ACTA MYCOLOGICA
Vol. 29 (2): 245-251
1994

Cellulolytic activity of some cellulose-decomposing fungi in salinized soils

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B a d r a n R. A. M.: *Cellulolytic activity of some cellulose-decomposing fungi in salinized soils.* Acta Mycol. 29 (2): 245-251, 1994.

Maximum evolution of CO_2 was marked in control soil inoculated by tested fungi but its rate decreased with the increasing salinity. The period of 10 days was most suitable for cellulose degradation by *A. niger* and *P. chrysogenum* and 15 days by *A. flavus* and *C. globosum* in control soil. High salinity levels affected greatly the cellulolytic activities of tested fungi. Carbon content of saline soils increased white the nitrogen content decreased.

INTRODUCTION

Soil salinity is the major problem in agriculture call over the world. Many methods including both chemical and organic matter amendments have been recommended for soil reclamations (H u s s a i n, 1969; M a l i k et al. 1979; Z a h r a n, 1991).

In Pakistan, S a n d h u and M a l i k (1975) proposed a plant succession scheme starting with a salt-tolerant grass and ending up with an economic crop to overcome the salinized soils. In this scheme, a salt-tolerant grass, *Diplachne fusca*, is used as the primary colonizer which is followed by relatively less salt-tolerant legume, *Sesbania aculeata*. Green manuring of both these plants and their subsequent decomposition anables the release of CO_2 which helps in the solubilization of $CaCO_3$ already present in the soils (M a l i k, S a n d h u, 1973 a, b).

Advantages of the reclamation procedure as outlined above are that, in addition to the release of CO_2, the stable organic matter fraction, so vital for the fertility of the soil, may be increased and soil structure improved.

In Egypt we have more locations (East bank of Kena Govern.) where the area of saline soil is increasing and becoming a threat to plant productivity. The Egyptian mycoflora of saline soils, including in deserts and along the coasts, have been surveyed (A b d e l - F a t t a h et al., 1977; A b d e l - H a f e z, 1991), but there is no

available information on the decomposition and humification occurring in these habitats.

The object of the present investigation is to study the role of individual cellulolytic fungi in the decomposition of plant residues (sugar cane straw) buried in artificially saline soils.

MATERIALS AND METHODS

Chemical analysis. The soil used in this study was a sandy-clay loam which had been tested for cultivation of sugar cane. Its composition was as follows: 60 % (ww.) sand, 21 % clay and 17 % silt. The organic carbon was 1.2 mg/100 g and nitrogen – 0.4 mg/100 g. The soil was artificially salinized by adding a mixture of Na_2SO_4, $CaCl_2$ and NaCl in a ratio 10 : 5.2 (by wt) to obtain salinities of electric conductivity (EC) 1.0, 1.5 and 2.0 ($Ohms^{-1}$).

Incubation of soil. The soil was prepared for incubation by passing it through a 2-mm sieve. Portions of 100 g of the soil were placed in 250-ml flasks, mixed throughtly with 5 g powdered plant material of sugar cane straw and brought to 60 % water-holding capacity. The plant material had 57.5 mg C/100 g and 2.7 mg N/100g. The flasks were closed with rubber stoppers fitted with a glass rod having a small cup at its end. These cups contained 5 ml of 0.5 M NaOH. For sterilized treatments, the flasks containing soil and plant mixture were autoclaved for 1 h at 15 Psi and 120°C as described previously by Malik, Bhatti and Kauser (1979).

The following species of fungi, *Aspergilllus flavus* Link, *A. niger* v. Tieghm, *Chaetomium globosum* Kunze: Fr. and *Penicillium chrysogenum* Thom which had been isolated previously from saline soils were selected for this study. Fungal growth for inoculation was prepared under sterile conditions as mentioned previously by Malik, Bhatti and Kauser (1979).

Fungal inoculum was prepared by growth the fungi in Petri dishes containing 20 g acid-washed sand which was moistened with 10 ml of Eggins and Pugh's cellulose medium (1962) having the following composition: KH_2PO_4 – 1.0 g, $(NH_4)_2SO_4$ – 0.5 g, KCl – 0.5 g, yeast extract – 0.5 g, $CaCl_2$ – 0.1 g, $MgSO_4 \cdot 7H_2O$ – 0.2 g, cellulose – 10 g and 1 litre distiled water. The pH was 6.7. Fungi were allowed to grow for 7 days on the sand medium at 28°C. At the end of the incubation period, the sand culture was mixed and 2 g were added to the sterilized soil and mixed throughly.

