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EFFECT OF SOME SALTS ON *IN VITRO* GROWTH OF *FUSARIUM SOLANI*

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Abstract

Effect of different salts viz., sea salt, NaCl , $CaCl_2$, $NaNO_3$, $MgSO_4$, $MgCl_2$ and KCl was studied on *in vitro* growth of *Fusarium solani* by food poison method. Growth of *F. solani* was enhanced as compared to control in potato sucrose agar (PSA) medium containing sea salt @ 20,000 ppm but suppressed where sea salt was used @ 60,000 ppm or more. No conidia were produced in 100,000 ppm of sea salt. Similarly, *F. solani* showed enhanced growth in NaCl at 10,000 and 20,000 ppm and declined with increasing conc. from 30,000 to 100,000 ppm. Growth of *F. solani* also increased significantly with increasing conc. of $MgSO_4$ up to 40,000 ppm but declined thereafter. Use of $NaNO_3$ @ 10,000 to 70,000 ppm showed significant increase in growth with massive cottony white mycelium as compared to control. Growth of *F. solani* declined gradually compared to control with increasing conc. of $MgCl_2$ from 20,000 ppm to 100,000 ppm. Similarly, use of KCl @ 20,000 to 40,000 ppm showed gradual decline in growth of *F. solani* whereas @ 50,000 ppm or more, growth started after 7-days of inoculation. Amendment of PSA with $CaCl_2$ showed gradual decline in growth of *F. solani* with increasing conc. of $CaCl_2$. Chlamydospores were produced only where $NaNO_3$ was used @ 10,000 to 40,000 ppm.

Introduction

Soil salinity is one of the important factors affecting agricultural production of Pakistan where more than 6 million hectares are affected (Anon., 1998). Soil salinity not only affects plant growth but also the soil microorganisms and can therefore enhance or suppress the incidence of plant infection by these microorganisms. Reports have been made where *Cercospora* leaf spot of peanut was favoured by soil salinity (Porter & Adamson, 1993). Similarly, NaCl treatment predisposed the citrus root tissues to infection by *Phytophthora citrophthora* (Sulistyowati, 1993). Chlamydospore germination of *Fusarium oxysporum* f.sp. *vasinfectum* was enhanced by salinity (Ragazzi & Vecchio, 1992), whereas, the hyphae of *Bipolaris sorokiniana* [*Cochliobolus sativus*] branched profusely while growing on a medium containing NaCl (Suryanarayanan & Janarthanam, 1985).

It is interesting to note that NaCl is also used for the control of *Gibberella fujikuroi* and *F. oxysporum*, the causal agents of root rot of *Asparagus* (Ragazzi & Vecchio, 1992). Similarly, the application of sodium chloride in *Asparagus* bed suppressed *Fusarium* crown and root rot caused by *F. oxysporum* and *F. proliferatum* (Elmer, 1990, 1992). Pre-sowing treatment of *Pennisetum typhoides* seeds with sodium chloride showed that with the increasing concentration of salt, the incidence of the green-ear disease caused by *Sclerospora graminicola* markedly decreased (Hedge & Karande, 1978). This paper describes the effect of different salts viz., sea salt, NaCl , $CaCl_2$, $NaNO_3$, $MgSO_4$, $MgCl_2$ and KCl on *in vitro* growth of *F. solani* that is known to cause wilt and root rot diseases of various crops in Pakistan thus adversely affecting the yield (Hafiz,1986; Ghaffar, 1988,1992).

Table 1. Electroconductivity of diffcrent salt solutions prepared in potato sucrose broth.

