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Effect of Some Boron Compounds to Corn Stalk Rot and Ear Rot

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Abstract In this study, it was aimed to determine the antifungal effect of some boron compouns (Di-sodium tetraborate, Boric acid, Di-sodium octaborate tetrahydrate and Borax decahydrate) against *Fusarium verticillioides* and *Fusarium pseudograminearum* in maize under *in-vitro* conditions. Boron compounds were applied in doses of 0.1, 0.2, 0.3, 0.4 and 0.5 g L^{-1}. PDA medium free of boron compounds was used as negative control and PDA medium containing commercial fungicide (80 g L^{-1} Triticonazole + 40 g L^{-1} Pyraclostrobin-BASF company) was used as positive control. The experiment was carried out in a randomized plot design with three replications. As a result of the study, the control (+) application of Di-sodium tetraborate was found to be 74.8% and 74.0% effective against *F. verticillioides* and *F. pseudograminearum*, respectively, while this effect was 62.9% and 56.8%, in 0.5 g L-1 application has been determined respectively. While boric acid showed 74.5% and 73.8% effects on control (+), *F verticillioides* and *F pseudograminearum*, respectively, this effect was 56.2% and 55.0% in 0.5 g L^{-1} application, respectively. Di-sodium octaborate tetrahydrate control (+) application inhibited mycelial growth of the *F. verticillioides* and *F. pseudograminearum* by 75.3% and 74.2%, respectively, while 0.5 g L^{-1} application inhibited 60.2% and 54.2%, respectively. In the control (+) application of Borax decahydrate, 75.5% and 75.6% effects were detected on *F. verticillioides* and *F. pseudograminearum*, respectively, while this effect was found to be 64.2% and 62.7%, respectively, in 0.5 g L^{-1} application. These results showed that Borax decahydrate can be used as an alternative to synthetic fungicides in the control of corn stalk rot and ear rot diseases.

Keywords Boron, *Fusarium verticillioides*, *Fusarium pseudograminearum*, *antifungal effect, corn*

1. Introduction

Grains have an important place among the cultivated agricultural products. Cereals are of great importance in terms of human and animal nutrition both in Turkey and in the world. Corn is the most produced grain after wheat and rice in the world, and its yield per unit area is about twice that of barley and wheat. In terms of corn cultivation area, China, USA, Brazil, India, Mexico, Argentina, Ukraine and Russia are the leading countries in the world. Turkey ranks 19th in terms of cultivation area and production. In Turkey, corn is cultivated on an area of 692 thousand hectares and 6.5 million tons of corn is produced [1].

Corn is used as a raw material both in human nutrition and in the starch, glucose, oil and feed industries. Corn grain is a very good energy source and has direct or indirect use in around 3500 other food and technological products, such as flour and starch, biscuits, rusk, crisps, and many other products of the food industry [2]. Corn is an important roughage used in animal nutrition as green and silage. While most of the world corn production is used as animal feed, the rate of use in human nutrition is higher in developing countries than in developed countries [3].

In corn production, biotic and abiotic stress factors cause direct and indirect crop losses. Due to fungal, bacterial and viral disease factors, which are biotic stress factors, approximately 10.9% cereal yield losses are observed in maize. Many Fusarium species cause significant yield losses by infecting the root, root collar, stalk rot and ear rot of cool season cereals such as wheat, barley, rye and oats and hot climate cereals such as maize, rice and sorghum. Due to these diseases, which are seen in every region where grain is grown in the world, there is a yield loss of 10-30% in Europe every year. It has been reported that the most important diseases that cause yield loss in corn cultivation all over the world are Fusarium stalk rot and ear rot [4, 5, 6]. One of the ear rot disease agents in corn is *Fusarium verticilloides* (Sacc) Nirenberg (Syn: *F. moniliforme)*. It is possible to see whitish-pink micelles of the pathogen individually or in groups on diseased ears. Pathogenic fungus can be found endophytically inside the plant without symptoms [7]. It has also been reported that the agent is transmitted by seeds [8, 9]. *F. verticilloides* is known to produce Fumonisin (FUM), Deoxynivenol (DON), and Zearalenone (ZEA) toxins in maize [10, 11, 12, 13]. Depending on the severity of the disease, the amount of toxins can reach levels that will harm human and animal health [14]. ZEN has an anabolic effect similar to estrogen, causing persistent öst-usa and infertility in animals at high doses [15].

