asit	H_3BO_3	B_2O_3	%56.25	5.13
		Safiyet	%99.90	
Boraks Dekahidrat	$Na_2B_4O_7.10H_2O$	B_2O_3	%36.50	9.19
		Safiyet	%99.90	

Antifungal effect of boron products on mycelial growth of fungal agents under in vitro conditions

The *in vitro* antifungal activity of the boron products used in the study was determined on Potato Dextrose Agar (PDA) media at different concentrations by inhibiting the mycelial growth of fungal agents (Kurt et al., 2020). Stock solutions containing different concentrations of boric acid (0.5%, 1, 1, 2, 3, 4, 5); borax (1%, 2, 4, 6, 8, 10) and boric acid + borax mixture (0.5%+1, 1+2, 2+4, 3+6, 4+8, 5+10) were used against *F. avanecaeum, G. candidum, F. solani* and *A. alternata* isolates. One ml of each of these stocks was mixed with 9 ml of PDA medium at 50 ^{o}C and poured into petri dishes. Mycelial discs of 5 mm diameter were cut with a cork borer from 7-day-old fungal cultures. These agar discs were placed in the centre of the Petri dishes. The mycelial part was in contact with the agar surface. The petri dishes were incubated at 25^{o}C for 5-7 days and the inhibition rates were calculated by measuring the radial growth (mm) of the fungal isolates on each petri dish. PDA medium without boron products was used as a control. The experiment was repeated twice with 3 Petri dishes for each concentration.

Antifungal effect of boron products on conidial germination of fungal agents under in vitro conditions

The antifungal effects of boron products on conidial germination of *F. avanecaeum, G. candidum, F. solani* and *A. alternata* isolates were also determined using stock doses. For this purpose, the different doses prepared as above were added to water agar (WA) media containing Petri plates. Conidial suspension was prepared from 7-day-old fresh fungal cultures.

To prepare the conidial suspension, sterile distilled water was added to the fungal isolates in the petri dish and the conidia were allowed to pass into the water with the help of a spoon and filtered using sterile cheesecloth. The concentration of the conidial suspension obtained was calculated using haemacytometer (Bright-line, HausserScientific, Horskam, PA, USA) and adjusted to 1x10^6 conidia/ml. From the prepared conidial suspensions, 100 µl were taken with a micropipette and transferred to WA Petri dishes containing different doses of boron products. After transfer, the suspension was spread on the medium using a sterile baguette. WA medium without boron products was used as control. The experiment was repeated twice with 3 petri dishes for each concentration. All treatments were incubated for 8 hours in the dark at 25^{o}C. After 8 hours, germinated and non-germinated conidia were counted under a light microscope. The spore was considered germinated when the length of the germ tube was 1.5 times the diameter of the conidia. For germination rate, 100 conidia were counted microscopically for each replicate at each concentration (Qin et al., 2003).

Antifungal effect of boron products on mycelial growth of fungal agents in liquid media under in vitro conditions

The antifungal effect of boron products on mycelial growth of *F. avanecaeum, G. candidum, F. solani* and *A. alternata* was also determined in liquid Potato Dextrose Broth (PDB) containing different concentrations of boron products (Figure 1). In the experiment, 4% for boric acid, 3% for borax and 1+2% for boric acid+borax were used, which gave the highest inhibition without blackening of the carrots. Five discs (5 mm in diameter) were cut with a cork borer from 7-day-old pure fungal cultures and into placed in liquid PDB media containing different doses and incubated in a rotary shaker incubator at 25 ^{o}C, 100 rpm for 7 days. PDB medium without boron treatment was used as control. Each treatment was carried out in 3 replicates. At the end of the incubation, the mycelia developed in the media were filtered using sterile cheese cloth. The mycelia remaining on the cheese cloth were weighed on a precision balance and recorded.