Concentration	Electroconductivity (ms/cm)						
%	Sea salt	NaCl	$CaCl_2$	$NaNO_3$	$MgSO_4$	$MgCl_2$	KCl
0	2.21	2.74	2.26	2.51	2.20	2.50	2.44
1	22.60	24.70	16.40	16.40	7.20	12.88	2.39
2	40.50	42.40	29.40	28.70	11.50	22.80	2.37
3	57.20	59.10	41.40	40.10	15.10	30.80	2.35
4	73.10	76.40	54.30	50.20	18.40	39.70	2.33
5	90.80	91.40	65.70	61.10	21.70	47.80	2.31
6	104.30	107.10	76.70	70.80	24.80	54.90	2.29
7	121.10	119.70	86.30	77.90	27.50	61.20	2.27
8	132.60	136.10	95.30	88.80	30.30	69.20	2.25
9	146.20	147.30	105.00	99.10	32.20	75.30	2.23
10	159.50	157.90	116.60	105.10	35.00	81.20	2.19

Materials and Methods

The isolate of *F. solani* selected for the present study was isolated from the seeds of sunflower by using standard blotter method (Anon., 1976). Food poison method (Nene & Thapliyal, 1979) was used to see the effect of sea salt on *in vitro* growth of *F. solani* where sea salt was added @ 0, 1, 10, 100, 1000, 10,000 and 100,000 ppm. The electroconductivity (EC) of each salt solution was recorded using an EC meter (Hanna HI8733) that appeared to be 2.19, 2.22, 2.24, 2.46, 4.3, 21.3 and 155.5 ms/cm, respectively.

In another set of experiments, sea salt as well as different salts commonly found in sea salt *viz.*, NaCl, KCl, $CaCl_2$, $NaNO_3$, $MgSO_4$, $MgCl_2$ were used where the salinity levels were adjusted to 0, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000 ppm. In each case the required amount of salt was added in 150ml of potato sucrose broth (PSB) and mixed thoroughly to get the desired concentration. The electroconductivity (EC) of each salt solution was recorded (Table 1). Three g of agar was then added in each flask containing 150ml of PSB with or without salt. The media were sterilized at 15 p.s.i. for 15 minutes and poured into 9 cm diameter Petri dishes @ 15ml per plate. There were 5 replicates for each treatment. After solidification, a 5mm diameter inoculum disc from an actively growing 7-days old culture of *F. solani* was placed in the center of each plate. The plates were incubated at room temperature (25-30^{0}C) and the diameter of fungal colonies recorded after each 24 hrs till the plates in any treatment were filled by the fungal growth. Microscopic slides were prepared from each treatment to see the effect of salts on conidia and chlamydospore production. All the experiments were repeated at least once.

Results

Effect on radial growth: Use of sea salt up to10,000 ppm concentration showed significant positive effect on *in vitro* growth of *F. solani*. In treatments where salt was used @ 100,000, growth was initiated very late and progressed very slowly (Fig. 1). In the