In Turkey, fungicides containing different active substances (sedaxana, triticonazole+pyraclostrobin) are used to prevent losses in corn due to stalk rot and ear rot [16]. Since fungicides cause the emergence of tolerant/resistant strains in pathogens and environmental pollution, it has revealed the need to develop alternative combat methods to chemical control in the control of disease agents [17]. Boron and its derivatives have been used in different fields for many years in the world. Boron is a small semiconductor element with only three valence electrons with properties intermediate between metals and nonmetals[18]. Turkey ranks first in the world with its 956 million tons of boron (B_2O_3) reserves [19]. Known boron deposits in Turkey, which has 73.6% of the world's boron reserves; It is located in Eskişehir-Kırka, Kütahya-Emet, Balıkesir-Bigadiç, Bursa-Kestelek. There are about 230 types of boron minerals in nature. Calcium or sodium borates such as colemanite (2CaO, $3B_2O_3$, $5H_2O$), ulexite (Na_2O, 2CaO, $5B_2O_3$, $16H_2O$), Tincal (Na_2O. B_2O_3, $10H_2O$) are the most important [20]. Boron products in Turkey; It is used in 36% glass, 31% ceramic, 9% cleaning-detergent, 7% agriculture, 4% glue and 14% other fields [21]. Other micronutrients such as boron, Zn and Mn are effective in some biochemical and physiological processes that are important in plant resistance and active/passive transport [22, 23]. Boron has many important roles in plants, including cell wall synthesis, lignification, carbohydrate transport, nutrient uptake, cell wall structure, cell division, carbohydrate metabolism, RNA metabolism, respiration, IAA metabolism, salinity stress, phenol metabolism, and membrane function [24, 25, 26].

The antifungal effects of some boron compounds against some fungal pathogens (*Fusarium solani*, *Eutypa lata*, *Monilinia laxa*, *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Fusarium sulphureum*, *Phytophthora nicotianae*, *Rhizoctonia solani*, *Penicillium expansum*) that cause significant losses in agricultural products have been detected in studies [27 28, 29, 30, 31, 32, 33, 34, 35]. In the literature review, no study was found on the determination of the effectiveness of boron compounds against corn stalk rot and ear rot. In this context, the study is the first of its kind. In this study, it was aimed to determine the antifungal effects of different concentrations of some boron compounds (Di-sodium tetraborate, Boric acid, Di- sodium octaborate tetrahydrate and Borax decahydrate) on mycelial growth of *F. verticillioides* and *F. pseudograminearum* under *in-vitro* conditions.

2. Materials and Methods

In the experiment, *F. verticillioides* and *F. pseudograminearum* isolates, isolated from corn and known for their virulence, have been supplied from the fungal culture collection of the General Directorate of Agricultural Research and Policies, Directorate of Plant Protection Central Research Institute. Fungal isolates were grown on potato dextrose agar medium (PDA-Difco) in a cooled incubator (25±1°C temperature; 12:12 dark and light conditions) and stored at +4^{o}C in the refrigerator.

The four boron compounds used in this study are listed in Table 1, along with their chemical composition and molecular weight. The boron compounds were supplied by the Eti Maden state-owned company in Turkey.

