Evaluation of Antifungal Activity of Sodium Salts against Onion Basal Rot Caused by *Fusarium oxysporum* f.sp. *cepae*

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Abstract

Türkkan M., Erper I. (2014): **Evaluation of antifungal activity of sodium salts against onion basal rot caused by** *Fusarium oxysporum* f.sp. *cepae*. Plant Protect. Sci., **50**: 19–25.

The efficacy of twelve sodium salts as possible alternatives to synthetic fungicides for the control of onion basal rot caused by *Fusarium oxysporum* f.sp. *cepae* was evaluated. *In vitro* tests showed that there were significant differences between the inhibitory effects of sodium salts on the mycelial growth ($P \leq 0.05$) and 2% (w/v) concentrations of sodium metabisulfite and sodium fluoride completely inhibited mycelial growth of the fungus, while other salts did not. Sodium metabisulfite and sodium phosphate monobasic had lower pH values than the other salts. Unlike sodium metabisulfite, sodium phosphate monobasic could not decrease the mycelial growth. The ED_{50}, minimum inhibition concentration (MIC), and minimum fungicidal concentration (MFC) values indicated that sodium metabisulfite was more inhibitory to the fungus compared to sodium fluoride. In soil tests, inhibitory effect of sodium metabisulfite on the fungus was higher than that of sodium fluoride, where sodium metabisulfite completely inhibited mycelial growth at even 0.4% concentration.

Keywords: *Fusarium oxysporum* f.sp. *cepae*; salts; inhibitory effect; alternative control

Onion (*Allium cepa* L.) is one of the most economically important crops in Turkey. According to FAO records in 2010, Turkey was the world's third largest onion producer with a total cultivation area of 85 784 ha and a production of 2295.193 t (FAOSTAT 2013). Onion production and quality are negatively affected by many foliar, bulb, and root fungal pathogens (Schwartz 2004). Fusarium basal rot caused by *Fusarium oxysporum* Schlechtend.: Fr. f.sp. *cepae* (H.N. Hans.) W.C. Snyder & H.N. Hans is one of the most destructive diseases of onion, which cause serious yield losses in Japan, the Netherlands, and the USA (Sumner 1995; Galván *et al*. 2008; Dissanayake *et al*. 2009). In Turkey, Türkkan and Karaca (2006) found that Fusarium basal rot is a common and severe disease in Amasya province where onions are intensively cultivated. In addition, *F. oxysporum* f.sp. *cepae* was the most prevalent pathogen isolated in the study, representing 86% of the total. The pathogen delayed seedling emergence and caused seedling damping-off as well as root and basal rot. The early symptoms of the disease in the field are curving, wilting, yellowing, and eventually dying back of the leaves from the tips. In addition, diseased bulbs are discoloured, and the infected tissue appears brown and watery when the bulbs are cut open (Sumner 1995).

Several agronomic practices have been implemented to control Fusarium basal rot, such as use of resistant onion cultivars, long crop rotation, solarisation, seed treatments with fungicides and soil fumigation (Özer & Köycü 1998; Cramer 2000). Although resistance to the disease is not complete, damage caused by the disease can be reduced by the use of resistant cultivars. Resistant onion cultivars have been reported in some countries (Cramer 2000). However, breeding of resistant onion cultivars against the disease is more difficult because of the variation of virulence of *F. oxysporum* f.sp. *cepae* isolates (Galván *et al*. 2008). It has been reported that seed treatments with fungicides such as benomyl, carbendazim, carboxin, maneb, methoxymehtyl mercury chloride, prochloraz, tebuconazole, and thiram reduced the disease on onions (Köycü & Özer 1997; Özer & Köycü 1998; Cramer 2000). The most effective control of