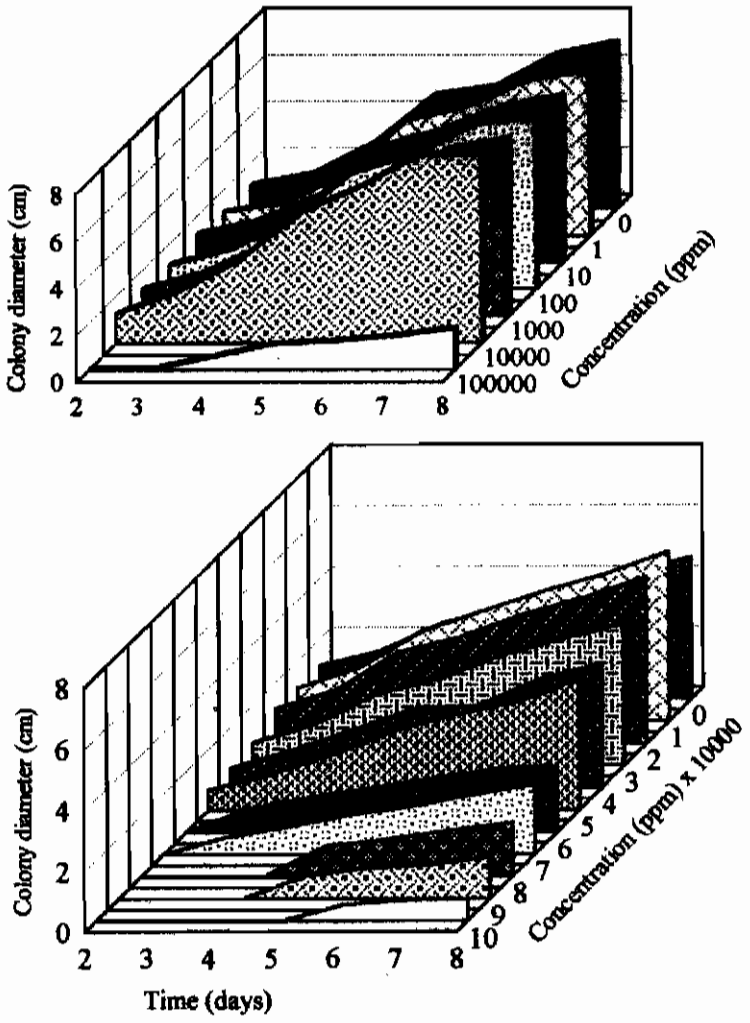

Fig. 1. Effect of sea salt on *in vitro* growth of *Fusarium solani*.

next experiments, where the concentration of sea salt was used @ 10,000 to 100,000 ppm, growth of *F. solani* was significantly greater than the control in treatments containing salt @ 10,000, 20,000 and 30,000 ppm. The growth was highest at 10,000 ppm and declined thereafter. A significant suppression in growth as compared to control was observed when salt was used @ 40,000 ppm or more (Fig. 1).

Effect of different salts found in sea salt on *in vitro* growth of *F. solani* is illustrated in Fig. 2. Use of NaCl @ 10,000 and 20,000 ppm showed enhanced growth of the fungus as compared to control. Growth of the fungus declined gradually with increasing concentration of NaCl from 30,000 to 100,000 ppm. Amendment of PSA with $CaCl_2$ showed a gradual decline in growth of *F. solani* with increasing conc. of $CaCl_2$ with no

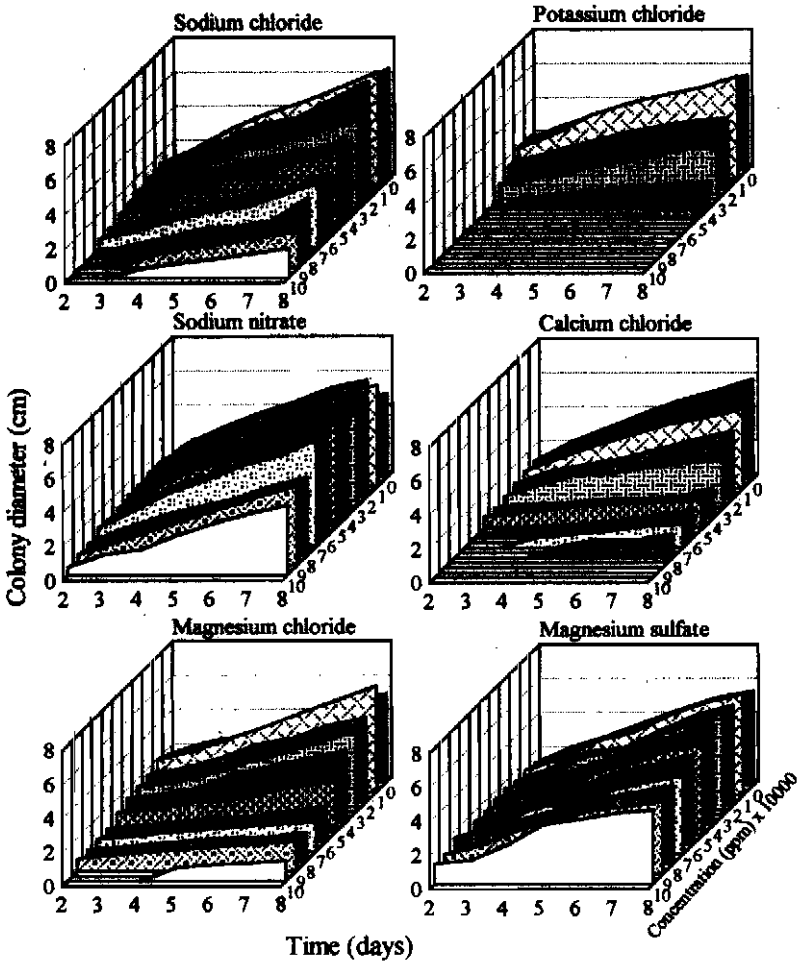

Fig. 2. Growth of *Fusarium solani* on media amended with different salts.

