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# Effect of salinity on biology of Saprolegnia ferax and Pythium ultimum

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*Saprolegnia ferax* and *Pythium ultimum* were cultivated on the leaves of *Bromus catharicus*. Different concentrations of NaCl were used in the medium. *S. ferax* appears to be more tolerant to salinity than *P. ultimum* in CMC-ase production and the ability for growth.

## INTRODUCTION

The effect of varying solute and water potential on occurrence, growth, sporulation, morphological characters, sexual or asexual reproduction and distribution of phytopathogenic zoosporic fungi *Saprolegnia*, *Achlya*, *Allomyces*, *Dictyuchus* and *Pythium* sp. have been well reviewed (Willoughby, 1962; Bremer, 1976; Duniway, 1979; Misra, 1982; El-Hissy, Khallil, 1989; Smith et al., 1990; Hassan, Fadl-Allah, 1991). Little attention, however, has been paid to the subtle interaction of growth and enzyme production under salt stress of both *Saprolegnia* sp. and *Pythium* sp. Hassan et al. (1990) reported that *Pythium graminicola* has a phytotoxic effects on the biochemical activities of unicellular green algae. El-Feki (1987) recorded that *Saprolegnia* caused a harmful effect to the fish tissues.

The present study was designed to investigate the effect of salinity on growth, osmotic pressure, organic acid content and CMC-ase production of *Saprolegnia ferax* and *Pythium ultimum*.

## MATERIALS AND METHODS

*Saprolegnia ferax* (Gruith) Thuret and *Pythium ultimum* Trow. Trow were isolated from water streams collected from El-Minia Governorate using the baiting techniuque described by Willoughby (1961) and Sparrow (1968). Bleached bromegrasse (*Bromus catharicus*) leaves were used as baits for isolating and cultivating these zoosporic fungi. According to Miller (1967) $M_3$ medium was used for growing *S. ferax* and *P. ultimum* at 21°C for 5 days then maintained on the same medium at 5°C and subcultured every one month.

The effect of salinity on growth in biomass was determined by colony day weight measurement. Erlenmeyer flasks (250 ml) contained 50 ml of sterile medium of the following composition: chlorophyll free bromegrasse leaves. 10 gm.; asparagine, 1.77 gm.; $K_2PO_4$ 1.0 gm.; $MgSO_4$ $7H_2O$ 0.5 gm. and $FeSO_4$ 0.01 gm/l with appropriate concentrations of NaCL (0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 %).

Flasks containing 50 ml of sterile medium were inoculated with two mycelial disks (0.7 cm. diam.) of each fungus obtained from the margin of 5 days old colonies grown on $M_3$ medium, and then incubated for 14 days at 21°C. Subsequently fungal mycelium and remaining cellulose (bromegrass leaves) were removed by filtrating through 3 layers of cheesecloth and the filtrates were assayed for electric conductivity using CM 25 WPA type Conductivity Mater. Osmotic pressures were determined by cryoscopic freezing point method (Walter, 1949). CMC-ase enzyme production was detected using viscometric technique (Abdel-Razik, 1970) and by reducing groups (Nelson, 1944; Somogyi, 1952). The changes in the total organic acid content of the nutrient media were due to the activity of the species at different NaCl concentration through incubation period at 21°C. This was determined from the difference in the (H) ion concentration according to ($H^+$) Logarithm x $10^{-7}$ M formula before and after incubation (Mert, Ekmekci, 1987). Mycelium was rinsed with distilled water, dried overnight at 90°C and the dry weight was determined. All experiments werte conducted in duplicate and repeated twice. The mean and standard errors were determined according to Mather (1972).

## RESULTS AND DISCUSSION

The vegetative growth and production of CMC-ase of *S. ferax* and *P. ultimum* in the incubation medium with different concentrations of NaCl were illustrated in Table 1. *S. ferax* appears to be more tolerant to salinity compared with *P. ultimum* in the vegetative growth and CMC-ase production. The vegetative growth of *P. ultimum* decreased with the rise in NaCl concentrations however, the growth of *S. ferax* slightly decreased at 21°C. No vegetative growth was observed in salinities exceeding 1.5 % in *P. ultimum*. These results were in accordance with those obtained

by H o h n k (1953) who reported that vegetative growth of *Pythiaceae* is not severely impaired by salinity stresses. The isolate of *P. imperfectum* was shown to grow within salinity range from 0.7 to 2.5 %. On the other hand. E l - H i s s y and K h a l l i l (1989) reported that no vegetative growth was observed in *S. ferax* in salinities exceeding 1.2 % at 16and 21°C. Our results revealed that growth was observed at tested NaCl con-centrations (0.3-1.8 %) at 21°C and this could be attributed to the type of used medium and strain of *S. ferax*.

