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Antifungal Effect of Various Salts Against *Fusarium oxysporum* f.sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onion

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ABSTRACT

This study evaluated the efficacy of 26 different salts as possible alternatives to synthetic fungicides for the control of *Fusarium oxysporum* f.sp. *cepae*, the causal agent of Fusarium basal rot. Preliminary evaluation of salt tests was performed *in vitro* using 2% concentration (w/v). When compared to control, potassium acetate, potassium chloride, potassium nitrate, potassium phosphate dibasic, sodium chloride, sodium sulfate and trisodium phosphate significantly enhanced the mycelial growth of the fungus; diammonium phosphate had no significant effect; 15 other salts reduced mycelial growth to some extent (16.61-83.44%) ($P \leq 0.05$); and ammonium bicarbonate, ammonium carbonate and sodium metabisulfite completely inhibited mycelial growth. No significant difference in the inhibitory effects of ammonium bicarbonate, ammonium carbonate and sodium metabisulfite was observed *in vitro* ($P \leq 0.05$); however, the ED_{50}, minimum inhibition concentration (MIC), and minimum fungicidal concentration (MFC) values indicated sodium metabisulfite to be more toxic to *F. oxysporum* f.sp. *cepae* than ammonium carbonate and ammonium bicarbonate. Soil tests showed that 0.4% sodium metabisulfite completely inhibited fungal growth, whereas only the highest concentrations of ammonium bicarbonate and ammonium carbonate tested (2%) were able to inhibit growth ($P \leq 0.05$). The present study also showed that *F. oxysporum* f.sp. *cepae* capable of growth in both acidic and basic environments. While the fungus showed uninhibited growth at pH values between 6-9, growth decreased significantly at both higher and lower pH values ($P \leq 0.05$) and was completely inhibited at pH 12.

Keywords: *Fusarium oxysporum* f.sp. *cepae;* Alternative control; Salts; Toxicity; pH

Soğan Dip Çürüklüğü Etmeni *Fusarium oxysporum* f.sp. *cepae*'ya Karşı Çeşitli Tuzların Antifungal Etkisi

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ÖZET

Bu çalışma, soğan dip çürüklüğü etmeni *Fusarium oxysporum* f.sp. *cepae*'nın kontrolü için sentetik fungusitlere alternatif olabilecek 26 farklı tuzun etkinliğini değerlendirmiştir. Tuzların ön değerlendirilmesi in vitro testlerde % 2'lik (w/v) konsantrasyonda gerçekleştirilmiştir. Kontrole kıyasla potasyum asetat, potasyum klorit, potasyum nitrat, potasyum fosfat dibazik, sodyum klorit, sodyum sülfat ve trisodyum fosfat fungusun gelişimini artırmış; diamonyum fosfat etkilememiş; diğer 15 tuz misel gelişimini bir derece azaltmış (% 16.61-83.44) ($P \leq 0.05$) ve amonyum bikarbonat, amonyum karbonat ve sodyum metabisülfit misel gelişimini tamamen engellemiştir. İn vitro'da amonyum bikarbonat, amonyum karbonat ve sodyum metabisülfitin engelleyici etkilerinde önemli bir farklılık gözlenmemiş ($P \leq 0.05$); ancak ED_{50}, minimum inhibisyon konsantrasyon (MIC) ve minimum fungisidal konsantrasyon (MFC) değerleri sodyum metabisülfitin *F. oxysporum* f.sp. *cepae* için amonyum bikarbonat ve amonyum karbonattan daha toksik olduğunu göstermiştir. Ayrıca, toprak testleri sodyum metabisülfitin % 0.4 konsantrasyonda fungus gelişimini tamamen engellediğini gösterir iken test edilen amonyum bikarbonat ve amonyum karbonatın yalnızca en yüksek konsantrasyonu (% 2) fungus gelişimini engelleyebilmiştir ($P \leq 0.05$). Çalışma *F. oxysporum* f.sp. *cepae*'nın hem asidik hem de bazik çevrede gelişebildiğini göstermiştir. Fungus pH 6 ile 9 arası değerlerde engellenmeksizin gelişirken, daha yüksek ve daha düşük pH değerlerinde gelişmesi önemli bir şekilde azalmış ($P \leq 0.05$) ve pH 12'de tamamen engellenmiştir.