the disease is soil fumigation with fumigants such as methyl bromide and chloropicrin (Jaworski *et al*. 1978; Köycü & Özer 1997; Sumner *et al*. 1997). However, because of the negative effects of these chemicals on both environment and public health, their usage has been banned in many countries (Fan *et al*. 2008). Therefore, there is a need to find alternative methods for the control of the disease. The use of efficient natural compounds such as organic and inorganic salts is one of the best alternatives. The salts including bicarbonate and carbonate have widely been used in the food industry as preservatives, pH regulators, and antimicrobial agents and are known to have low mammalian toxicity (Olivier *et al*. 1998). These salts are generally recognised as safe (GRAS) by the United States Food and Drug Administration (FDA) (FDA 2009). In addition, several of them were reported to have broad inhibitory effect against a range of fungal plant pathogens including *Penicillium griseofulvum* and *Fusarium graminearum* (DePasquale & Montville 1990), *Aspergillus ochraceus*, *F. graminearium,* and *P. griseofulvum* (Montville & Shih 1991), *Sphaerotheca fuliginea*, *Didymella bryoniae*, *Alternaria cucumerina*, *Ulocladium cucurbitae*, and *Colletotrichum orbiculare* (Ziv & Zitter 1992), *Botrytis cinerea* (Palmer *et al*. 1997), *Helminthosporium solani* (Olivier *et al*. 1998), *F. oxysporum* f.sp. *cyclaminis* (Elmer 2002), *F. sambucinum* (Mecteau *et al*. 2002), *P. digitatum* and *P. italicum* (Palou *et al*. 2002), *A. alternata*, *B. cinerea*, *F. solani* var. *coeruleum*, *Phytophthora erythroseptica*, *P. infestans*, *Verticillium albo-atrum*, and *V. dahliae* (Mills *et al*. 2004), *F. solani* var. *coeruleum*, (Mecteau *et al.* 2008), *F. oxysporum* f.sp. *melonis*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Arslan *et al*. 2009), and *A. solani*, *F. solani*, *F. oxysporum* and *Pythium* sp. (Abdel-Kader et al 2012).

The objective of this study was to evaluate the efficacy of sodium salts for the control of *F. oxysporum* f.sp. *cepae*. At first, inhibitory effects of the salts on the mycelial growth of the fungus were determined by *in vitro* tests, then the salts with higher inhibitory effects were used in soil tests. In the study, effect of pH on the mycelial growth of the pathogen was also evaluated.

MATERIAL AND METHODS

Fungal isolate. The isolate of *Fusarium oxysporum* f.sp. *cepae* used in this study was obtained from the culture collection of the Department of Plant Protection, Faculty of Agriculture, University of Ankara. The isolate was maintained on potato dextrose agar (PDA; Merck, Darmstadt, Germany). The PDA slants were stored at 4°C and served as stock cultures for further use. The present study was carried out in 2012.

Chemicals. All salts were purchased from Merck Chemicals (Merck, Darmstadt, Germany) except sodium metabisulfite (J.T.Baker; Deventer, the Netherlands). The salts used in this study were listed in Table 1.

Effect of sodium salts on mycelial growth. The effect of sodium salts on mycelial growth of *F. oxysporum* f.sp. *cepae* were assayed according to Mecteau *et al.* (2002) with a slight modification. The salts (2% w/v) were added to autoclaved and cooled PDA medium at 50°C, and then the pH of each salt treatment was determined by pH meter (Hanna HI 2211; Hanna Intruments, Kehl, Germany). Medium was dispensed aseptically into 7-cm-diameter Petri plates. Plates with unamended PDA were used as

Table 1. Salts used in the study

No.	Salts	Chemical formula	Molecular weight (g/mol)	Company
1	sodium acetate	$C_2H_3NaO_2$	82.03	Merck (Germany)
2	sodium bicarbonate	$NaHCO_3$	84.01	Merck (Germany)
3	sodium carbonate	Na_2CO_3	105.99	Merck (Germany)
4	sodium chloride	NaCl	58.44	Merck (Germany)
5	sodium citrate dihydrate	$Na_3C_6H_5O_7 \cdot 2H_2O$	294.10	Merck (Germany)
6	sodium fluoride	NaF	41.99	Merck (Germany)
7	sodium metabisulfite	$Na_2S_2O_5$	190.11	J.T. Baker (the Netherlands)
8	sodium phosphate monobasic	$NaH_2PO_4 \cdot H_2O$	137.99	Merck (Germany)
9	sodium phosphate dibasic	Na_2HPO_4	141.96	Merck (Germany)
10	sodium sulfate	Na_2SO_4	142.04	Merck (Germany)
11	sodium thiosulfate	$Na_2S_2O_3$	158.11	Merck (Germany)
12	trisodium phosphate dodecahydrate	$Na_3PO_4 \cdot 12H_2O$	380.18	Merck (Germany)