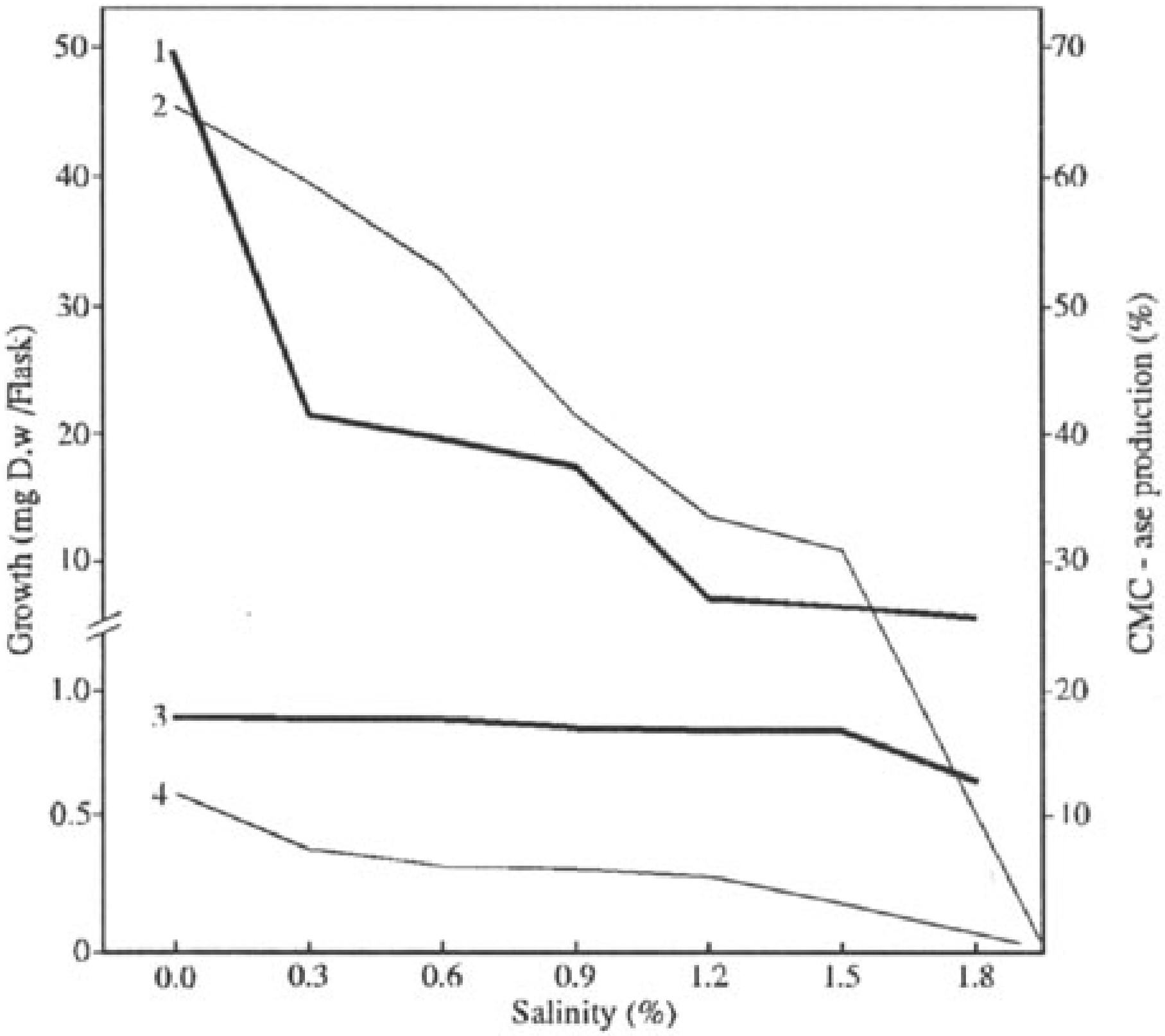
T a b l e 1

The correlations between salinity, osmotic pressure, mycelial dry weight and CMC-ase production in *Saprolegnia ferax* and *Pythium ultimum* after incubation at 21°C for 14 days