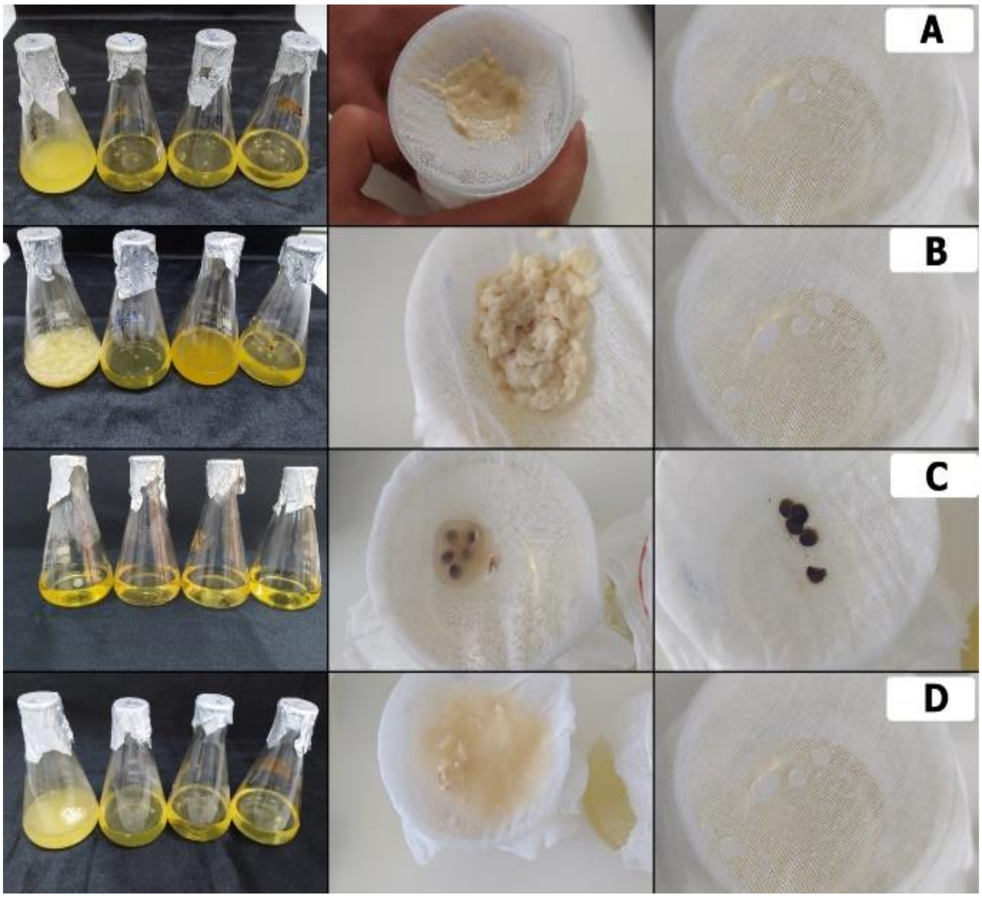

Figure 1. Antifungal effect of different boron applications on mycelial growth of A) *Geotrichum candidum*, B) *Fusarium avenaceum,* C) *Alternaria alternata,* D) *Fusarium solani in liquid PDB medium* (arrows)

Şekil 1. Farklı bor uygulamalarının sıvı PDB besi yerinde A) Geotrichum candidum, B) Fusarium avenaceum, C) Alternaria alternata, D) Fusarium solani izolatlarının misel gelişimi üzerine antifungal etkisi (ok)

Protective and therapeutic efficacy of boron products in preventing disease development under controlled conditions

Only *F. solani* isolate was used in this experiment. The protective and curative (therapeutic) ability of different boron applications on carrot fruit rot caused by the disease agent was determined. Fresh healthy carrots used in the experiment were sliced and placed in the sterile petri plates. In the experiment, 4% for boric acid, 3% for borax, 1+2% for boric acid+borax were used as boron products, which did not blacken the carrots and gave the highest inhibition in *in vitro* experiments. The experiment was carried out in two stages.

In the first stage (protective effect), disinfected carrot slices were sprayed with different concentrations of boron products and then 7-day-old fungal isolates were cut with a cork-borer and 5 mm discs were placed in the middle of the carrot slices. Three carrot slices were placed in each petri dish, sterile blotting paper was used to create a moist environment in the petri dishes, the blotting paper was moistened with sterile distilled water and the petri dishes were sealed tightly with parafilm.

In the second stage (therapeutic), discs of the pathogen were first placed on the carrot slices and after 48 hours of incubation boron products at the concentrations were sprayed to cover the carrot slices. For the positive control, the pathogen was inoculated and for the negative control, a disc was taken from the pathogen-free PDA medium and placed on the carrot slices. Both stages were incubated at room temperature for 7 days and mycelial growth on carrots was observed and recorded.

Statistical analysis

In vitro experiments were performed using the randomised plot design with 3 replicates at each concentration. In the experiments conducted under controlled conditions, the percentage effects of the data were determined in the randomised plot experimental design, and all data obtained were subjected to analysis of variance by one-way ANOVA using the SPSS statistical software program. Differences between treatments were compared using Duncan's Multiple Comparison Test at 5% significance level.

RESULTS and DISCUSSIONS

Antifungal effect of boron products on mycelial growth of fungal agents under in vitro conditions

The antifungal effect of boron products on the mycelial growth of fungal agents under *in vitro* conditions was determined on PDA medium containing different doses of boric acid, borax and their mixtures (Figure 2). While the mycelial growth of *G. candidum* and *F. solani* was completely inhibited in the media tested containing different doses of boric acid, borax and their mixtures, the mycelial growth of *F. avenaceaum* and *A. alternata* isolates was not completely inhibited at any of the doses tested (Table 2). The mycelial growth of the *G. candidum* isolate was inhibited at 0.3% doses of boric acid and 0.4% doses of borax, while the mycelial growth of the *F. solani* isolate was 100% inhibited at 0.5% doses of boric acid and 0.2% doses of borax. In petri dishes containing a mixture of boric acid and borax, mycelial growth of *G. candidum* and *F. solani* isolates was 100% suppressed at the dose of 0.1%+0.2% (Table 2, Figure 3).