control. Mycelial disks (5 mm in diameter) from 7-day-old fungal cultures were placed in the center of the plates with 10 ml of PDA. Plates were then sealed with Parafilm and incubated at 24 ± 1°C. Mycelial growth was measured daily at two perpendicular colony diameters until the growth in the control plates reached the edge of the plates. Mycelial growth values were converted into 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 plates, respectively. Each treatment was replicated 3 times and the experiment was repeated twice.

Effect of pH on mycelial growth. Effect of pH on mycelial growth of *F. oxysporum* f.sp. *cepae* was examined by adjusting PDA at 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0 [with 1.0N NaOH (Riedel-de Haen AG, Buchs SG, Switzerland), or HCI (Merck, Darmstadt, Germany)]. Mycelial disks (5 mm) from 7-day-old fungal cultures were transferred to 9-cm-diameter Petri plates. Plates were then sealed with Parafilm. After incubation at 24 ± 1°C for 7 days, mycelial growth was determined by measuring colony diameter. Each treatment was replicated 3 times and the experiment was repeated twice.

ED_{50}, MIC, and MFC values of sodium salts. Concentrations of the salts that caused 50% reduction (ED_{50}) in the mycelial growth of *F. oxysporum* f.sp. *cepae* were calculated by probit analysis (IBM SPSS Statistics, Version 19; SPSS Inc., IBM Company, Chicago, USA). Mycelial growth was determined in PDA amended with 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0% (w/v) concentrations of the salts, as described above. The minimum inhibition concentration (MIC) that completely inhibited the mycelial growth was determined by using the probit analysis. The minimum fungicidal concentration (MFC) was also determined by parallel experiments.

Inhibitory effect of the salts was determined by using the method of Thompson (1989) and Tripathi *et al.* (2004). The inhibited fungal discs with no growth were taken from the salts-amended Petri plates, reinoculated separately into the fresh medium, and revival of their growth was observed for 9 days at 24 ± 1°C. The concentration that completely inhibits the fungi and irreversibly when transferred to fresh medium was stated as MFC.

Soil tests. In soil tests, cornmeal-sand medium was used to evaluate the efficacy of sodium salts that completely inhibited the mycelial growth at 2% concentration of the salts in the previous study. The medium was prepared as described by Arslan *et al.* (2009). The ratio of sand to corn was 1:8 and 45 g of medium was placed in glass 7-cm-diamater Petri plates. Petri plates including the medium were sterilised in an oven at 130°C for 5 hours. Mycelial disks (5 mm in diameter) from 7-day-old fungal cultures grown on PDA medium were placed at the centre of cornmeal-sand medium into a 0.5 cm depth. Salt concentrations (previously described) were prepared in sterile distilled water and 10 ml of each solution was homogeneously added to cornmeal-sand medium. After incubation at 24 ± 1°C in the dark for 7 days, image of the mycelial growth area was copied to a transparent paper with a 5 cm bar placed on the lids of the Petri plates. Images were then scanned into a digital format using a Mustek 1200 UB Plus desktop scanner (Mustek Systems, Inc., China), and the final versions of scanned images were saved as bmp 24-bit file. Mycelial growth area was measured by using public domain software (Digimizer, Version 4.0.0.0 for MS Windows 2005–2011; MedCalc Software bvba, Ostend, Belgium). The ratio of mycelial growth on salt amended medium to that of control was determined as percentage inhibition. Treatment was replicated 3 times for each concentration of the salts and repeated twice.

