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EFFECT OF SEA SALT ON *IN VITRO* GROWTH OF *SCLEROTINIA SCLEROTIORUM*

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Abstract

Use of sea salt @ 1, 10, 100, 1000 and 10,000 ppm in potato sucrose agar did not show any significant suppression or promotion in growth of *Sclerotinia sclerotiorum*. In treatment where sea salt was used @ 100,000 ppm, the growth of the fungus started after 7 days, progressed slowly and filled the Petri dish after 15 days but no sclerotia were produced. Number of sclerotia and mean sclerotial weight increased with increase in concentration of salt in the medium. Sclerotial viability in control and sea salt @ 1, 10, 100, 1000 and 10,000 ppm treatments was found to be 100%.

Introduction

Soil salinity is one of the important factors that affect agricultural production of Pakistan where more than six million hectares are affected (Anon., 1998). Besides affecting the plant growth, soil salinity also affects soil microorganisms and can therefore enhance or suppress the infection of plant by these microorganisms. It has been reported that *Cercospora* leaf spot of peanut was favored by soil salinity (Porter & Adamson, 1993). Similarly, NaCl treatment predisposed the citrus root tissues to infection by *Phytophthora citrophthora* (Sulistyowati, 1993). Salinity enhanced the growth and chalamydospore germination of *Fusarium oxysporum* f.sp. *vasinfectum* (Ragazzi & Vecchio, 1992), whereas, the hyphae of *Bipolaris sorokiniana* [*Cochliobolus sativus*] branched profusely on a medium containing NaCl as compared to control (Suryanarayanan & Janarthanam, 1985). A significant promotion in growth of *Fusarium solani* by sea salt and NaCl was also observed (Firdous & Shahzad, 2001).

The present paper describes the effect of sea salt on *in vitro* growth of *Sclerotinia sclerotiorum* that is known to attack a large number of plant species in different parts of the world (Domsch *et al*., 1980). In Pakistan, it is reported to cause wilt of flax (Mirza & Ilyas, 1984), safflower (Mirza *et al.*, 1995), head rot of sunflower (Mirza & Yasmin, 1984; Bhutta *et al*., 1995), foot rot of wheat (Kishwar *et al*., 1992).

Materials and Methods

The culture of *Sclerotinia sclerotiorum* used in this study was obtained from Karachi University Culture Collection (KUCC). Food poison method (Nene & Thapliyal, 1979) was used to see the effect of sea salt on *in vitro* growth of *S. sclerotiorum* where oven dried sea salt was used @ 0, 1, 10, 100, 1000, 10,000 and 100,000 ppm. 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) using an EC meter (Hanna HI8733). Three g agar was then added in each flask containing 150ml of PSB with or without salt. The potato sucrose agar (PSA) media were sterilized at 15 p.s.i for 20 minutes and poured into 9cm diam., Petri dishes @ 15ml per plate. Antibiotics viz., penicillin (@100,000 units L^{-1}) and streptomycin (@ 0.2 g L^{-1}) were added to the medium just before pouring to avoid bacterial contamination. A 5mm diam. inoculum disc was placed in the centre of each Petri dish. There were 10 replicates for each treatment.

Table 1. Electroconductivity (EC) of sea salt solutions prepared in potato sucrose broth.

Salt Concentration (ppm)	0	1	10	100	1000	10000	100000
Electroconductivity(ms/cm)	1.70	1.66	1.63	1.86	3.67	21.0	151.7

In another comparable set, mature sclerotia of *S. sclerotiorum* were used instead of mycelial disc as the source of inoculum. The sclerotia were surface sterilized with 5% commercial liquid bleach (sodium hypochlorite) and washed thoroughly with sterilized distilled water before plating it in the centre of each plate. The plates were incubated at room temperature (24-28°C) and the diameter of fungal colonies was recorded after 24 h till the plates in any treatment were filled by the fungal growth. Number of sclerotia and mean sclerotial weight in each treatment were also recorded. The viability of sclerotia was determined by plating 30 sclerotia in PSA plates (10 scl. plate^{-1}) and incubating at room temperature for 5 days.