Anahtar Kelimeler: *Fusarium oxysporum* f.sp. *cepae*; Alternatif mücadele; Tuzlar; Toksisite; pH

1. Inroduction

Onion (*Allium cepa* L.) is one of the many economically important vegetable crops grown in Turkey. In 2010, the total area of onion cultivation in Turkey was 85.784 ha, production volume was 2.295.193 tons, and the rate yield was 40.29 kg per hectare (FAO 2013). 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 devastating diseases of onion and causes important yield losses in all growing areas of the world (Brayford 1996). The pathogen delays seedling emergence and causes damping-off on onion seedlings as well as root and basal rot. Early symptoms of the disease include curving, wilting, yellowing and eventually dying-back of the leaves from the tips. In addition, diseased bulbs are discolored and the infected tissue appears brown and watery when the bulbs are cut open (Sumner 1995).

Various agronomic practices have been used to control Fusarium basal rot, including the use of resistant onion cultivars, long crop rotation, solarisation, treatments of seed with fungicides and soil fumigation (Özer & Köycü 1998; Cramer 2000). Although the use of the pathogen-resistant cultivars may reduce the damage caused by *F. oxysporum* f.sp. *cepae*, variations in the aggressiveness and genetic structure of the pathogen make it difficult to breed resistant cultivars (Turkkan & Karaca 2006; Galván et al 2008; Bayraktar 2010). Seed treatments with fungicides such as benomyl, carbendazim, carboxin, maneb, methoxymehtyl mercury chloride, prochloraz, tebuconazole and thiram have been shown to reduce the disease on onion (Köycü & Özer 1997; Özer & Köycü 1998; Cramer 2000). Soil fumigation with fumigants such as methyl bromide and chloropicrin is the most effective method for controlling the disease (Jaworski et al 1978; Köycü & Özer 1997; Sumner et al 1997). However, the negative effects of these chemicals on both the environmental and public health has led to their banning in many countries (Fan et al 2008). The use of natural coumponds such as organic and inorganic salts represent one of the best alternative methods for controlling the disease. Organic and inorganic salts have low mammalian toxicity and are widely used in the food industry as preservatives, pH regulators and antimicrobial agents (Olivier et al 1998). Their antifungal effects have also been demonstrated on numerous pathogens including, *Botrytis cinerea*

(Palmer et al 1997), *Monilinia fructicola* (Biggs et al 1997), *Helminthosporium solani* (Olivier et al 1998), *Fusarium oxysporum* f.sp. *asparagi* and *F. proliferatum* (Reid et al 2001), *F. oxysporum* f.sp. *cyclaminis* (Elmer 2002), *F. sambicinum* (Mecteau et al 2002), *Alternaria 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 aim of the present study was to evaluate the efficacy of different salts in controlling *F. oxysporum* f.sp. *cepae*. *In vitro* testing was performed to determine the fungistatic effects of 26 salts, and those with the highest toxicity were evaluated in soil tests. Also, the effects of pH on mycelial growth of *F. oxysporum* f.sp. *cepae* were investigated in this study.

2. Material and Methods

2.1. Fungal isolate

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

2.2. Chemicals

The salts used in this study were purchased from Merck Chemicals (Darmstadt, Germany), Sigma-Aldrich (Seelze, Germany), J.T.Baker (Deventer, The Netherlands), Fluka Chemical (Steinheim, Germany) and Carlo Erba Reagenti (Milan, Italy) (Table 1).

2.3. Effect of the salts on mycelial growth

The effect of salts on *F. oxysporum* f.sp. *cepae* mycelial growth was assayed according to Mecteau et al (2002) with a slight modification. Salts (2%, w/v) were added to autoclaved and cooled PDA medium at 50 °C, and the pH of each treatment was measured using a pH meter (Hanna HI 2211, Hanna Instruments, Germany). The medium was dispensed aseptically into 7-cm-diameter Petri plates. Plates with unamended PDA were used as control. Mycelial disks of 5 mm in dia. from 7-day-old fungal cultures were placed in the center of the plates with 10 mL of PDA. The 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 Petri plates. Mycelial growth values were converted into the inhibition percentage of mycelial growth inhibition (MGI) in relation to controls using the formula,

$$MGI(\%) = [\frac{dc - dt}{dc}] \times 100$$

where dc and dt represent mycelial growth diameters in controls and amended Petri plates, respectively. Each treatment was replicated 3 times and the experiment was repeated twice.