To determine the pH of the cornmeal-sand medium, 45 g of the medium was randomly sampled from each treatment and 20 ml of deionised water was added to each sample. The pH value was determined by inserting the pH electrode to the sample.

Statistical analysis. Results obtained from the present study were separately subjected to Analysis of Variance (One-Way ANOVA) using the IBM SPSS Statistics Program, and significant differences between the means were determined by using Tukey's *HSD* test ($P \leq 0.05$).

RESULTS

In this study, twelve salts were evaluated for their inhibitory activity against *F. oxysporum* f.sp. *cepae*. At 2%, sodium fluoride and sodium metabisulfite completely inhibited the mycelial growth of the fungus. There was no significant difference between the inhibitory effects of sodium fluoride and sodium metabisulfite ($P \leq 0.05$) (Figure 1). Sodium carbonate reduced the mycelial growth by 80.66% in comparison with the control. There were significant differences between sodium carbonate and four other salts ($P \leq 0.05$) – sodium acetate, sodium bicarbonate, sodium citrate dihydrate, and sodium phosphate dibasic inhibited mycelial growth to a smaller extent (14.39–54.21%). Sodium chloride, so-

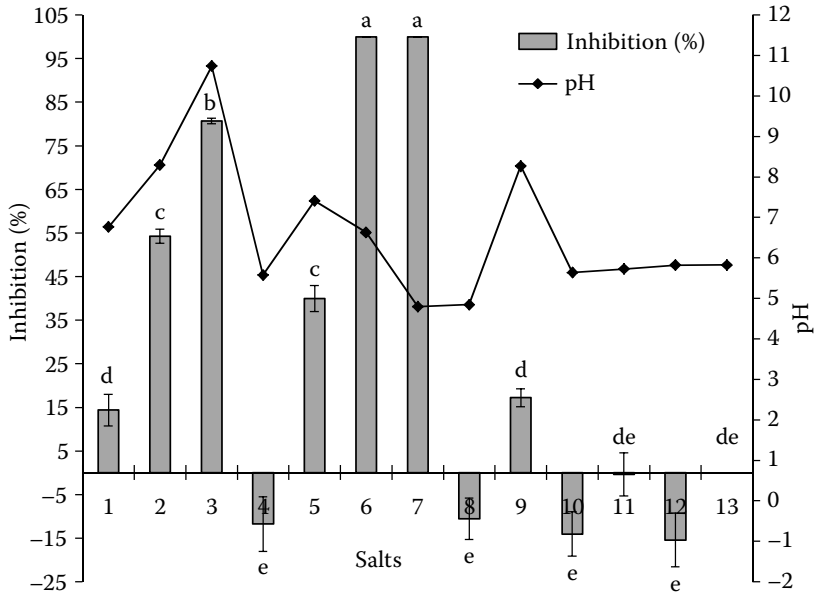

Figure 1. Effect on mycelial growth of *Fusarium oxysporum* f.sp. *cepae* at 2% concentration of salts (order of the salts is given in Table 1, control is represented by 13)

dium phosphate monobasic, sodium sulfate, sodium thiosulfate and trisodium phosphate dodecahydrate had no inhibitory effect on the mycelial growth of the fungus. Moreover, they stimulated the growth of the fungus. Effects of sodium chloride, sodium phosphate monobasic, sodium sulfate, and trisodium phosphate dodecahydrate were statistically different from control ($P \leq 0.05$), while sodium thiosulfate was in the same group with control ($P \leq 0.05$).

At 2% concentration of the salts, it was determined that pH values ranged from 4.80 to 10.73. The pH value of sodium carbonate was the highest with 10.73, followed by sodium bicarbonate (8.30), and sodium phosphate dibasic (8.27) (Figure 1). The minumum pH values were 4.80 and 4.85, which belonged to sodium metabisulfite and sodium phosphate monobasic, respectively.