Table 1: Boron compounds used in the study

Compounds	Chemical formula	Molecular weight (g/mol)	Company
Di-sodium tetraborate	$Na_2B_4O_7$	201.27	Eti Maden, Ankara, Turkey
Borik acid	H_3BO_3	61.83	Eti Maden, Ankara, Turkey
Di-sodium octaborate tetrahydrate	$Na_2B_8O_{13}.4H_2O$	412.52	Eti Maden, Ankara, Turkey
Borax decahydrate	$Na_2B_4O_7.10H_2O$	381.4	Eti Maden, Ankara, Turkey

Different concentrations of boron compounds were added to the PDA medium, which was sterilized in an autoclave at 121°C for 15 minutes and cooled (Table 2). The sterilized mixture was poured into each sterile petri dish (10 cm diameter) as 25 ml. After keeping the petri dishes overnight at room conditions, discs with a five mm diameter cork borer were taken from the ends of the 7-day-old fungal isolates, which were previously grown in PDA medium, and placed in the middle of petri dishes containing boron salts + PDA medium. After inoculation, the petri dishes were sealed with parafilm and incubated at 25±1°C. PDA medium free of boron compounds was used as negative control (0 g L^{-1}), while licensed fungicide (80 g L^{-1} Triticonazole + 40 g L^{-1} Pyraclostrobin- BASF company) was used at recommended dose against corn stalk rot and ear rot as positive control. After inoculation, the petri dishes were covered with parafilm and on the 10^{th} day, fungal colony diameters were measured and recorded. The measurement of the colony diameter was made by measuring the diameter of the fungus colony in separate directions perpendicular to each other [36]. The percent inhibition of different doses of boron compounds was calculated by comparing the mycelial growth in the boron compounds containing Petri dish with that of the negative control Petri dish [37].

$$MGI\ (\%) = [(dc-dt)/dc] \times 100$$

In the formula; MGI: Mycelial growth inhibition rate (%), dc: Represents the mycelial growth diameter of the control Petri dish (mm), dt: Represents the mycelial growth diameter of the amended Petri dish (mm). All experiments were conducted twice, with three replicates per treatment in a completely randomized plot design.

Table 2: Treatment of boron compounds against *F. verticillioides* and *F. pseudograminearum*

	Application	Doses (g L^{-1})
Boron compounds	Di-sodium tetraborate	0.1
	Borik acid	0.2
	Di-sodium octaborate tetrahydrate	0.3
	Borax decahydrate	0.4
		0.5

Statistical analyses of the data were performed with the JMP IN packet statistic program (SAS Institute, Carry, NC, 13.0 PC version). Analysis of variance (one-way ANOVA) was carried out to determine the effects of the treatments. When the treatment effects were statistically significantly ($p \leq 0.01$), the Duncan's multiple range test was used for means separations.

3. Results and Discussion

In the study conducted *in-vitro,* effects of five different concentrations of boron compounds (Di-sodium tetraborate, Boric acid, Di-sodium octaborate tetrahydrate and Borax decahydrate) on mycelial growth and % inhibition rates of *F. verticillioides* and *F. pseudograminearum* are given in Table 3.

The inhibitory effect of different concentrations of boron compounds on mycelial growth of *F. verticillioides* and *F. pseudograminearum* was found to be statistically significant ($P \leq 0.01$) compared to control (-). Boron compounds used in the study inhibited mycelial growth of both pathogens at different levels depending on the dose increase. Di-sodium tetraborate control (+) application showed 74.8% and 74.0% effect against *F. verticillioides* and *F. pseudograminearum*, respectively, while high dose (0.5 g L^{-1}) application showed 62.9% and 56.8% effect. Other doses of Di-sodium tetraborate inhibited mycelial growth of *F. verticillioides* by 13.3%-55.1%, and by 13.3%-42.6% of *F. pseudograminearum*. Boric acid control (+) application showed 74.5% and 73.8% effects against *F. verticillioides* and *F. pseudograminearum*, respectively, while in high dose (0.5 g L^{-1}) application, the effect against *F. verticillioides* and *F. pseudograminearum* was 56.2% and 55.0% respectively. In other doses of Boric acid, 16.3%-44.2% inhibition was detected in mycelial growth of *F. verticillioides*, while