2.4. Effect of pH on mycelial growth

Mycelial growth of *F. oxysporum* f.sp. *cepae* was examined on adjusted PDA at pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 using 1.0 N NaOH (Riedel-de Haen AG, Buchs SG, Switzerland) or HCI (Merck, Darmstadt, Germany). The Petri plates containing unmodified PDA were used as controls. Mycelial disks of 5 mm in dia. from 7-day-old fungal cultures were placed in 9-cm-dia. plates that were then sealed with Parafilm and incubated at 24±1 °C for 7 days. Mycelial growth was determined by measuring colony diameter. Each experiment was replicated 3 times and repeated twice.

2.5. ED_{50}, MIC and MFC values of the salts

Probit analysis was used to calculate concentrations of salts causing 50% reduction (ED_{50}) in mycelial growth of *F. oxysporum* f.sp. *cepae* (StatPlus, AnalystSoft Inc.). Mycelial growth was determined in PDA amended with salts at concentrations of

Table 1- Salts used in the study

Çizelge 1- Çalışmada kullanılan tuzlar

No	*Compounds*	*Chemical formule*	*Molecular weight (g mol^{-1})*	*Company*
1	Ammonium acetate	$C_2H_3O_2NH_4$	77.08	Merck (Darmstadt, Germany)
2	Ammonium bicarbonate	$(NH_4)HCO_3$	79.07	Sigma-Aldrich (Seelze, Germany)
3	Ammonium carbonate	$(NH_4)_2CO_3$	96.06	Merck (Darmstadt, Germany)
4	Ammonium chloride	NH_4Cl	53.49	Merck (Darmstadt, Germany)
5	Ammonium molybdate	$Mo_7O_{24}6(NH_4)4(H_2O)$	1235.86	Fluka Chemical (Steinheim, Germany)
6	Ammonium nitrate	$(NH_4)(NO_3)$	80.05	Merck (Darmstadt, Germany)
7	Ammonium sulfate	$(NH_4)_2SO_4$	132.14	Merck (Darmstadt, Germany)
8	Diammonium phosphate	$(NH_4)_2HPO_4$	132.07	Merck (Darmstadt, Germany)
9	Calcium acetate	$C_4H_6CaO_4$	158.17	Merck (Darmstadt, Germany)
10	Calcium chloride	$CaCl_2$	110.98	Merck (Darmstadt, Germany)
11	Potassium acetate	CH_3CO_2K	98.15	Carlo Erba Reagenti (Milan, Italy)
12	Potassium bicarbonate	$KHCO_3$	100.12	J.T.Baker (Deventer, The Netherlands)
13	Potassium carbonate	K_2CO_3	138.21	J.T.Baker (Deventer, The Netherlands)
14	Potassium chloride	KCl	74.55	Merck (Darmstadt, Germany)
15	Potassium nitrate	KNO_3	101.10	Merck (Darmstadt, Germany)
16	Potassium phosphate dibasic	K_2HPO_4	174.20	Merck (Darmstadt, Germany)
17	Potassium sulfate	K_2SO_4	174.26	Merck (Darmstadt, Germany)
18	Sodium acetate trihydrate	$C_2H_3NaO_2.3H_2O$	136.08	Merck (Darmstadt, Germany)
19	Sodium bicarbonate	$NaHCO_3$	84.01	Sigma-Aldrich (Seelze, Germany)
20	Sodium carbonate	Na_2CO_3	105.99	Sigma-Aldrich (Seelze, Germany)
21	Sodium chloride	NaCl	58.44	Merck (Darmstadt, Germany)
22	Sodium citrate dihydrate	$C_6H_5O_7Na_3.2H_2O$	294.10	Merck (Darmstadt, Germany)
23	Sodium metabisulphite	$Na_2S_2O_5$	190.11	Merck (Darmstadt, Germany)
24	Sodium phosphate dibasic	$Na_2HPO_4.7H_2O$	268.06	Merck (Darmstadt, Germany)
25	Sodium sulfate	Na_2SO_4	142.04	Merck (Darmstadt, Germany)
26	Trisodium phosphate	Na_3PO_4	163.94	Merck (Darmstadt, Germany)

0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0% (w/v) as described above. Probit analysis was performed to determine the minimum inhibition concentration (MIC) value that completely inhibited mycelial growth.