| Salinity % | *Saprolegnia ferax* | | | | | *Pythium ultimum* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | EC mhos x $x 10^{-3}$ | OP atm. | growth (mg Dw/f) | CMC-ase production | | EC mhos x $x 10^{-3}$ | OP atm. | growth (mg Dw/f) | CMC-ase production | |
| | | | | A | B | | | | A | B |
| control | 1.70 | 6.25 | 0.93 | 12.1 | 0.70 | 2.40 | 0.93 | 48.6 | 64.4 | 0.16 |
| 0.3 | 6.85 | 3.71 | 0.86 | 6.7 | 0.04 | 6.70 | 3.09 | 23.5 | 58.4 | 0.14 |
| 0.6 | 0.12 | 4.45 | 0.88 | 5.9 | 0.03 | 0.14 | 4.21 | 19.9 | 55.3 | 0.11 |
| 0.9 | 0.17 | 7.41 | 0.85 | 5.0 | 0.01 | 0.21 | 8.90 | 17.4 | 41.3 | 0.09 |
| 1.2 | 0.22 | 10.01 | 0.83 | 4.6 | 0.00 | 0.20 | 10.38 | 4.6 | 35.4 | 0.06 |
| 1.5 | 0.26 | 12.36 | 0.78 | 3.1 | 0.00 | 0.29 | 12.36 | 2.5 | 30.0 | 0.05 |
| 1.8 | 0.33 | 10.01 | 0.64 | 0.0 | 0.00 | 0.40 | 16.91 | 0.0 | 0.0 | 0.00 |

In the filtrates of *S. ferax* and *P. ultimum* (Fig. 1), the production of CMC-ase was inhibited with the rise of NaCl concentrations in the incubation medium. Also, osmotic pressure of filtrates of both *S. ferax* and *P. ultimum* increased with the rise in NaCl (Table 1). The two extreme factors, salinity and osmotic pressure of the incubation medium affected the vegetative growth and enzyme production of these zoosporic fungi. Electric conductivity of the filtrates was stimulated at low concentration of NaCl (0.3 %), were as high concentrations exerted inhibition compared to the control.

After 14 days of incubation at 21°C in the liquid incubation medium the total organic acid content were in the as well as the changes in the $H^+$ ion concentrations of NaCl and osmotic pressures were similar in the incubation medium of the two fungal species *S. ferax* and *P. ultimum*; the total organic acid content decreased compared to the control. In the control experiment, the total organic acid content of filtrates of tested species changes in the opposite direction, under low and high osmotic pressure of incubation medium (Tab. 2). Our results were in accordance with the findings of M e r t and E k m e k c i (1987) in case of *A. flavus*. The lack of an increase in the total organic acid content of the two fungal species can be attributed to the reduction in the growth rate particularly in *P. ultimum*.




*Pythium*: 1 – growth, 2 – CMC-ase; *Saprolegnia*: 3 – growth, 4 – CMC-ase

Fig. 1. The correlation between salinity, growth and CMC-ase production of *Pythium ultimum* and *Saprolegnia ferax* at 21°C after 14 days

Table 2

Variations of ($H^+$) in *Saprolegnia ferax* and *Pythium ultimum* in the medium with different salinity and osmotic pressures after incubation for 14 days at 21°C

| Salinity % | *Saprolegnia ferax* | | *Pythium ultimum* | |
|---|---|---|---|---|
| | OP atm. | ($H^+$) ($\times 10^{-7}$) | OP atm. | ($H^+$) ($\times 10^{-7}$) |
| control | 0.25 | -12.50 | 9.03 | -12.53 |
| 0.3 | 3.71 | -39.70 | 3.09 | -39.69 |
| 0.6 | 4.45 | -34.65 | 4.21 | -39.68 |
| 0.9 | 7.41 | -39.65 | 8.90 | -39.73 |
| 1.2 | 10.01 | -39.65 | 10.90 | -39.69 |
| 1.5 | 12.36 | -39.00 | 12.36 | -34.80 |
| 1.8 | 10.01 | -39.30 | 16.91 | -39.71 |

The present results revealed that sodium chloride salt could be osmotically toxic, but the effect of these synergistic factors my differ depending on the fungal species to another.

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