Results

Effect on radial growth: There was no significant difference in the growth of the fungus from mycelium discs in treatments containing salt @ 1-10,000ppm as compared to control and the plates were filled within 4 days (Fig. 1,2). In treatments containing salt @ 100,000 ppm, *S. sclerotiorum* started to grow after 7 days, progressed slowly and filled the plate after 15 days (Fig. 1). In treatments where sclerotia were used as inoculum, growth started after 2 days and plates were filled with fungal mycelium within 5 days with no significant difference between the treatments containing salt upto 10,000 ppm (Fig. 2). Plates containing salt @100,000 ppm showed a delay in sclerotial germination where growth started after 6 days and the plates were filled after 15 days.

Effect on sclerotia production: There was an increase in the number of sclerotia produced in almost all the salt concentrations as compared to control. Generally, the number of sclerotia was greater in cultures grown from mycelial disc as compared to the cultures grown from the sclerotia. No sclerotia were produced in treatment containing salt @ 100,000 ppm (Fig. 3).

Effect on weight of sclerotia: Mean sclerotial weight also increased in all the salt concentration as compared to control with highest sclerotial weight in treatments containing salt @ 1000 and 10,000 ppm (Fig. 4). Viability of sclerotia produced in different treatments was found to be 100% when plated on PSA medium.

Discussion

There are reports where salinity favoured the growth of *Cercospora* leaf spot of peanut (Porter & Adamson, 1993), chlamydospore germination of *F. oxysporum* f.sp. *vasinfectum* (Ragazzi & Vecchio, 1992). Similarly, the growth of *Fusarium solani* was promoted by sea salt and NaCl (Firdous & Shahzad, 2001), whereas, the hyphae of *Bipolaris sorokiniana* [*Cochliobolus sativus*] branched profusely when grown in a medium containing NaCl (Suryanarayanan & Janarthanam, 1985). The application of NaCl on peach trees infected with *Leucostroma* promoted winter-die-back of canaopy shoots and thus greatly reduced the fruit yield percentage (Northover, 1987). It is also

Fig.1. Growth of *Sclerotinia sclerotiorum* in different sea salt treatments.
From right: top row: Control, Salt @ 1, 10 and 100 ppm.
Bottom row: Salt @ 1000, 10,000 and 100,000 ppm.

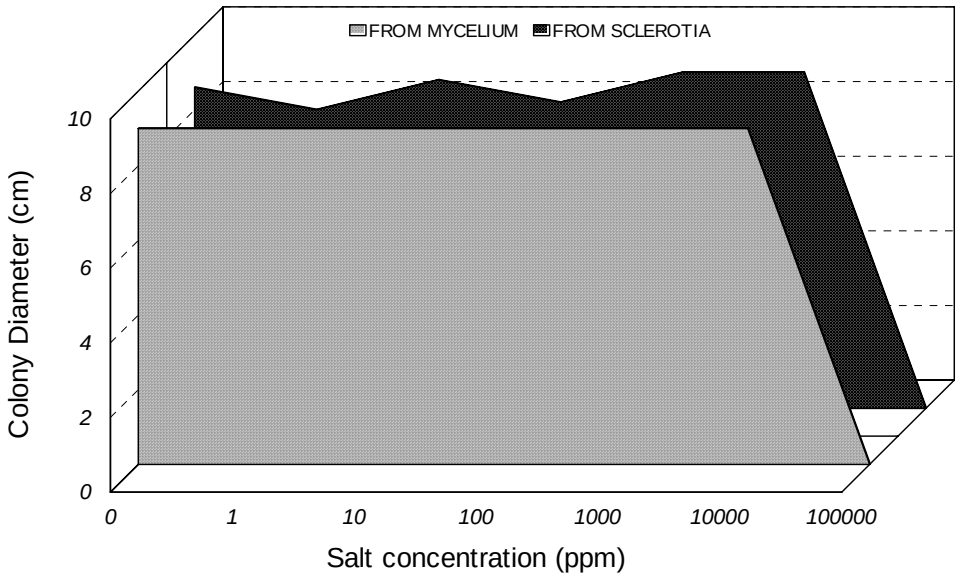

Fig. 2. Growth of *Sclerotinia sclerotiorum* in different sea salt treatments after 4 days of incubation.
*= Growth in sea salt @ 100,000 treatment started after 7 days.