13.0%-40.7% inhibition in mycelial growth of *F. pseudograminearum* was determined. Di-sodium octaborate tetrahydrate control (+) application inhibited mycelial growth of *F verticillioides* and *F pseudograminearum* by 75.3% and 74.2%, respectively, while high dose (0.5 g L^{-1}) application inhibitited mycelial growth of *F verticillioides* and *F pseudograminearum* by 60.2% and of 54.2% respectively. Other doses of Di-sodium octaborate tetrahydrate compound inhibited mycelial growth of *F. verticillioides* by 15.2%-44.2%, and mycelial growth of *F. pseudograminearum* by 13.7%-41.7%. While the effect of Borax decahydrate control (+) application on mycelial growth of *F. verticillioides* and *F. pseudograminearum* was 75.5% and 75.6%, respectively, the effect of high dose (0.5 g L^{-1}) application on *F. verticillioides* and *F. pseudograminearum* was detected 64.2% and 62.7%, respectively. Other doses of Borax decahydrate compound inhibited mycelial growth of *F. verticillioides* by 17.2%-46.0%, and mycelial growth of *F. pseudograminearum* by 14.6%-42.5%. *In-vitro* applications showed that Borax decahydrate compound was the most effective boron compound in inhibiting mycelial growth of *F. verticillioides* and *F. pseudograminearum* compared to Di-sodium tetraborate, Boric acid and Di-sodium octaborate tetrahydrate compounds (Table 3).

0.5 g L^{-1} dose of Borax decahydrate, one of the boron compounds used in the study, showed an antifungal effect close to the control (+) application against *F. verticillioides* and *F. pseudograminearum*. While the antifungal effects of other boron compounds (Di-sodium tetraborate, Boric acid and Di-sodium octaborate tetrahydrate) varied depending on the pathogen and dose increase, they were found close to each other. In this context, it has been reported that Borax has an antifungal effect by causing the cytoplasmic contents to come out and the death of the pathogen due to the damage to the cell membrane of the fungus [38]. Yılmaz [39] stated that different forms of boron are involved in the Quorum Sensing mechanism, which is responsible for the formation of the antimicrobial effect. It is also known that boron compounds disrupt protein synthesis in microorganisms and the activities of serine-protease, β-lactamase and aminoacyl tRNA synthetase enzymes [40].

In the studies carried out to determine the effects of boron compounds and different fungal pathogens, which are problems in different plants, on mycelial growth; Webster and Dixon [41] reported that 20 and 30 ppm doses of boron significantly reduced the growth of *Plasmodiophora brassicae* in cabbages. Qin et al. [42] determined that potassium tetraborate (PTB) had an antifungal effect on *P. expansum*, and a concentration of 0.1% reduced spore germination by about 12% compared to the control. Thomidis and Exadaktylou [29] found that Power B (B 20% w/w, FARMA-CHEM SA) and Borax (B 20% w/w, Moscholios Chemicals SA)], compounds used against the monilial disease agent *M. laxa*, have antifungal effect and doses of 750 µg mL^{-1} of borax and 1000 µg mM^{-1} of Power B completely inhibited the mycelial growth of the pathogen. Li et al. [32] in their study to determine the antifungal effect of potassium tetraborate ($K_2B_4O_7$) and borax ($Na_2B_4O_7$) against *F. sulphureum*, a potato dry rot agent, found a positive relationship between increasing boron concentrations and inhibition of mycelial growth. reported 100% inhibition at high concentration (20 g L^{-1}). Shi et al. [43] reported that 20 mM concentration of PTB against the anthracnose agent *C. gloeosporioides* in mango fruits inhibited spore germination by 72%. Türkkan et al. [44] found that 10, 25, 25, 50, 50 and 75 mM concentrations of ammonium carbonate, ammonium bicarbonate, sodium carbonate, sodium bicarbonate, potassium carbonate and potassium bicarbonate completely inhibited the mycelial growth of *B. cinerea in-vitro*. They determined that there was a positive correlation between increasing doses of etidote-67 and borax decahydrate used against *P. expansum* and their inhibitory effects, and mycelial growth of *P. expansum* was completely inhibited in 0.25% [34]. Yildirim et al. [35] reported that *in-vitro*, 0.125% concentration of boric acid reduced mycelial growth of *P. expansum* by 68.88% and completely inhibited it at higher concentrations (0.25%, 0.50, 1.00% and 2.00%).