Salt toxicity (fungistatic/fungicidal) was determined according to Thompson (1989) and Tripathi et al (2004). The inhibited fungal discs with no growth were taken from the salts amended Petri plates, and then re-inoculated separately into the fresh medium, and revival of their growth was observed for 9 days at 24±1 °C. The concentration that inhibited the fungi completely and irreversibly after transference to fresh medium was recorded as the minimum fungicidal concentration (MFC) value.

2.6. Soil tests

Soil tests were performed using a cornmeal-sand medium to further evaluate those salts that had completely inhibited mycelial growth at 2% concentrations *in vitro*. The medium was prepared

as described by Arslan et al (2009) using a 1:8 ratio of sand to corn. Medium (45 g) was placed in glass 7-cm-dia. Petri plates and sterilized in an oven (Ecocell LSIS-B2V/EC111, MMM Group, Germany) at 130 °C for 5 h. Mycelial disks (5-mm dia.) were taken from 7-day-old fungal cultures grown on PDA medium and placed in the center of cornmeal-sand medium at a depth of 0.5 cm. Salt concentrations (described above) were prepared using sterile distilled water, 10 mL of solution was added to each Petri plate, and plates were incubated in the dark at 24±1 °C for 7 days. Images of fungal mycelial growth were photocopied on transparent paper, with a 5 cm scale placed on the lid of each plates. Images were then digitally scanned using a Mustek 1200 UB Plus desktop scanner (Mustek, China) and saved as bmp 24 bit files. Surface areas were digitally measured by using Digimizer (Version 4.0, Belgium). Inhibition of mycelial growth was expressed as a percentage of mycelia growth of each sample in comparison to the control.

In addition, pH values of the cornmeal-sand medium were determined by randomly sampling 45 g of medium per treatment replicate. Measurements were performed by inserting the electrode of the pH meter into the sample after adding 20 mL of deionized water.

2.7. Statistical analysis

Results were separately subjected to one-way analysis of variance (ANOVA) using the IBM SPSS Statistics Program (Version 19, Property of SPSS, Inc., IBM Company, USA). Significant differences between means were determined using the Tukey–HSD test ($P \leq 0.05$).

3. Results and Discussion

This study evaluated the inhibitory activity of 26 inorganic and organic salts against *Fusarium oxysporum* f.sp. *cepae in vitro* at 2% concentration. Ammonium bicarbonate, ammonium carbonate and sodium metabisulfite completely inhibited mycelial growth of the fungus whereas potassium sulfate and sodium carbonate strongly reduced mycelial growth. Sodium citrate dihydrate, sodium bicarbonate, ammonium molybdate, potassium carbonate inhibited mycelial growth to a lesser extent (43.65-68.10%) (Figure 1).