Mycelial growth of *A. alternata* and *F. avenaceum* isolates was not completely inhibited in the media containing the highest doses of boric acid (0.5%), borax (1.0%) and their mixtures (0.5%+1.0%). At the highest dose of boric acid (0.5%), mycelial growth of the *A. alternata* isolate was 41.18% and that of *F. avenaceum* was 60.78%; mycelial growth of the *A. alternata* isolate was 60.78% and that of *F. avenaceum* by 80.78% at the 1.0% dose of borax; the mycelial growth of *A. alternata* isolate was inhibited by 51.37% and that of *F. avenaceum* isolate by 91.37% at the 0.5%+1.0% dose of the mixtures (Table 2, Figure 3).

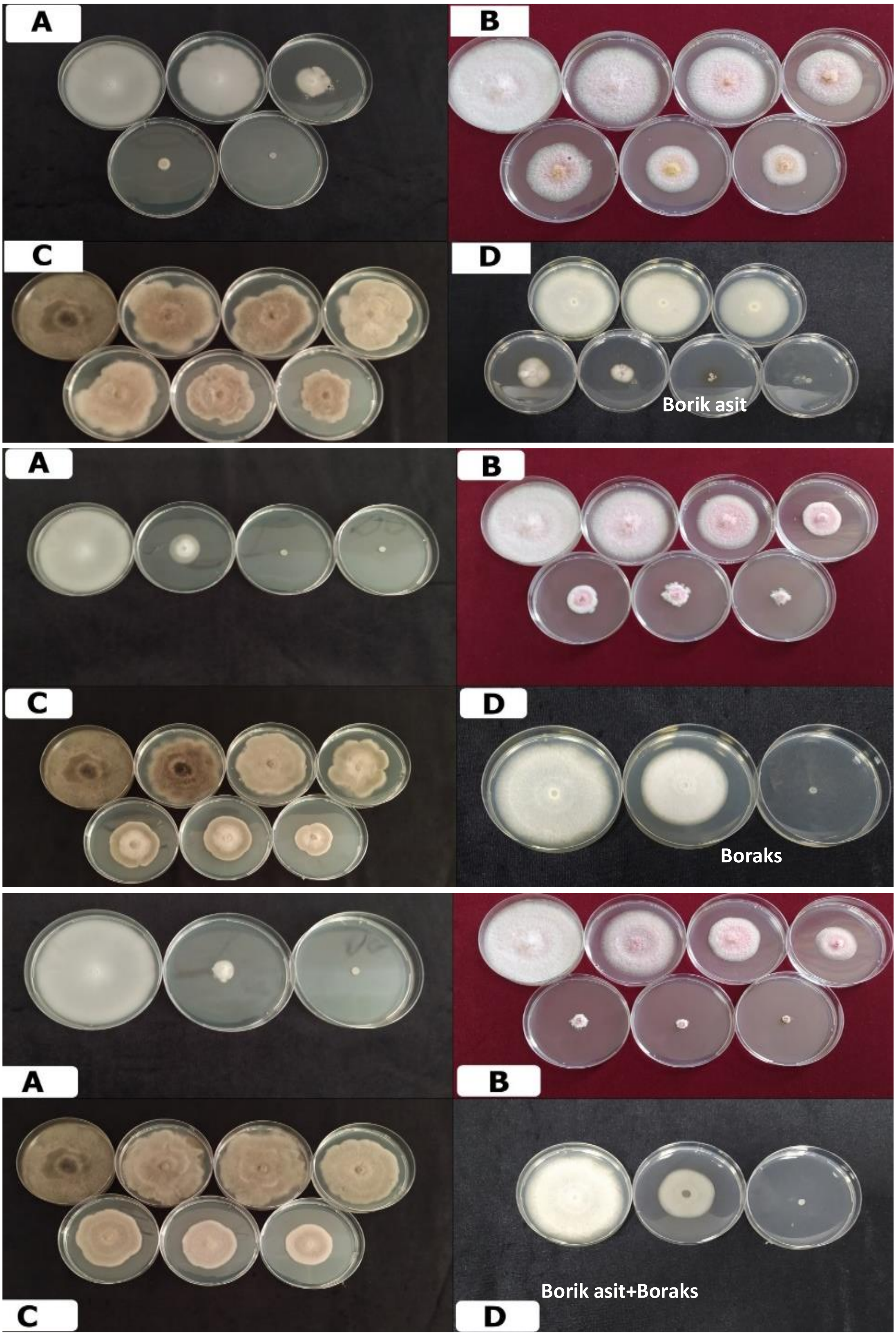

Figure 2. Inhibition of mycelial growth of *A) Geotrichum candidum, B) Fusarium avenaceum, C) Alternaria alternata, D) Fusarium solani* isolates in petri dishes containing different doses of boric acid, borax and their mixtures