growth of the fungus where the salt was used @ 80,000 ppm or more. Use of $NaNO_3$ @ 10,000 to 70,000 ppm showed significant increase in growth as compared to control with a massive cottony growth of the fungus in $NaNO_3$ amended as compared to non-amended plates. The highest growth was observed in 20,000 ppm, and there was no significant suppression in growth as compared to control even when $NaNO_3$ was used @ 100,000 ppm. Where $MgSO_4$ was used, the growth of *F.solani* increased significantly with the increasing concentration of salt up to 40,000 ppm but declined thereafter. However, the fungus was still able to grow nicely even at the highest salt concentration. Growth of *F. solani* in $MgCl_2$ amended PSA was highest in 10,000 ppm treatment as compared to control which gradually declined with the increasing concentration of $MgCl_2$ with significant suppression in growth when the salt was used @ 50,000 ppm or more. In $MgCl_2$ @ 100,000 ppm treatment, the fungus started to grow after 5th day of inoculation and progressed very slowly. Similarly, amendment of PSA with KCl showed slight

increase in growth in 10,000 ppm treatment which gradually declined thereafter with no growth on media containing KCl salt @ 60,000 ppm or more. In 50,000 ppm treatment the growth started 7 days after inoculation and was negligible at the end of the experiment.

Effect on conidial size: Different salts showed different effects on the size of conidia (Table 2). Length of conidia was significantly affected when sea salt was used @ 60,000 ppm or more. No conidia were produced in 100,000 ppm treatment. However, length of conidia was maximum where NaCl was used @ 60,000 and 70,000 ppm and than decreased gradually. Conidia were formed even in salt @100,000 ppm treatment. There was no significant effect of $CaCl_2$ on the size of conidia where $CaCl_2$ was used up to 70,000 ppm, however, no conidia were produced in 80,000 ppm or more $CaCl_2$ concentration. Similarly, the size of conidia was not much affected in different concentrations of $NaNO_3$, however, the maximum size of conidia was observed where $NaNO_3$ was used @ 30,000 and 40,000 ppm. No significant effect of $MgSO_4$ on the size of conidia was observed in any concentration used. It was interesting to note that the size of conidia reduced with the increasing concentration of $MgCl_2$ with significant reduction when the salt was used @ 90,000 ppm or more. Similarly, the use of KCl also showed gradual decline in size of conidia up to 50,000 ppm salt treatment as compared to control. No conidia were produced where KCl was used @ 60,000 ppm or more.

Effect on chlamydospore production: No chlamydospores were produced in the control, sea salt , NaCl, $CaCl_2$, $MgSO_4$, $MgCl_2$ and KCl after 8 days of incubation. The chlamydospores were only observed in media containing $NaNO_3$ @ 10,000 ppm to 40,000 ppm treatments.

Discussion

In the present study, growth of *F. solani* was enhanced where sea salt was used @ 20,000 ppm. Such similar reports have been made where salinity favoured the *Cercospora* leaf spot of peanut (Porter & Adamson, 1993) and chlamydospore germination of *F. oxysporum* f.sp. *vasinfectum* (Ragazzi & Vecchio, 1992). Peach trees infected by *Leucostroma* when treated with NaCl suffered winter-die-back of canopy shoots and greatly reduced fruit yield percentage (Northover, 1987). Similarly, NaCl treatment predisposed the citrus root tissues to infection by *Phytophthora citrophthora* (Sulistyowati, 1993), whereas, the hyphae of *Bipolaris sorokiniana* [*Cochliobolus sativus*] branched profusely while growing on a medium containing NaCl (Suryanarayanan & Janarthanam, 1985). It has also been reported that most species of *Penicillium* and *Aspergillus* were able to grow in the presence of 20% or more of NaCl, whereas, most Basidiomycetes were unable to withstand more than 2% NaCl (Tresner & Hayes, 1971).