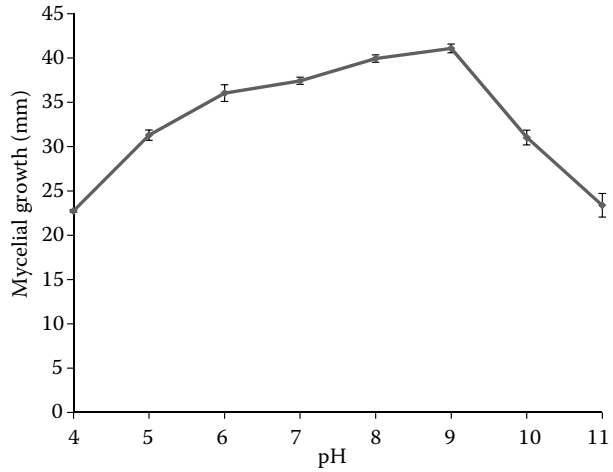

Figure 2. Effect of pH on mycelial growth of *Fusarium oxysporum* f.sp. *cepae*

The results of this study showed that *F. oxysporum* f.sp. *cepae* could grow both on acidic and basic pH (Figure 2). Variations of pH (6–9) did not negatively affect the mycelial growth of the fungus. Moreover, pH variations (7–9) showed a stimulating effect on the mycelial growth when compared to the control (34.83 mm, pH 5.83). There were statistically significant differences among mycelial growth at different pHs and control ($P \leq 0.05$). The higher (10–11) and lower (4–5) pH values significantly reduced the mycelial growth when compared to the control ($P \leq 0.05$).

Sodium metabisulfite was the most inhibitory salt against *F. oxysporum* f.sp. *cepae*, followed by sodium

Table 2. ED_{50}, MIC, and MFC values (%, w/v) of sodium salts inhibiting mycelial growth of *Fusarium oxysporum* f.sp. *cepae*

Salts	ED_{50}	MIC	MFC
Sodium acetate	> 2	> 2	> 2
Sodium bicarbonate	> 2	> 2	> 2
Sodium carbonate	0.82	> 2	> 2
Sodium chloride	nd	nd	> 2
Sodium citrate	> 2	> 2	> 2
Sodium fluoride	0.30	0.62	0.80
Sodium metabisulfite	< 0.05	0.135	0.20
Sodium phosphate monobasic	nd	nd	> 2
Sodium phosphate dibasic	> 2	> 2	> 2
Sodium sulfate	nd	nd	> 2
Sodium thiosulfate	nd	nd	> 2
Trisodium phosphate	nd	nd	> 2

nd – not determined

Table 3. Effect of the sodium fluoride and sodium metabisulfite on *Fusarium oxysporum* fsp. *cepae* in soil tests

Salts	Concentration (% w/v)	pH	Inhibition (%)
Control	0.00	5.64	0.00^{f}
Sodium fluoride	0.05	5.43	3.48^{f}
	0.10	5.43	5.06^{f}
	0.20	5.48	6.00^{f}
	0.40	5.52	9.88^{f}
	0.60	5.53	10.89^{f}
	0.80	5.62	53.12^{c}
	1.00	5.65	75.65^{b}
	1.50	5.97	100.00^{a}
	2.00	6.34	100.00^{a}
Sodium metabisulfite	0.05	5.59	12.89^{ef}
	0.10	5.54	30.83^{de}
	0.20	5.50	47.27^{cd}
	0.40	5.40	100.00^{a}
	0.60	5.34	100.00^{a}
	0.80	5.30	100.00^{a}
	1.00	5.29	100.00^{a}
	1.50	5.26	100.00^{a}
	2.00	5.13	100.00^{a}

fluoride, and sodium carbonate, respectively (Table 2). ED_{50} values of four other salts (sodium acetate, sodium bicarbonate, sodium citrate dihydrate, and sodium phosphate dibasic) had a much higher value than 2%. For the salts that stimulated mycelial growth of the fungus, ED_{50} values could not be determined. MIC values obtained from the study were compatible with ED_{50} values. Inhibitory efficacy of sodium metabisulfite was higher than that of sodium fluoride. Fungal discs from the medium amended with 0.2–2.0% of sodium metabisulfite did not grow on the fresh medium during 9 days. However, fungal discs from the medium amended with 0.6% concentration of sodium fluoride grew on the fresh medium on the first day, while those from the medium with 0.8–2.0% concentration of the same salt did not.