Duplicate flasks were kept for each fungus. Similar flasks amended with 5 g powdered sugar cane straw were kept as controls. They were incubated at 30°C for 30 days. Every five days, CO_2 evolved and cellulase activity were determined as described by Malik, Bhatti and Kauser (1979), at the end of the incubation period (30 days), the organic carbon and nitrogen contents were determined.

Determination of carbon and nitrogen content. The soil was air dried and ground to pass through a 0.2 mm sieve after 30 days. A portion

(20 g) of this soil was extracted with 200 ml of a mixture of 0.1 N NaOH and 0.1 M Na-pyrophosphate in a conical flask for 1 h on a reciprocal shaker. The flasks were left overnight and the supernatant was then separated by centrofugation. The residue after alkali extraction is termed as humin. A 100 ml portion of the supernatant was acidified to pH 2.0 with concentrated H_2SO_4, kept in oven at 90°C for 30 min. and then left overnight. It was centrifuged to separate the precipitate. The supernatant is termed as fulvic acid whereas the dark-coloured precipitate is humic acid. The precipitate was redissolved in 0.1 N NaOH to make the volume to 50 ml. A 20 ml sample of this humic acid solution was taken for N estimation by Kjeldahl method. Another 20 ml were taken for carbon estimation. Organic carbon in humic acid, fulvic acid and humin was estimated by adding 8 ml H_2SO_4 and 5 ml 2 N $K_2Cr_2O_7$ to 20 ml of humic or fulvic acid fraction and 1 g air dried and powdered humin fraction, keeping the reaction in ice bath. The mixture was kept in oven at 110°C for 1.5 h along with a blank prepared similarly. The volume was made to 50 ml with distilled water. Absorbance was noted at 590 nm. Absorbance of a standard containing 5 mg C (glucose) treated as before was also noted.

The carbon content in the samples was calculated by comparing with the absorbance of the standard.

RESULTS AND DISCUSSION

After 5 days of incubation CO_2 evolution was estimated in both control and salinized soil. A rapid increase of CO_2 evolution occurred in the control. Maximum evolution of CO_2 was recorded in the control inoculated with *C. globosum*. On the other hand, CO_2 evolution decreased slightly in salinized soil and it was more pronounced in soil with EC 2.0 (Fig. 1). Malik, Bhatti and Kauser (1979) showed that the increasing salinity had an inhibitory effect on CO_2 production in soil.

The results obtained indicated that the control had the maximum production of cellulase between 5 and 15 days (Fig. 1). Cellulase activity decreased sharply with the increasing salinity levels of treated soils (Fig. 2) Mandels and Reese (1965), Malik, Bhatti and Kauser (1979) showed that in addition to the inhibitory effect of salinity, the exhausting of substrate and production of glucose in sufficient equantities result in the inhibition of cellulase.

It is evident that, the carbon content increased and was more pronounced in soil amended with high salinity level at EC 1.5 and EC 2.0 (Table 1). However, the nitrogen content decreased slightly. On the other hand the soil treated wits *A. flavus* (control) showed a remarkable amount of nitrogen (3.4 mg/100 g of soil). These results may be due to the retardation of CO_2 evolution, therefore the added sugar cane straw might accumulate and thus increase the organic carbon. Moreover, cellulolytic activities occurred even at high salinity levels and the continous liberation of glucose or reducing sugars may also result in the increase of the organic carbon in salinized soil. The nitrogen content in the treated soil decreased and this may be attributed to the exhaustion of nitrogen during the formation of the fungal cells and

to the loss of nitrogen from soil in the form of ammonia. M a l i k, B h a t t i and K a u s e r (1979) showed that salinized soil inoculated with C. globosum produced maximum carbon and nitrogen contents. There is evidence that fungi can synthesize polyphenols which polymerize to form organic acids (M a r t i n, H a i d e r, 1967). Previously much has been written regarding fungal tolerance of high salt environments especially by marine organisms (K a n w a r, B h u m b l a, 1969). Similar studies have also been conducted by K u l i c (1968); but there have been no reports regarding the effect of salts on the activities of fungi in soil. However, some authors (L a u r a, 1973; E l - S h a k e e r et al., 1976) have studied the effect of salt on the decomposition of organic matter in soil and observed a decrease in the rate of decomposition of organic matter due to salts.