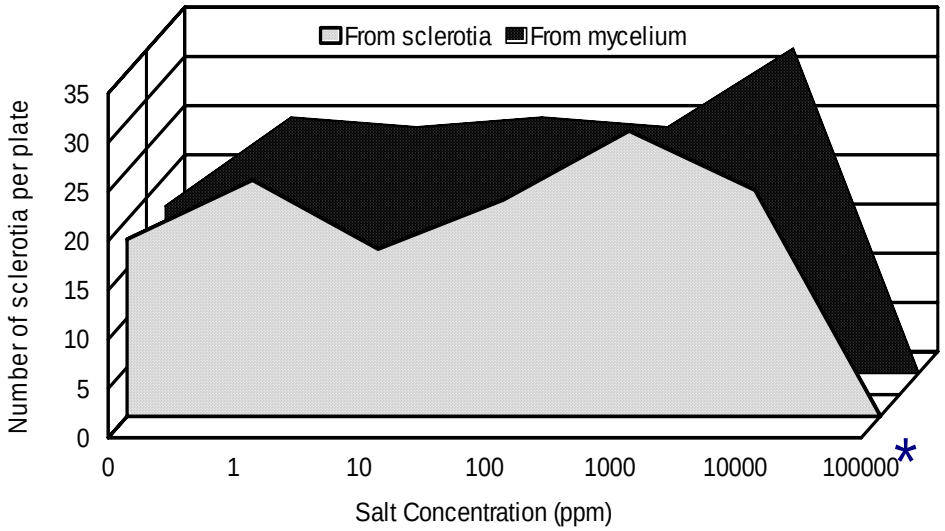

Fig. 3. Number of sclerotia produced by *Sclerotinia sclerotiorum* in different sea salt treatments after 15 days of incubation.

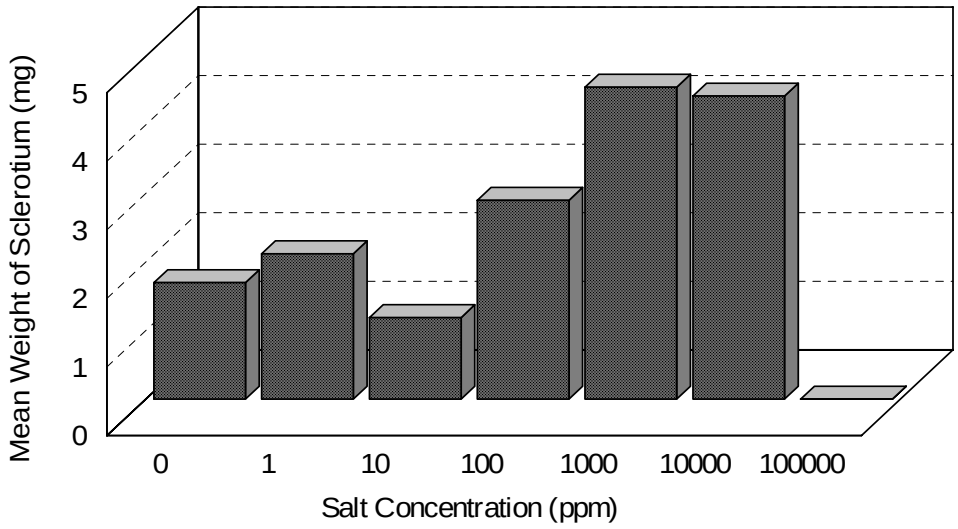

Fig. 4. Mean sclerotial weight in different sea salt treatments.

reported that NaCl predisposed the citrus roots to infection by *Phytophthora citrophthora* (Sulistyowati, 1993). Although, during the present investigation, growth of *S. sclerotiorum* was not promoted by sea salt as compared to control, but there was no adverse effect of salt on growth of *S. sclerotiorum*. It would suggest that the fungus can tolerate a high level of salt that could suppress the growth of almost all the crop plants. Even if the salinity level is within the tolerance limit of the host, the stress exerted by salt on the host could increase the adverse effect of the pathogen that is not affected by the salt. Greater number of sclerotia of much higher size, as reflected by increased sclerotial weight, could also support the survival of the pathogen under saline condition.

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). A 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). However, the results of the present studies would suggest that salt application in *S. sclerotiorum* infested soils should be done cautiously since any over-treatment would affect the host and can predispose it to infection by *S. sclerotiorum.* Effect of salt on pathogenisity of *S. sclerotiorum* needs elucidation.

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