Table 3: Antifungal effect of some boron compounds on mycelial growth of *F. verticillioides and F. pseudograminearum*

Compounds	Doses	*F. Verticillioides*		*F. Pseudograminearum*	
	(g L^{-1})	Mycelial growth (mm)[1]	MGI (%)	Mycelial growth (mm)[1]	MGI (%)
Di-sodium tetraborate	0	40.7 a*	0.0	40.1 a*	0.0
	0.1	35.3 b	13.3	34.8 b	13.3
	0.2	30.8 c	24.3	30.3 c	24.5
	0.3	27.3 d	33.0	26.9 d	33.0

	0.4	18.3 e	55.1	23.0 e	42.6
	0.5	15.1 f	62.9	17.3 f	56.8
	Control (+)	10.3 g	74.8	10.4 g	74.0
	$CV_{(0.01)}$	2.02		1.34	
Borik acid	0	40.7 a	0.0	40.3 a	0.0
	0.1	34.0 b	16.3	35.0 b	13.0
	0.2	29.9 c	26.6	30.7 c	23.8
	0.3	26.4 d	35.0	27.5 d	31.6
	0.4	22.7 e	44.2	23.9 e	40.7
	0.5	17.8 f	56.2	18.1 f	55.0
	Control (+)	10.4 g	74.5	10.5 g	73.8
	$CV_{(0.01)}$	1.14		1.08	
Di-sodium octaborate tetrahydrate	0	40.8 a	0.0	40.5 a	0.0
	0.1	34.6 b	15.2	34.9 b	13.7
	0.2	30.7 c	24.8	30.8 c	24.0
	0.3	27.4 d	32.8	27.6 d	31.8
	0.4	22.8 e	44.2	23.6 e	41.7
	0.5	16.3 f	60.2	18.5 f	54.2
	Control (+)	10.1 g	75.3	10.4 g	74.2
	$CV_{(0.01)}$	1.01		1.51	
Borax decahydrate	0	40.8 a	0.0	40.4 a	0.0
	0.1	33.8 b	17.2	34.5 b	14.6
	0.2	29.9 c	26.7	30.5 c	24.5
	0.3	26.3 d	35.6	26.7 d	34.0
	0.4	22.0 e	46.0	23.3 e	42.5
	0.5	14.6 f	64.2	15.1 f	62.7
	Control (+)	10.0 g	75.5	10.3 g	75.6
	$CV_{(0.01)}$	1.09		1.02	

[1]The mean radial mycelial growth of *F.verticillioides* and *F. pseudograminearum* were determined at ten days after inoculation. Each observation is based on three replicate plates. Arcsine transformation was performed prior to statistical analysis. *Mean values followed by different letters within the column are significantly different according to Duncan Test (P≤0.01). Control (+): 80 g l^{-1} Triticonazole + 40 g l^{-1} Pyraclostrobin-BASF company. MGI: Mycelial growth inhibition rate

4. Conclusion

Turkey has the world's largest boron resources. For this reason, the use of boron compounds against plant pathogens is of great importance, both because of its low cost and because it is an environmentally friendly product. In the study carried out under *in-vitro* conditions, the efficacy of different boron compounds in the control of *F. verticillioides* and *F. pseudograminearum* was determined for the first time. In the study, it was determined that among the compounds of Di-sodium tetraborate, Boric acid, Di-sodium octaborate tetrahydrate and Borax decahydrate, especially Borax decahydrate compound was the most effective boron compound against *F. verticillioides* and *F. pseudograminearum.* The highest antifungal effect against both pathogens was obtained from the control (+) application of Borax decahydrate and the high dose (0.5 g L^{-1}) application. However, the excess of boron compounds can cause biochemical, physiological and anatomical disorders in plants. Therefore, detailed studies should be carried out to determine the efficacy of boron in combination with other broad-spectrum organic and inorganic compounds, which have antimicrobial properties, under *in-vivo* conditions in the control of corn stalk rot and ear rot diseases.

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