Sodium metabisulfite strontgly inhibited the mycelial growth of *F. oxysporum* f.sp. *cepae* when compared with sodium fluoride ($P \leq 0.05$) (Table 3). Even a 0.4% concentration of sodium metabisulfite completely inhibited the mycelial growth. Moreover, there was no significant difference between the effects of 0.4 and 2.0% concentrations of sodium metabisulfite ($P \leq 0.05$). However, sodium fluoride inhibited the fungus at a higher concentration (1.5%). There was no significant difference between 0.2% concentration of sodium metabisulfite and 0.8% concentration of sodium fluoride ($P \leq 0.05$).

DISCUSSION

Fusarium basal rot caused by *F. oxysporum* f.sp. *cepae* is one of the most devastating diseases of onion and causes serious yield losses in all growing areas of the world. The main source of the disease was determined as contaminated seeds and soil (Köycü & Özer 1997). Fungicide seed treatments and soil fumigation were reported to reduce the disease incidence (Jaworski *et al.* 1978; Köycü & Özer 1997; Sumner *et al.* 1997; Özer & Köycü 1998; Cramer 2000). However, usage of these chemicals for the control of the disease is not economical and causes the development of fungicide-resistant strains of the pathogen. In addition, these chemicals have adverse effects on environment and human health (Özer & Köycü 1998; Fan *et al.* 2008). In many studies performed to develop alternative strategies for the control of plant diseases, researchers tested the organic and inorganic salts for their effects on plant diseases. They showed that the salts may eventually be used in the control of plant diseases.

The present study showed that the mycelial growth of *F. oxysporum* f.sp. *cepae* is strongly affected by only three out of twelve sodium salts tested. Among them, sodium fluoride and sodium metabisulfite completely inhibited the mycelial growth of the fungus, whereas sodium carbonate reduced mycelial growth by 80.66%. These results are in agreement with those of Mecteau *et al.* (2002, 2008), who reported that sodium benzoate and sodium metabisulfite completely inhibited mycelial growth of *F. sambicinum* and *F. solani* var. *coereuleum*, and had a strong inhibitory activity on spor germination of *F. sambicinum*. Similarly, the mycelial growth and spore germination of *Alternaria alternata*, *Botrytis cinerea*, *F. solani* var. *coeruleum*, *Phytophthora erythroseptica*, *P. infestans*, *Verticillium albo-atrum*, and *V. dahliae* were strongly limited by sodium metabisulfite and propyl-paraben (Mills *et al.* 2004). Arslan *et al.* (2009) determined that the inhibitory effect of sodium carbonate on the mycelial growth of *F. oxysporum* f.sp. *melonis* was higher than that of sodium bicarbonate and sodium citrate. In the present study, sodium acetate, sodium bicarbonate, sodium citrate dihydrate, and sodium phosphate dibasic relatively reduced the mycelial growth of *F. oxysporum* f.sp. *cepae*. The five other salts (sodium chloride, sodium phosphate monobasic, sodium sulfate, sodium thiosulfate, and trisodium phosphate

dodecahydrate) did not inhibit the mycelial growth. On the contrary, stimulatory effects were observed on the mycelial growth of the fungus cultured on PDA amended with these salts. The results are compatible with those of Palmer *et al*. (1997), who reported that out of 26 tested salts, sodium chloride, sodium phosphate monobasic, sodium sulfate, and sodium thiosulfate did not affect the mycelial growth of *Botrytis cinerea*. Moreover, sodium phosphate monobasic was determined to provide additional nutrients for the development of the pathogen. Elmer (2002) also found that when 0.25 and 0.50 g/l concentrations of sodium chloride were applied to potting mix, final disease severity of Fusarium wilt caused by *F. oxysporum* f.sp. *cyclaminis* was not reduced. However, in contrast to our results, trisodium phosphate dodecahydrate was reported to completely inhibit the mycelial growth of *F. sambicinum* and *F. solani* var. *coereuleum* at 0.2M (7.6%) concentration on PDA (Mecteau *et al*. 2002, 2008). This implied that the sensitivity of *Fusarium* spp. to trisodium phosphate dodecahydrate may vary. Maybe, the higher concentrations of the salt might negatively affect the mycelial growth of *F. oxysporum* f.sp. *cepae*.