Şekil 2. Farklı dozlarda borik asit, boraks ve karışımları içeren petrilerde A) Geotrichum candidum, B) Fusarium avenaceum, C) Alternaria alternata, D) Fusarium solani izolatlarının misel gelişimlerinin engellenmesi

Table 2. Mycelial growth (mm) of fungal disease agents in petri dishes containing different doses of boric acid, borax and their mixtures

Çizelge 2. Farklı dozlarda borik asit, boraks ve karışımları içeren petrilerde fungal hastalık etmenlerin misel gelişimi (mm)

		Fungal agents and mycelail growth (mm)			
	Doses (%)	***G. candidum***	***F. solani***	***F. avenaceum***	***A. alternata***
Boric acid	**0.00**	85.00e*	85.00g	85.00f	85.00e
	0.05	75.00d	74.00f	67.67e	78.33d
	0.1	33.00c	68.00e	63.33d	69.33c
	0.2	11.67b	33.00d	49.67c	68.00c
	0.3	0.00a	20.00c	47.67c	67.67c
	0.4	0.00a	8.00b	39.67b	57.67b
	0.5	0.00a	0.00a	33.33a	50.00a
Borax	**0.0**	85.00d	85.00c	85.00g	85.00g
	0.1	30.67c	55.33b	72.33f	76.00f
	0.2	7.67b	0.00a	53.00e	69.67e
	0.4	0.00a	0.00a	32.33d	64.00d
	0.6	0.00a	0.00a	25.67c	50.00c
	0.8	0.00a	0.00a	21.33b	43.67b
	1.0	0.00a	0.00a	16.33a	33.33a
Boric acid + Borax	**0.0**	85.00c	85.00c	85.00g	85.00g
	0.05+0.1	19.33b	40.33b	61.33f	78.00f
	0.1+0.2	0.00a	0.00a	44.67e	79.00e
	0.2+0.4	0.00a	0.00a	33.67d	68.67d
	0.3+0.6	0.00a	0.00a	15.67c	60.33c
	0.4+0.8	0.00a	0.00a	11.33b	50.00b
	0.5+1	0.00a	0.00a	7.33a	41.33a

** Different small letters next to the mean values in the each column indicate that the difference between the doses is statistically significant according to Duncan's Multiple Comparison Test (P<0.05).

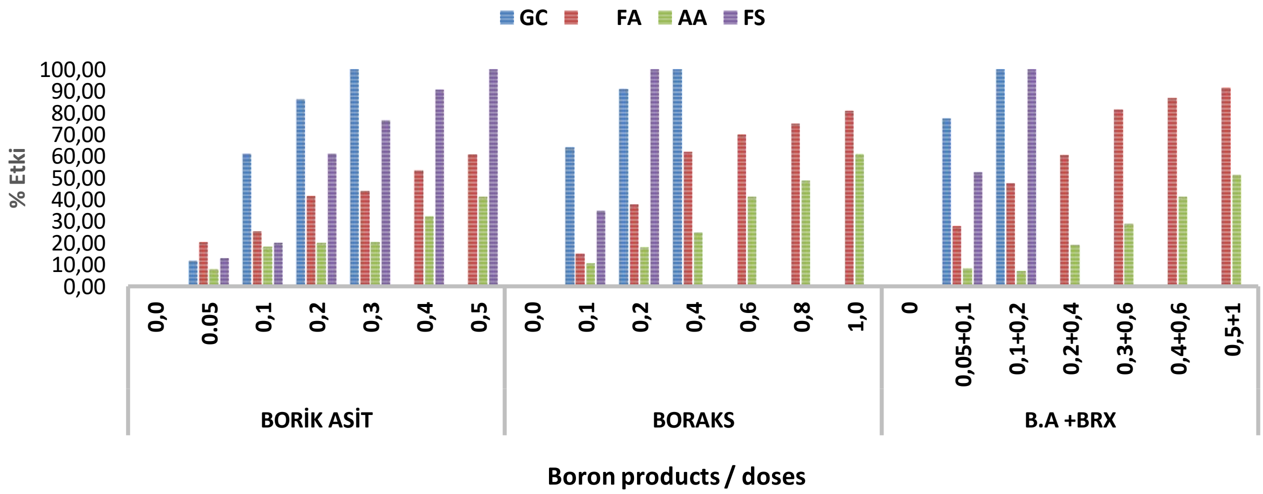

Figure 3. Antifungal activity of boron products at different doses on % inhibition of mycelial growth of *Geotrichum candidum (GC), Fusarium avenaceum (FA), Alternaria alternata (AA) and Fusarium solani (FS)*

Şekil 3. Bor ürünlerinin farklı dozlarda Geotrichum candidum (GC), Fusarium avenaceum (FA), Alternaria alternata (AA) ve Fusarium solani (FS) misel gelişiminin % engellemesi üzerine olan antifungal etkinlikleri

Antifungal effect of boron products on conidial germination of fungal agents under in vitro conditions

The conidial germination of *G. candidum, F. solani* and *F. avenaceum* isolates was inhibited by 100% at different doses in the media to which different boron products and mixtures were added (Table 3). *A. alternata* conidia were inhibited by 100% at the highest dose of boric acid (0.5%). However, *A. alternata* conidia were inhibited by 79% at the highest doses of borax and its mixtures used (Table 3, Figure 4).