The results reported here indicate that increasing salinity has an inhibitory effect on CO_2 evolution and cellulase activity. However, this effect varied in different fungi and the degree of soil salinization. On the other hand the organic carbon content increased in high salt-treated soil whereas the nitrogen content decreased.

T a b l e 1

Organic carbon and nitrogen contents per 100 mg dry of control and salinized soil inoculated with different fungal species for 30 days

Fungi and treatments	O. C.	N
Aspergillus flavus		
C	5.5	3.4
$S_{1.0}$	94.1	2.0
$S_{1.5}$	102.5	1.5
$S_{2.0}$	102.0	0.5
Aspergillus niger		
C	2.5	2.5
$S_{1.0}$	72.2	2.0
$S_{1.5}$	111.5	2.0
$S_{2.0}$	111.0	0.5
Chaetomium globosum		
C	14.4	2.1
$S_{1.0}$	113.5	1.5
$S_{1.5}$	113.0	1.0
$S_{2.0}$	121.5	1.0
Penicillium chrysogenum		
C	4.2	1.5
$S_{1.0}$	122.8	1.0
$S_{1.5}$	132.5	0.8
$S_{2.0}$	140.0	0.2

C — The control; S — 1.0 – Salinized soil with EC 1.0, S — 1.5 – Salinized soil with EC 1.5, S — 2.0 – Salinized soil with EC 2.0; O. C. — Organic carbon content (mg\100 g of soil; N — Nitrogen content (mg\100 g of soil).

Fig. 1. Evolution of CO_2 from control and salinized soil inoculated with four types of fungi at different salinity levels amended with 5 % material of sugar cane straw
1 – the control; Salinized soil with: 2 – $EC_{1.0}$, 3 – $EC_{1.5}$, 4 – $EC_{2.0}$