The results from the study demonstrated that *F. oxysporum* f.sp. *cepae* grew both on acidic and basic pH. The pH values of the salts at 2% were different from each other. Sodium metabisulfite had the lowest pH value (4.80), followed by 4.85 (sodium phosphate monobasic), and 5.58 (sodium chloride), respectively. As mentioned above, sodium metabisulfite completely inhibited the mycelial growth of the fungus, whereas sodium phosphate monobasic, that had similar pH value, enhanced the mycelial growth of the fungus. These results showed that its inhibitory effect was independent from pH. The results of previous studies are in agreement with the finding that pH values of organic and inorganic salts had a minor role in their inhibitory effects (Mecteau *et al.* 2002). DePasquale and Montville (1990) also reported that pH alone was not responsible for the antifungal activity of ammonium bicarbonate against *F. graminearum*.

In the present study, the salt that had the most inhibitory effect on *F. oxysporum* f.sp. *cepae* was sodium metabisulfite, followed by sodium fluoride, sodium carbonate, sodium bicarbonate, sodium citrate dihydrate, sodium acetate, and sodium phosphate dibasic, respectively. Similar findings were reported by previous researchers. Mecteau *et al.* (2008) found that the mycelial growth of *F. solani* var. *coeruleum* appeared to be particularly sensitive to potassium sorbate (1.8mM), aluminium chloride (3.7mM), sodium metabisulfite (4.2mM), sodium benzoate (9.8mM), and aluminium acetate (18.6mM). Mecteau *et al.* (2002) showed that aluminium acetate, aluminium lactate, aluminium chloride, sodium benzoate, sodium metabisulfite, and potassium sorbate had higher inhibitory effect on spores of *F. sambucinum*, whereas sodium carbonate and sodium citrate had lower inhibitory effect. Sodium carbonate was determined to have a higher fungicidal activity against *F. oxysporum* f.sp. *melonis* compared with sodium bicarbonate and sodium citrate (Arslan *et al.* 2009). ED_{50} values of the remaining salts could not be determined in the study because of their stimulating effect on mycelial development. We observed that MIC values were compatible with ED_{50} values. Sodium metabisulfite had the higher inhibitory effect against *F. oxysporum* f.sp. *cepae* as compared with sodium fluoride. Moreover, the MFC value (0.2%) of sodium metabisulfite was lower than that of sodium fluoride (0.8%). MFC values of the salts that did not completely inhibit mycelial growth of the fungus could not be determined.

In soil tests, sodium metabisulfite also showed a much higher antifungal activity to the fungus when compared to sodium fluoride. Arslan *et al*. (2009) reported that potassium sorbate completely inhibited *F. oxysporum* f.sp. *melonis* at 0.4%, but ammonium bicarbonate did not do so.

The present study showed that the use of sodium metabisulfite and sodium fluoride could be an alternative treatment in reducing disease severity of onion basal rot caused by *F. oxysporum* f.sp. *cepae*. For the control of the disease sodium metabisulfite could potentially be used alone or in combination with other safe treatments. However, sodium fluoride is not a compound that is GRAS by the FDA. Therefore, there is a need to evaluate the effect of the compound on seeds and seedlings of onion. These problems should be eliminated with further studies before their use in practice.

Acknowledgement. The authors thank Prof Dr Gürsel Karaca and Assoc Prof Dr Miray Sökmen for their careful proofreading.

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Received for publication February 8, 2013
Accepted after corrections June 13, 2013

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