However, our results contradict the reports made by Ragazzai & Vecchio (1992) where NaCl was used for the control of *Gibberella fujikuroi* and *F. oxysporum*, the causal agents of root rot of *Asparagus*. Similarly, suppression in *Asparagus* crown and root rot caused by *F. oxysporum* and *F. proliferatum* by the application of sodium chloride

Table 2. Size of conidia of *Fusarium solani* in different salt treatments.

Concentration (%)	Size of conidia (µm)							
	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
	Sea salt		NaCl		$CaCl_2$		$NaNO_3$	
0	17.27(7.5 - 25)*	3.275(2.5 - 5)	11.56(5 - 22.5)	2.9(2.75 - 3)	11.75 (4 - 22.5)	3.34 (3 - 3.75)	10 (5 - 17.5)	2.85 (2.75 - 3)
1	15 (7.5 - 25)	4.2(3 - 5)	14.2(5 - 22.5)	3.30(3.5 - 5)	12.6 (5 - 22.5)	3..29 (2.75 - 4)	8.94 (3 - 17.5)	2.91(2.75 - 3.25)
2	15.03(2.75- 30)	3.875(2.75 - 5)	12.8(5 - 20)	3.17 (2.75 - 5)	12.72 (5 - 20)	3.34(2.75 - 3.75)	10.3 (5 - 20)	2.96(2.75 - 3.25)
3	14.68 (5 - 22.5)	3.03(2.5 - 3.75)	13.86(5 - 22.5)	3.79(3 - 5)	14.3 (5 - 25)	3.34(2.75 - 3.75)	17.7 (5 - 25)	3.4 (3 - 4.75)
4	14.15(5 - 22.5)	4.16(3.75 - 5)	12.3(5.62 - 22.5)	4 (3.25 - 4.25)	10.17 (4.25 - 15)	3.88 (3 - 4.5)	17.7(5 - 25)	3.27(2.75 - 3.75)
5	14.71(3 - 25)	3.60(3 - 4.5)	15.8(4.5 - 25)	3 .55(3 - 3.75)	9.85 (4 - 20)	3.22(2.75 - 3.5)	10.43(4.5 - 17.5)	2.97 (2.75 - 3.5)
6	11.6(3 - 25)	3.5(3 - 4.5)	15.95(5 - 27.5)	3.43 (3 - 4.5)	10.17 (4.25 - 20)	2.77 (3 - 3.75)	8.2 (5 - 12.5)	2.85 (2.75 - 3)
7	11.33(3 - 20)	3.25 (3 - 3.75)	17.08 (5 -27)	3.16 (3 - 4)	11.07 (3 - 17.5)	3.2 (3 - 3.5)	10.37 (4.5 - 15)	2.85 (2.75 - 3.25)
8	11.6(3 - 17.5)	2.95(2.75 - 3)	18.58 (5 - 25.5)	3.11 (2.5 - 3.25)	0	0	8.97 (3.75 - 15)	2.95 (3 - 3.75)
9	13.33(5 - 20)	3.36(3 - 4.5)	11.57 (5 - 22.5)	2.95 (2.75 - 3.25)	0	0	9.44 (3.75 - 15)	3.30 (3 - 3.75)
10	0	0	9.75 (5 - 17.5)	3.07 (2.75 - 3.75)	0	0	8.75 (3.75 - 15)	3 (2.5 - 3.75)
	$MgSO_4$		$MgCl_2$		KCl			
0	11.56 (7.5 -17.5)	2.90 (3.25 - 3.75)	12.39 (5 - 25)	2.85 (2.5 - 3.25)	15.75 (5 - 25)	3 (2.5 - 3.5)		
1	10.3 (4.5 - 17.5)	2.35 (2.5 - 3)	12.7 (5 - 22.5)	2.66 (2.25 - 3.5)	13.25 (7.5 - 25)	3 (2.5 - 3.35)		
2	10.6 (5 - 17.5)	2.75 (2.25 - 3.25)	11.67 (5 - 17.5)	2.83 (2.25 - 3)	11.5 (5 - 22.5)	3.02 (2.5 - 3.25)		
3	11.38 (5 - 20)	2.49 (2.25 - 3.25)	11.66 (5 - 17.5)	2.83 (2.25 - 3)	11.87 (5 - 20)	2.92 (2.75 - 3.25)		
4	9.77 (5 - 12.5)	2.47 (2.25 - 3)	10.45 (5 - 15)	2.70 (2.25 3)	10.3 (5 - 20)	2.75 (2.25 - 3)		
5	9.61 (5 - 17.5)	2.53 (2.25 - 3)	11.75 (5 - 17.5)	2.72 (2.5 - 3.25)	13.3 (5 - 20)	2.52 (2.25 - 2.5)		
6	9.6 (5 - 20)	2.37 (2.25 - 2.5)	10.1 (5 - 15)	2.75 (2.5 - 3.25)	0	0		
7	10.55 (5 - 17.5)	1.55 (2.25 - 2.75)	10.3 (5 - 10)	2.75 (2.5 - 3)	0	0		
8	8.07 (3.25 - 17.5)	2.49 (2.25 - 2.75)	10.4 (5 - 10)	2.26 (2.5 - 2.75)	0	0		
9	9.56 (3 - 20)	2.40 (2.25 - 2.75)	6.87 (5 - 10)	2.87 (2.5 - 3)	0	0		
10	10.8 (5 - 17.5)	2.38 (2.25 - 2.75)	6.80 (4.5 - 10)	2.27 (2.25 - 3)	0	0		