The germination of conidia of *G. candidum, F. solani, F. avenacearum* and *A. alternata* isolates was completely prevented at 0.2%, 0.4%, 0.5% and 0.5% doses of boric acid, respectively (Table 3). The doses of borax that completely prevented the germination of conidia of *G. candidum, F. solani* and *F. avenacearum* isolates were 0.2%, 0.4% and 0.4%, respectively (Table 3). The doses of boric acid and borax mixtures that completely inhibited the germination of conidia of *G. candidum, F. solani* and *F. avenacearum* isolates were determined to be 0.05+0.1%, 0.1+0.2% and 0.4+0.8%, respectively (Table 3).

Borax (1.0%) and its mixtures (0.5+1.0%) were only able to inhibit conidial germination of the A. alternata isolate by 79% at the highest doses used (Table 3, Figure 4).

Table 3. Conidial germination (%) of fungal disease agents in petri dishes containing different doses of boric acid, borax and their mixtures

Çizelge 3. Farklı dozlarda borik asit, boraks ve karışımları içeren petrilerde fungal hastalık etmenlerin konidi çimlenmesi (%)

		Fungal agents			
	Doses (%)	***G. candidum***	***F. solani***	***F. avenaceum***	***A. alternata***
Boric acid	**0.00**	100.00c*	100.00f	100.00d	100.00g
	0.05	100.00c	67.00e	100.00d	77.00f
	0.1	61.66b	51.66d	100.00d	63.00e
	0.2	0.00a	32.00c	100.00d	54.33d
	0.3	0.00a	14.00b	71.00c	45.00c
	0.4	0.00a	0.00a	41.00b	39.33b
	0.5	0.00a	0.00a	0.00a	0.00a
Borax	**0.0**	100.00c	100.00c	100.00b	100.00g
	0.1	25.00b	100.00c	100.00b	91.00f
	0.2	0.00a	26.33b	100.00b	71.00e
	0.4	0.00a	0.00a	0.00a	65.00d
	0.6	0.00a	0.00a	0.00a	31.33c
	0.8	0.00a	0.00a	0.00a	25.66b
	1.0	0.00a	0.00a	0.00a	21.00a
Boric acid + Borax	**0.0**	100.00b	100.00c	100.00f	100.00f
	0.05+0.1	0.00a	27.66b	98.00e	100.00f
	0.1+0.2	0.00a	0.00a	25.00d	84.33e
	0.2+0.4	0.00a	0.00a	10.66c	56.66d
	0.3+0.6	0.00a	0.00a	4.00b	30.33c
	0.4+0.8	0.00a	0.00a	0.00a	27.66b
	0.5+1	0.00a	0.00a	0.00a	21.00a

** Different small letters next to the mean values in the column indicate that the difference between the doses is statistically significant according to Duncan's Multiple Comparison Test (P<0.05).

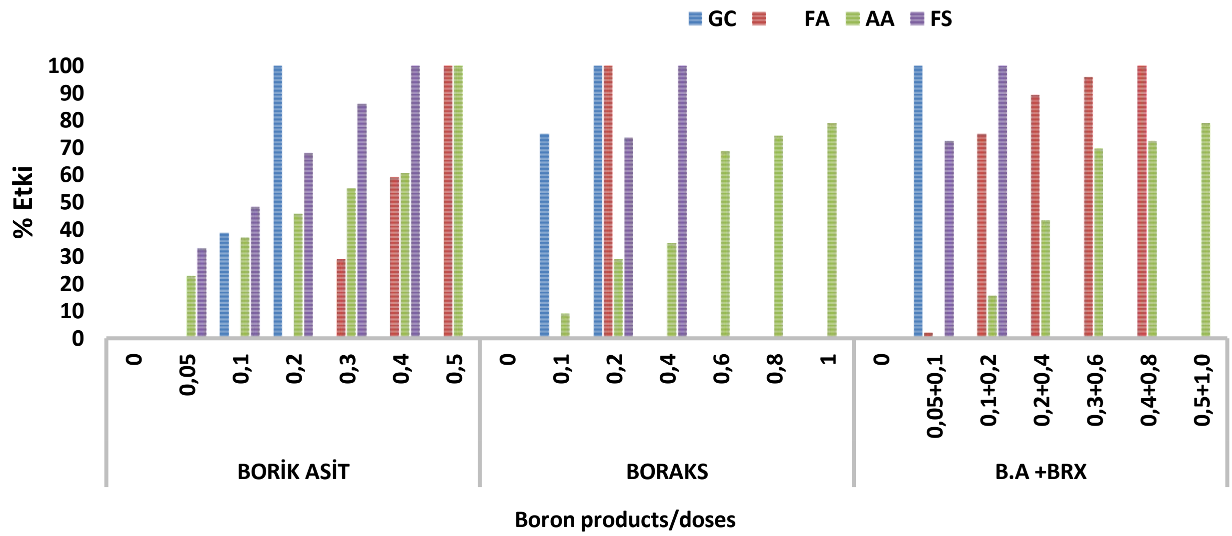

Figure 4. Antifungal activity of boron products at different doses on % inhibition of conidial germination of *Geotrichum candidum (GC), Fusarium avenaceum (FA), Alternaria alternata (AA) and Fusarium solani (FS)*