The differences in the inhibitory effects of the salts were in general statistically significant ($P \leq 0.05$). Although sodium carbonate had a grater inhibitory effect than potassium sulfate, the difference between the two was not statistically significant ($P \leq 0.05$). These results are in line with those reported by previous studies. Arslan et al (2009) reported that mycelial growth of *F. oxysporum* f.sp. *melonis* was totally inhibited by ammonium bicarbonate, potassium benzoate, potassium sorbate and sodium benzoate, but not by sodium carbonate, sodium bicarbonate, or sodium citrate dihydrate. Mills et al (2004) determined that mycelial growth and spore germination of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani* var. *coeruleum*, *Phytophthora erythroseptica*, *P. infestans*, *Verticillium albo-atrum*, and *V. dahliae* were strongly limited by sodium metabisulfite and propyl-paraben. The present study revealed that 10 salts (ammonium acetate, ammonium chloride, ammonium nitrate, ammonium sulfate, calcium acetate, calcium chloride, diammonium phosphate, potassium bicarbonate, sodium acetate trihydrate and sodium phosphate dibasic) inhibited the mycelial growth of *F. oxysporum* f.sp. *cepae* to a certain extent (6.22 and 31.62%). Diammonium phosphate, had the lowest inhibitory effect, which did not differ significantly from the control ($P \leq 0.05$). No statistically significant differences were observed among ammonium acetate, ammonium nitrate, calcium acetate, calcium chloride and potassium bicarbonate or between ammonium chloride and sodium phosphate dibasic ($P \leq 0.05$), all of which had significantly higher inhibitory effects than diammonium phosphate and the control. These results are in line with those of previous studies. Abdel-Kader et al (2012) found that calcium chloride concentrations of 1% and 2% gradually reduced mycelial growth of *F. solani* and *F. oxysporum* by 22.2% and 33.3%, respectively,

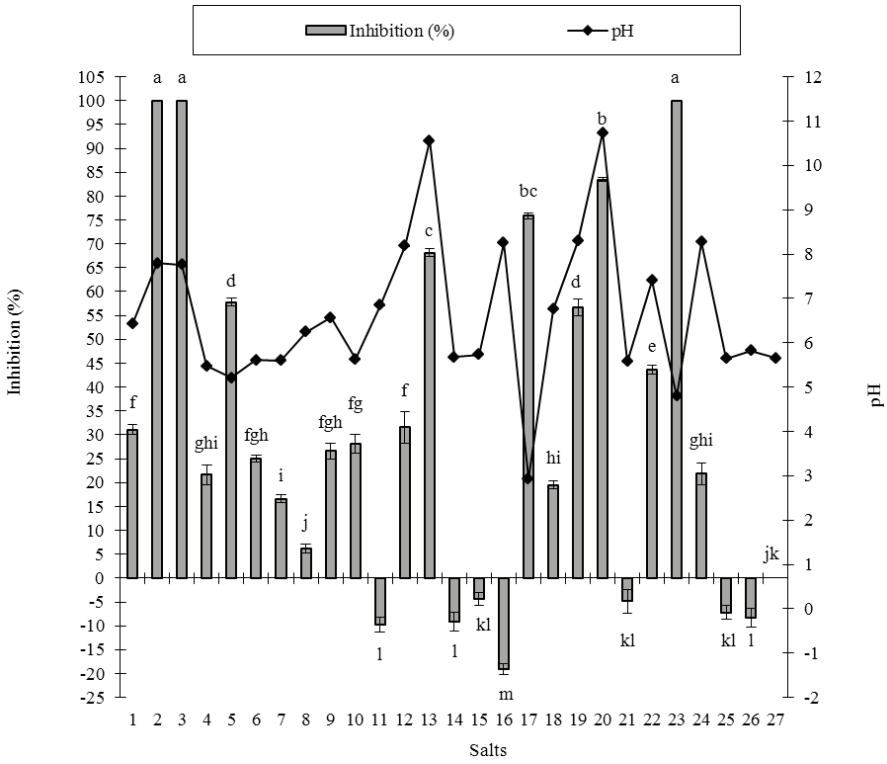

Figure 1- Effects of salts at 2% concentration on mycelial growth of *Fusarium oxysporum* f.sp. *cepae* (See Table 1 for salt details, 27=control)

Şekil 1- % 2 konsantrasyonda Fusarium oxysporum f.sp. cepae'nın miselyal gelişimi üzerine tuzların etkileri (Tuzlar için Çizelge 1'e bakınız, 27=kontrol)