* = Mean (maximum - minimum)

(Elmer, 1990, 1992) and a gradual suppression in the incidence of the green-ear disease of *Pennisetum typhoides* caused by *Sclerospora graminicola* as a result of pre-sowing seed treatment (Hedge & Karande, 1978) with increasing concentration of sodium chloride are not in agreement with our results. It could be attributed to variable salt tolerance by different fungi as discussed above.

Tabak & Cooke (1968) reported that nitrogen stimulates the formation of thick cottony white colony of *F.solani* which corroborates well with our results where use of $NaNO_3$ showed similar effects. Such similar results have been reported by de Assis *et al.*, (1997) where NO_3 was found to be a good source of nitrogen for *F. solani*. Use of $MgSO_4$ provided maximum growth by *F. solani* during the present studies. The role of Mg^{++} in activating the enzyme system is well known that could be attained to such effects. It has been reported that the growth of *Aspergillus niger* was proportional to the magnesium content of the medium provided as sulphate at about 0.001 M (24 mg/l). In contrast *Phycomyces blakesleanus* tolerated as high as 820 mg/l, however, *Penicillium glaucum*, *Botrytis cinerea* and *Alternaria tenuis* were not able to grow without magnesium (Cochrane, 1958).

It has been reported that K and Mg promote the growth of fungi (de Assis *et al.*, 1997). Our results showed that salts where chloride was present e.g., KCl, $CaCl_2$, $MgCl_2$ suppressed the growth of *F. solani*, whereas the salts without chloride i.e., $MgSO_4$ and $NaNO_3$ enhanced the growth. It would appear that the toxicity of chloride suppressed the favourable effects of these ions during the present study. These results support the findings of Windels *et al.*, (1991) and Goos *et al.*, (1989) who reported that field experiments with chloride fertilizers (KCl and $CaCl_2$) sometimes reduced common root rot severity, but mostly resulted in little effect on yield, lower plant nitrate and protein concentrations, and increased plant weight.

The results of the present study would suggest that *F. solani* is capable of surviving under highly saline conditions and its growth is promoted by various salts. The levels of salt concentration supporting the growth of *F. solani* are much higher than those tolerated by most of the crop plants. It would appear that in saline soils, the fungus could be one of the factors limiting the survival of crop plants. Salinity may also decrease the efficacy of any control measure adopted for the control of *F. solani* in saline soil that should be looked into.

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