Şekil 4. Bor ürünlerinin farklı dozlarda Geotrichum candidum (GC), Fusarium avenaceum (FA), Alternaria alternata (AA) ve Fusarium solani (FS) konidi çimlenmesinin % engellemesi üzerine olan antifungal etkinlikleri

The results obtained from the efficacy studies of boron products on the inhibition of conidial germination of fungi are considered to be in agreement with the previously published studies (Thomidis and Exadaktylou, 2010; Li et al., 2012) which reported that the prevention of fruit rot development is probably due to the direct toxic activity of boron on conidia as well as the promotion of defence mechanisms in the treated plants. In a similar study, when the effects of different boron products on arthroconidia germination of *G. citri-aurantii* were investigated, the highest antifungal effect (92.7% inhitions) was obtained with borax application which was followed by boric acid+borax mixture with 90.3% effect. The lowest antifungal effect (31.2% inhibition) was found in boric acid application (Gür, 2015).

Antifungal effect of boron products on mycelial growth of fungal agents in liquid media under in vitro conditions

The antifungal effect on the inhibition of mycelial growth of *F. avenaceum, G. candidum, F. solani* and *A. alternata* isolates was also determined in liquid PDB media containing different doses of boron products and mixtures (Table 4, Figure 5). Different boron products and mixtures completely inhibited the mycelial growth of *G. candidum* and *F. solani* isolates at the doses applied. These treatments inhibited the mycelial growth of *F. avenaceum* isolate by 99.6% and *A. alternata* isolate by 90.5-96.1% (Table 4, Figure 5).

Table 4. Antifungal effect of different boron applications on mycelial growth (mg) in liquid PDB medium

Çizelge 4. Farklı bor uygulamalarının sıvı PDB besi yerinde misel gelişimi (mg) üzerine antifungal etkisi

	Fungal isolates adn mycelial growth (mg)			
Treatments	***G. candidum***	***F. solani***	***F. avenaceum***	***A. alternata***
Control	1730.0b*	7000.0b	7880.0b	2310.0d
%0.5 Boric acid	0.0a	0.0a	20.0a	80.0b
%1.0 Borax	0.0a	0.0a	30.0a	40.0a
%0.5 Boric acid + %1.0 Borax	0.0a	0.0a	30.0a	90.0b

** Different small letters next to the mean values in the column indicate that the difference between the doses is statistically significant according to Duncan’s Multiple Comparison Test (P<0.05).

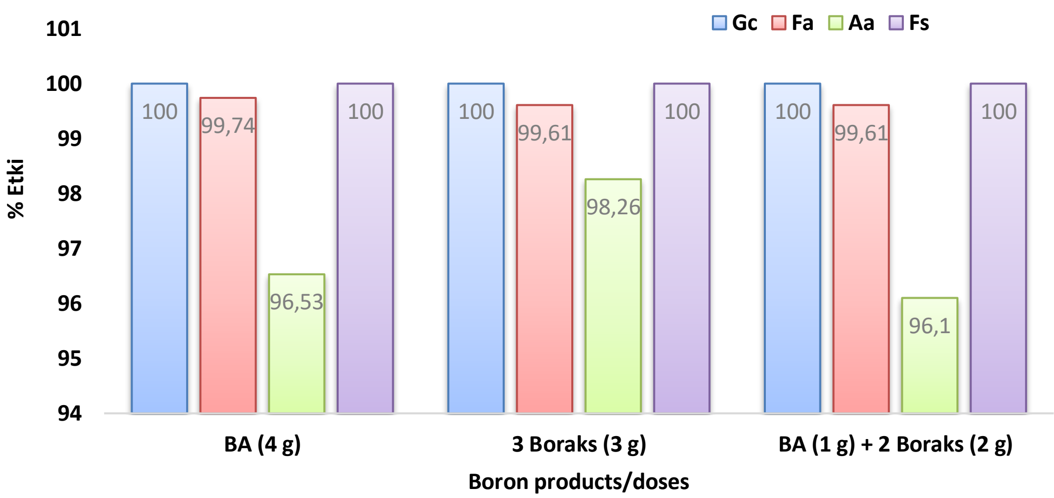

Figure 5. Antifungal activity of boron products on % inhibition of mycelial growth of *Geotrichum candidum (Gc), Fusarium avenaceum (Fa), Alternaria alternata (Aa) and Fusarium solani (Fs) in liquid PDB medium*
Şekil 5. Bor ürünlerinin sıvı PDP besi yeri içerisinde Geotrichum candidum (GC), Fusarium avenaceum (FA), Alternaria alternata (AA) ve Fusarium solani (FS) misel gelişimin % engellemesi üzerine olan antifungal etkinlikleri