whereas no further reductions were observed at a higher concentration (4%). Bigss et al (1997) reported that calcium acetate and calcium chloride did not inhibit growth of *Monilinia fructicola* on PDA as strongly as 4 other calcium salts (calcium oxide, calcium propionate, calcium pyrophosphate, calcium cilicate). However, Reid et al (2001) reported that sodium chloride was more effective than other chloride salts (calcium chloride, ammonium chloride and manganese chloride) in controlling Fusarium crown and root rot caused by *F. oxysporum* f.sp. *asparagi* and *F. proliferatum*. This contrasts with the finding of the present study showing calcium and ammonium chloride to be more effective than sodium chloride in inhibiting mycelial growth of *F. oxysporum* f.sp. *cepae*. Moreover, the present study found that sodium chloride, sodium sulfate, trisodium phosphate, potassium acetate, potassium chloride, potassium nitrate and potassium phosphate dibasic did not reduced the mycelial growth of *F. oxysporum* f.sp. *cepae*, but in fact had a stimulatory effect on mycelial growth when compared to the control ($P \leq 0.05$). Potassium phosphate dibasic had the greatest stimulatory effect, followed by potassium acetate, potassium chloride and trisodium phosphate. No differences were found in the effects of potassium nitrate, sodium chloride and sodium sulfate ($P \leq 0.05$). This is in line with previous studies such as Elmer (2002), which found no reductions in the severity of Fusarium wilt caused by *F. oxysporum* f.sp. *cyclaminis* when 0.25 g and 0.50 gL^{-1} concentrations of sodium chloride were

applied to potting mix. Similarly, Palmer et al (1997) reported that out of 26 salts tested, sodium chloride, sodium phosphate monobasic, sodium sulfate and sodium thiosulfate had no effect on the mycelial growth of *Botrytis cinerea*. Moreover, sodium phosphate monobasic was found to provide additional nutrients for development of pathogen. In contrast to the findings of the present study, Mecteau et al (2002, 2008) reported that trisodium phosphate completely inhibited mycelial growth of *F. sambicinum* and *F. solani* var. *coereuleum* at 0.2 M (7.6%) on PDA. This implies that the sensitivity of *Fusarium* spp. to trisodium phosphate may vary and that higher concentrations of the salt may be required to completely inhibit mycelial growth of *F. oxysporum* f.sp. *cepae*.

In terms of pH, the present study found pH values to range from 2.94 (potassium sulfate) to 10.75 (sodium carbonate) at 2% salt concentrations (Figure 1). Whereas 23 salts had pH values that differed significantly from the control (5.65), the pH values of 3 salts (calcium chloride, potassium chloride and sodium sulfate) did not differ significantly from the control ($P \leq 0.05$). However, calcium chloride inhibited mycelial growth (28.18%), both potassium chloride and sodium sulfate stimulated mycelial growth (-9.19% and -7.19%, respectively). Ammonium bicarbonate, ammonium carbonate and sodium metabisulfite had pH values of 7.80, 7.76 and 4.83, respectively, and all 3 salts completely inhibited mycelial growth (Figure 1). Overall, this study showed that *F. oxysporum* f.sp. *cepae* was able to grow both in acidic and basic environments (Figure 2). While no negative effects were observed with pH values ranging from 6 to 9, mycelial growth was significantly inhibited at both higher and lower pH values ($P \leq 0.05$) and halted completely at a pH of 12. Moreover, no significant differences were found between mycelial growth of the fungus at pH 5, 10 and the unmodified pH (5.65) ($P \leq 0.05$). In other words, toxic effects of salts appear to be pH-independent. In line with this finding, a previous study also found that the pH values of organic and inorganic salts have a minor role in their toxic effects (Mecteau et al 2002).

Of the various salts examined in the present study, sodium metabisulfite had the most toxic effect on the mycelial growth of *F. oxysporum*