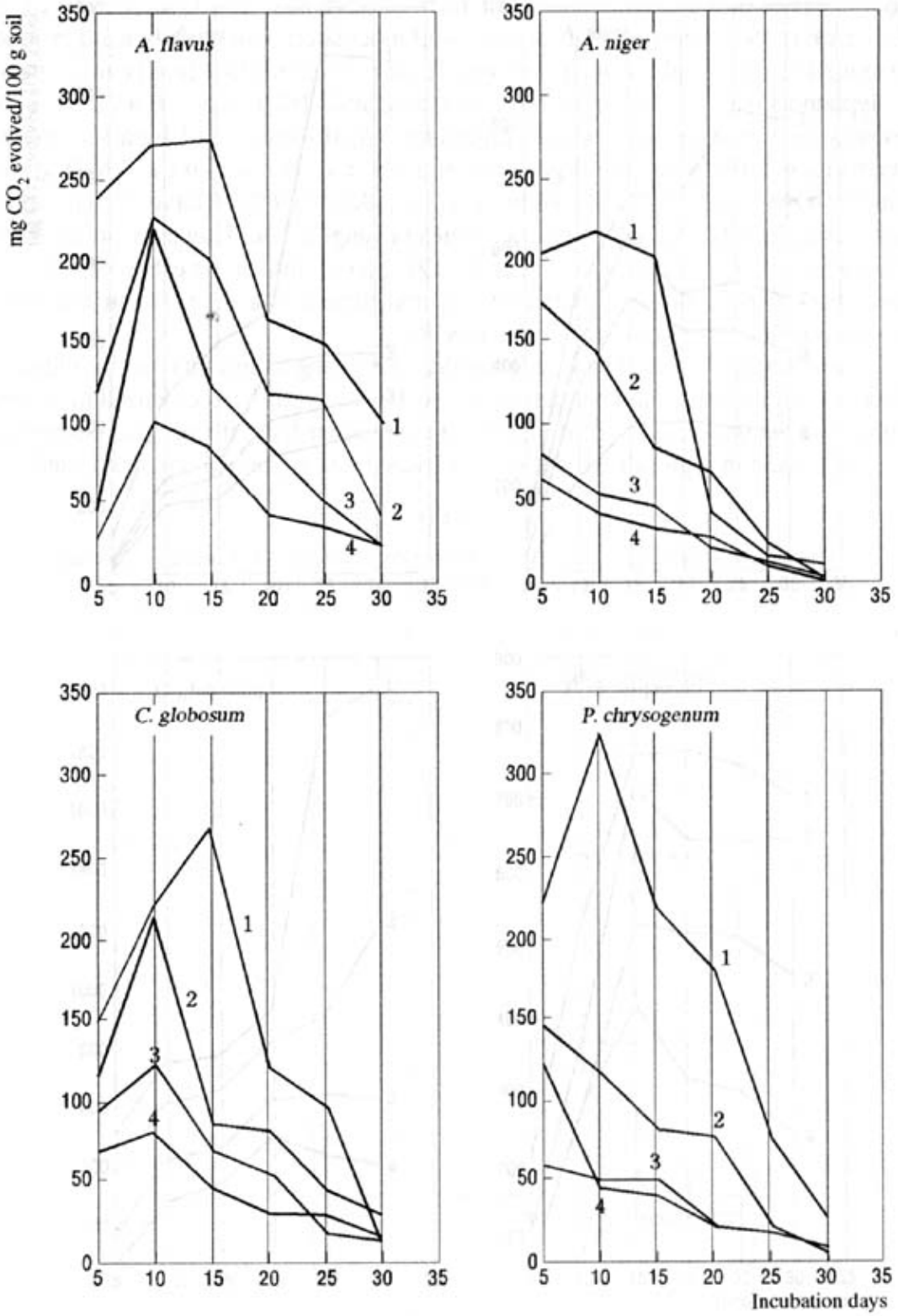

Fig. 2. Cellulase activity from control and salinized soil inoculated with four types of fungi at different salinity levels amended with 5 % material of sugar cane straw
1 – the control; Salinized soil with: 2 – $EC_{1.0}$, 3 – $EC_{1.5}$, 4 – $EC_{2.0}$

REFERENCES

Abdel-Fattah H.M., Moubasher A.H., Abdel-Hafez S.I.I. 1977. Studies on mycoflora of salt marshes in Egypt. I. Mycopathologia 61: 19-26.

Abdel-Hafez S.I.I., 1991. Soil Fungi of mangrove at Egyptian red sea coast and Glycerol accumulation by four species. Bull. Fac. Sci. Assiut Univ. 20 (2-D): 137-160.

Eggins H.O.W., Pugh G.J.F., 1962. Isolation of cellulose decomposing fungi from the soil. Nature 193: 94-95. London.

El-Shakweer M.H.A., Gomah A.M., Barkat M.A., Abdel-Ghaffar A.S., 1976. Effects of alkali salts on decomposition of plant residues. pp. 204-212. [In:] Proceed. intern. symp. on soil organic matter stud. Braunschweig, Sept. 6-10. Vol. I. IAEA, Vienna.

Haider H., Martin J.P., 1967. Synthesis and transformation of phenolic compounds by *Epicoccum nigrum* in relation to humic acid formation. Soil. Sci. Amer. Proc. 31: 766-772.

Hussain M., 1969. Reclamation of saline and alkali soils in West Pakistan. Directorate Land reclamation, West Pakistan, Lahore. Res. Pull. 11: 1-19.

Kulic M.M., 1968. Osmophilic strains of some *Aspergillus* species. Mycologia 60: 961-964.

Laura R.D., 1973. Effect of sodium carbonate on carbon and nitrogen mineralization of organic matter to soil. Geoderma 9: 15-26.

Malik K.A., Bhatti N.A., Kauser F., 1979. Effect of soil salinity on decomposition and humification of organic matter by some cellulolytic fungi. Mycologia 71: 811-820.

Malik K.A., Sandhu G.R., 1973 a. Decomposition of organic matter in saline soil by fungi. Mycopath. Mycol. Appl. 50: 339-357.

Malik K.A., Sandhu G.R., 1973 b. Some studies on the fungi of Kallar-grass *Diplachne fusca* compost. Pakistan J. Bot. 5: 57-64.

Mendels M., Reese E.T., 1965. Inhibition of cellulases. Annual. Rev. Phytopat. 3: 85-101.

Martin J.P., Richard S.J., Haider K., 1967. Properties and decomposition and binding acting in soil of humic acid synthesized by *Epicoccum nigrum*. Soil Sci. Soc. Amer. Proc. 31: 657-662.

Sandhu G.R., Malik K.A., 1975. Plant succession. A key to the utilization of saline soils. Nucleus 12: 35-38.

Zahran H.H., 1991. Conditions for successful *Rhizobium* – legume symbiosis in saline environments. Biol. Fertil. Soils 12: 73-80.