Determination of protective and therapeutic properties of boron products under controlled conditions

The protective and therapeutic efficacy of different boron products in preventing disease symptoms caused by *F. solani* was determined at different doses (Figure 6). On carrot slices treated with boric acid, borax and the mixture, the suppression of disease development was higher in the protective efficacy compared to the therapeutic efficacy (Table 5, Figure 7). Disease symptoms caused by *F. solani* were prevented by 35.5% by borax, 57.41% by boric acid and 54.85% by the mixture on carrot slices where protective efficacy was tested (Figure 7). Disease symptoms caused by *F. solani* were prevented by 9.6% by borax, by 17.42% by boric acid and by 22.5% by the mixture on carrot slices where therapeutic efficacy was tested (Figure 7).

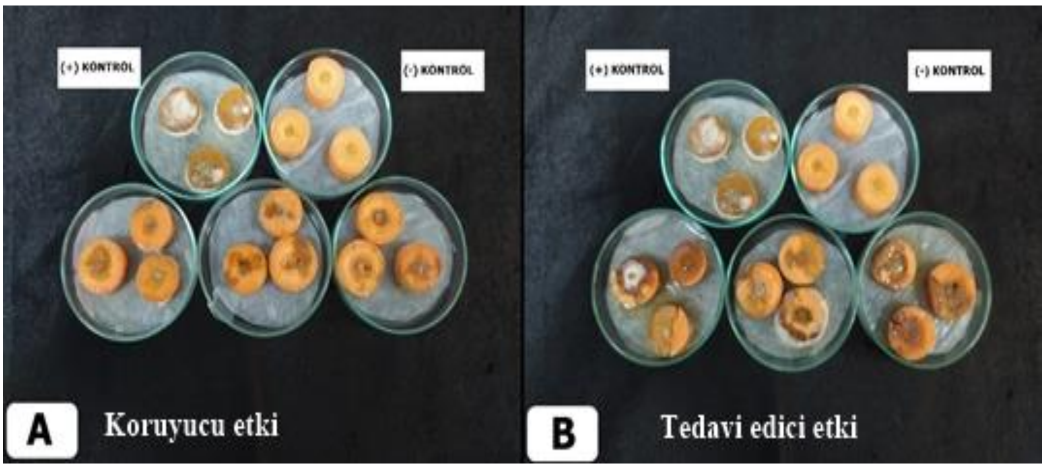

Figure 6. Lesion development caused by *Fusarium solani* in carrot slices where different doses of boron products were applied (A: protective effect; B: therapeutic effect)
Şekil 6. Bor ürünlerinin farklı dozlarının uygulandığı havuç dilimlerinde Fusarium solani' nin oluşturduğu lezyon gelişimi (A: koruyucu etki; B: tedavi edici etki)

Table 5. Protective and therapeutic effects of different boron products on the inhibition of lesion development (mm) caused by *Fusarium solani* infection on carrot slices

Çizelge 5. Farklı bor ürünlerinin havuç dilimleri üzerinde Fusarium solani enfeksiyonu sonucu ortaya çıkan lezyon gelişiminin (mm) engellenmesi üzerine olan koruyucu ve tedavi edici etkisi

Doses (%)	**Protective**	**Therapeutic**
0.0	25.83c*	25.83c
Boric acid (4 gr)	11.00a	21.33a
Boric acid (1 gr) + Borax (2 gr)	11.66a	20.00a
Borax (3 gr)	16.66b	23.33b

* Different small letters next to the mean values in the column indicate that the difference between the doses is statistically significant according to Duncan's Multiple Comparison Test (P<0.05).