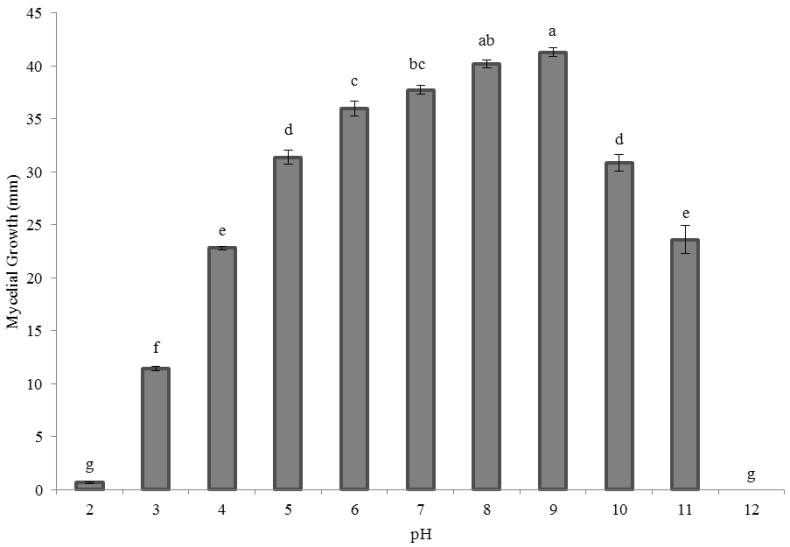

Figure 2- Effects of pH on mycelial growth of *Fusarium oxysporum* f.sp. *cepae*

Şekil 2- Fusarium oxysporum f.sp. cepae'nın miselyal gelişimi üzerine pH'nın etkileri

f.sp. *cepae*, followed by ammonium bicarbonate, ammonium carbonate, ammonium molibdate, potassium sulfate, sodium carbonate and potassium carbonate, respectively. ED_{50} values of 10 other salts (ammonium acetate, ammonium chloride, ammonium nitrate, calcium acetate, calcium chloride, potassium bicarbonate, sodium acetate trihydrate, sodium bicarbonate, sodium citrate dihydrate and sodium phosphate dibasic) were above 2%. ED_{50} values were not calculated for those salts found to stimulate mycelial growth (Table 2).

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

Çizelge 2- Fusarium oxysporum f.sp. cepae'nın miselyal gelişimini engelleyen tuzların ED_{50}, MIC, and MFC değerleri(%, w/v)

Compounds	*ED_{50}*	*MIC*	*MFC*
Ammonium acetate	> 2	> 2	> 2
Ammonium bicarbonate	0.19	0.57	0.80
Ammonium carbonate	0.22	0.55	0.60
Ammonium chloride	> 2	> 2	> 2
Ammonium molybdate	0.49	> 2	> 2
Ammonium nitrate	> 2	> 2	> 2
Ammonium sulfate	ND	ND	> 2
Diammonium phosphate	ND	ND	> 2
Calcium acetate	> 2	> 2	> 2
Calcium chloride	> 2	> 2	> 2
Potassium acetate	ND	ND	> 2
Potassium bicarbonate	> 2	> 2	> 2
Potassium carbonate	1.67	> 2	> 2
Potassium chloride	ND	ND	> 2
Potassium nitrate	ND	ND	> 2
Potassium phosphate dibasic	ND	ND	> 2
Potassium sulfate	0.64	> 2	> 2
Sodium acetate trihydrate	> 2	> 2	> 2
Sodium bicarbonate	> 2	> 2	> 2
Sodium carbonate	0.80	> 2	> 2
Sodium chloride	ND	ND	> 2
Sodium citrate dihydrate	> 2	> 2	> 2
Sodium metabisulphite	< 0.05	0.31	0.20
Sodium phosphate dibasic	> 2	> 2	> 2
Sodium sulfate	ND	ND	> 2
Trisodium phosphate	ND	ND	> 2