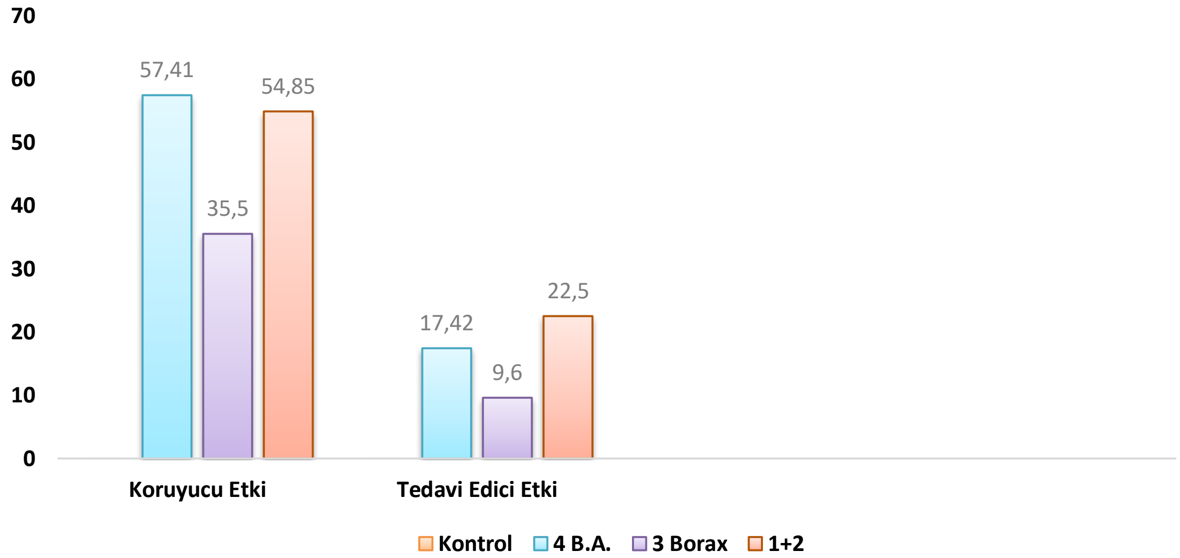

Figure 7. Protective and therapeutic effects of different boron products on the inhibition of lesion development (%) caused by *Fusarium solani* infection on carrot slices

Şekil 7. Farklı bor ürünlerinin havuç dilimleri üzerinde Fusarium solani enfeksiyonu sonucu ortaya çıkan lezyon gelişiminin (%) engellenmesi üzerine olan koruyucu ve tedavi edici etkisi

In previous studies, *in vitro* mycelial growth of *G. candidum* on carrot was completely inhibited by ammonium bicarbonate and carbonate; calcium oxide; potassium benzoate, carbonate and sorbate; sodium benzoate, carbonate and fluoride (2% w/v). Potassium bicarbonate and sodium bicarbonate also reduced mycelial growth by 77.78% and 90.60%, respectively (Türkkan, 2019). The antifungal activity of boron products against citrus sour rot disease caused by *G. citri-aurantii* was investigated under *in vitro* and semi *in vivo* conditions (Gür, 2015). In this study, 15 g, 30 g and 45 g doses of Etidot-67 and a single dose of boric acid + borax mixture were used as treatments. The highest efficacy was observed with 97.8% in the 45 g dose of Etidot-67 with boric acid + borax mixture (30+60g). Li et al. (2012) investigated the therapeutic and protective activities of borax and potassium tetraborate against dry rot disease in potato tubers. According to their results, although borax and potassium tetraborate had significant therapeutic effect, they had no protective activity against the potato tuber dry rot agent *Fusarium sulphureum*. In a study investigating the potential of boron to promote defense mechanism(s) in plants (Thomidis & Exadaktylou, 2010), it was reported that boron chemical reduced the susceptibility of fruit to infection by *Monilinia* conidia, resulting in the reduction of small cracks in the cuticle tissue on the fruit surface. It was also reported that the fruit infection rate by *Monilinia laxa* decreased with increasing doses of boron applied to the leaves. In addition, it was suggested that the percentage of fruit infection caused by fungi belonging to the genus *Monilinia* could probably be reduced at the highest level (below the toxicity level) by maintaining the boron content in the fruit. In a related study, it was reported that boron applied after flowering reduced the incidence of *Gloeosporium* rot in apples during storage (Wojcik et al., 1997).

In this study, the antifungal activities of boron products were investigated under *in vitro* and controlled semi *in vivo* conditions in the control of *G. candidum, A. alternata, F. solani,* and *F. avenaceum,* fungal disease agents causing significant post-harvest losses in carrot. It was found that the antifungal activity of boron products increased significantly in parallel with increasing doses in the inhibition of mycelial growth and conidial germination of the tested disease agents. Compared to the single application of boron products, the antifungal activities of the mixtures were realized at higher rates at lower doses. In semi *in vivo* studies, no statistical difference was observed between the efficacy of boric acid applied as a single product and when applied as a mixture. The protective efficacy of the applications was found to be more effective than the therapeutic efficacy.

When all the results are evaluated together, boric acid and borax alone or boric acid + borax mixtures can be a reliable, environmentally friendly alternative to pesticides in packing houses and warehouses because they are effective against rot in stored carrots. However, since high concentrations cause blackening of carrots, it would be better to use boron products together with antagonistic bacterial isolates (Gedik, 2019), potassium salts (Kurt et al. 2018) or essential oils. Turkey has rich resources of boron products. As boron products are cheaper and more reliable than pesticides, they will have an important place in the future in the control of postharvest fungal pathogens.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between them.

AUTHOR'S CONTRIBUTIONS

The authors declare that they have contributed equally to the study.

STATEMENT OF ETHICS CONSENT

This article does not require ethical approval as there are no studies with human or animal subjects.

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