ND: Not determined

MIC values were consistent with ED_{50} values in all the salts tested, with the exception of ammonium bicarbonate. Moreover, ammonium bicarbonate had a lower ED_{50} value than ammonium carbonate, whereas ammonium carbonate had lower MIC value (0.55%). However, sodium metabisulfite had the lowest MIC value with 0.31% (Table 2). These results are supported by previous studies. Mecteau et al (2002) found that aluminium acetate, aluminium lactate, aluminium chloride, sodium benzoate, sodium metabisulfite and potassium sorbate had a higher toxic effect, and sodium carbonate and sodium citrate dihydrate had a lower toxic effect on *Fusarium sambucinum* spores. Ammonium bicarbonate showed greater fungucidal activity against *F. oxysporum* f.sp. *melonis* than potassium carbonate, potassium bicarbonate, sodium bicarbonate, sodium carbonate and sodium citrate dihydrate (Arslan et al 2009). Similarly, Montville & Shih (1991) observed that ammonium bicarbonate at 1 and 2% completely inhibited monocultures of *F. graminearium*, *Aspergillus ochraceus* and *Penicillium griseofulvum* on cracked corn. The present study showed sodium metabisulfite to have a higher fungistatic effect than ammonium bicarbonate and ammonium carbonate (Table 2). Whereas fungal discs taken from medium amended with 0.2–2.0% of sodium metabisulfite did not grow in the fresh medium for 9 days, discs taken from medium amended with 0.6% ammonium carbonate grew in fresh medium after 4 days, discs taken from medium amended with ammonium bicarbonate did not grow for 9 days. In contrast, Arslan et al (2009) reported ammonium bicarbonate to have an MFC value of 1% for *F. oxysporum* f.sp. *melonis* and an ED_{50} value of 0.35%, which was nearly twice as high as the value reported in the present study (ED_{50}=0.19). These results indicate that *F. oxysporum* f.sp. *cepae* is more sensitive to ammonium bicarbonate than *F. oxysporum* f.sp. *melonis*. In soil tests, sodium metabisulfite had a higher toxic effect on *F. oxysporum* f.sp. *cepae* than ammonium bicarbonate and ammonium carbonate (Table 3). Sodium metabisulfite at a 0.4% concentration completely inhibited the fungus, whereas ammonium bicarbonate and ammonium

Table 3- Effects of ammonium bicarbonate, ammonium carbonate and sodium metabisulfite on *Fusarium oxysporum* f.sp. *cepae* in soil tests

Çizelge 3- Toprak testlerinde Fusarium oxysporum f.sp. cepae üzerine amonyum bikarbonat, amonyum karbonat ve sodyum metabisülfitin etkileri

Salts	*Concentration (% w/v)*	*pH*	*Inhibition (%)*
Control	0.00	5.64 k	0.00 g
Ammonium bicarbonate	0.05	5.97 j	35.35 def
	0.10	6.24 i	45.98 cdef
	0.20	6.60 h	59.06 bcde
	0.40	6.82 g	87.10 ab
	0.60	7.03 f	87.51 ab
	0.80	7.30 de	94.54 ab
	1.00	7.38 cd	93.99 ab
	1.50	7.52 b	96.68 ab
	2.00	7.62 a	100.00 a
Ammonium carbonate	0.05	6.00 j	19.42 fg
	0.10	6.30 i	29.97 efg
	0.20	6.62 h	50.19 cdef
	0.40	6.87 g	59.89 bcde
	0.60	7.10 f	67.92 abcd
	0.80	7.25 e	78.47 abc
	1.00	7.30 de	88.38 ab
	1.50	7.42 c	93.17 a
	2.00	7.53 ab	100.00 a
Sodium meta-bisulphite	0.05	5.56 kl	17.86 fg
	0.10	5.52 l	31.93 efg
	0.20	5.48 l	48.08 cdef
	0.40	5.38 m	100.00 a
	0.60	5.33 mn	100.00 a
	0.80	5.29 mn	100.00 a
	1.00	5.27 n	100.00 a
	1.50	5.24 n	100.00 a
	2.00	5.08 o	100.00 a

carbonate managed to control the fungus only at the highest concentration used in the study (2%). Differences in the inhibitory effects of the three salts were statistically significant ($P \leq 0.05$). However, no significant differences were observed between 0.4 and 2% concentrations of sodium metabisulfite and ammonium bicarbonate ($P \leq 0.05$). In vitro and soil tests had similar results in terms of pH values; both ammonium bicarbonate and ammonium carbonate increased soil pH, whereas sodium metabisulfite reduced soil pH. Similarly, Arslan et al (2009) reported that ammonium bicarbonate at a 2% concentration increased the pH of soil and completely inhibited *F. oxysporum* f.sp. *melonis*.

4. Conclusions

The results of present study indicate that sodium metabisulfite, ammonium bicarbonate and ammonium carbonate, used alone or in combination with other safe treatments, may be appropriate alternatives for reducing the severity of Fusarium basal rot caused by *Fusarium oxysporum* f.sp. *cepae*. Each of these salts are "Generally Recognized as Safe (GRAS)" by the United States Food and Drug Administration (FDA). However, this was a preliminary study, and subsequent evaluations of the effects of these salts on onion seeds and seedlings are required before their use can be recommended.

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