J. Indian bot. Soc., **65**, 95—100, 1986

EFFECT OF TANNINS ON THE GROWTH OF *CHAETOMIUM CUPREUM*[1]

S. N. SIVASWAMY AND A. MAHADEVAN

Centre of Advanced Study in Botany, University of Madras, Madras

ABSTRACT

Growth of *Chaetomium cupreum* was maximum at pH 3, but in presence of tannins maximum growth occurred at pH 4 and above. Myrobalan tannin promoted growth at pH 5. Gallic acid at 15 mM, tannic acid, 1 mM, wattle tannin, 0.3% and 12 mM catechin were most effective in favouring growth. With glucose, the tannins enhanced mycelial growth of the fungus. In presence of tannins, urea and sodium nitrate at 35 mM induced profuse growth. Toxicity of urea and sodium nitrate declined in presence of tannins. *C. cupreum* accumulated protein in wattle tannin medium. Protein level declined in medium containing urea and sodium nitrate.

INTRODUCTION

Tannins are recalcitrant molecules and resist microbial attack. Condensed tannins are more resistant than hydrolysable tannins (Haslam, 1981) and are toxic to a variety of microorganisms. However, a few fungi slowly degrade tannins (Mahadevan, *et al.*, 1982a), but in presence of additional carbon or nitrogen source, tannins are readily oxidized (Sivaswamy, 1982 ; Mahadevan *et al.*, 1982b). The additional carbon or nitrogen source induces copious production of tannase (Nishira, 1962 ; Ganga *et al.*, 1977).

By using enrichment techniques, we isolated *Chaetomium cupreum* from rice field soils which utilized tannin derivatives. In this paper, we report on the effect of model tannin substances, carbon and nitrogen sources on the growth of *C. cupreum*.

MATERIALS AND METHODS

C. cupreum was cultured on Czapek's-Dox medium replacing sucrose with glucose. Solutions of wattle and myrobalan tannins, catechin, gallic acid and tannic acid (first dissolved in 2-3 mL of alcohol) were prepared in water, sterilized through millipore filter, pore size 0.45 μ separately and aseptically added to the sterilized medium to get the desired final concentration.

C. cupreum was inoculated into 100 mL. medium using 8 mm mycelial disc obtained from Czapek's-Dox plates. After 15 day incubation at room temperature (29C), the mycelium was filtered and dry weight was measured.

To estimate mycelial protein, the mycalium was macerated with equal volume (w/v) of Tris-HCl buffer, pH 3.0, in presence of acid washed sand. The ground

1. Accepted for publication on November 30, 1984.

Financial assistance from the Indian Council of Agricultural Research, New Delhi is gratefully acknowledged.

mycelium was centrifuged for 20 min at 3,000 g. The supernatant was collected and diluted twice. One mL of mycelial extract was added to 2 mL of 10% TCA. After 30 min, the solution was centrifuged for 20 min at 3,000 g. The pellet was dissolved in 1.5 mL of M/10 NaOH solution. Protein was measured in 1.5 mL sample using Lowry's method (Lowry *et al.*, 1951). Standards prepared from bovine serum albumin were used to prepare a standard curve.

RESULTS

pH influencing tannin activity :

Growth of *C. cupreum* in medium was influenced by pH. At pH 3, the growth of *C. cupreum* was maximum but not in presence of tannins (Table III). Although tannins were inhibitory at pH 3, growth improved at higher pH. Gallic acid was inhibitory at both pH 3 and 7.

Tannin concentration and mycelial growth :

Gallic acid at 5 to 20 mM enhanced the growth of *C. cupreum* : 15 mM being most effective. Tannic acid was markedly inhibitory to growth (Table II). Wattle tannin promoted growth. Catechin up to 12 mM promoted mycelial growth and at 16 mM, reduced the growth.

Effect of tannins with glucose :

The fungus utilized glucose up to a concn of 416.7 mM (Table III). With gallic acid, glucose at 166.7 mM induced maximum growth of *C. cupreum* but higher concn of glucose reduced it. Glucose along with tannic acid improved fungal growth and 250 mM was most effective. With wattle tannin, glucose, 250 mM favoured substantial growth. Catechin and glucose enhanced the growth of *C. cupreum* which increased up to 416.7 mM.

Tannin with nitrogen sources on C. cupreum :

Sodium nitrate promoted the growth of *C. cupreum* up to a concentration of 150 mM, beyond which it was toxic. Urea was toxic even at 70 mM. Surprisingly, toxicity of urea with sodium nitrate declined (Table IV). Only at 150 mM, were the combined nitrogen sources toxic to *C. cupreum*. With gallic acid, sodium nitrate at 150 mM favoured maximum growth. Sodium nitrate and urea in

TABLE I

EFFECT OF pH ON THE GROWTH OF *C. cupreum*

pH	Control	Catechin (12 mM)	Wattle tannin (0.3%)	Myrobalan tannin (0.3%)	Gallic acid (15 mM)
		Dry weight of mycelium (mg)			
3	303	123	125	57	91
4	297	703	538	89	413
5	190	556	395	544	424
6	217	733	350	277	335
7	179	508	365	280	132

TABLE II

EFFECT OF TANNINS ON *C. cupreum*

Gallic acid		Tannic acid		Wattle tannin		Catechin	
Concn (mM)	Dry weight (mg)	Concn (mM)	Dry weight (mg)	Concn (%)	Dry weight (mg)	Concn (mM)	Dry weight (mg)
Control	205	Control	223	Control	187	Control	116
5	280	1	144	0.2	294	4	166
10	326	2	102	0.3	378	8	154
15	445	4	124	0.4	375	12	288
20	214	6	106	0.5	332	16	253

TABLE III

EFFECT OF GLUCOSE AND TANNINS ON *C. cupreum*

Concn of glucose (mM)	Control	Gallic acid (15 mM)	Tannic acid (1 mM)	Wattle tannin (0.3%)	Catechin (12 mM)
		Dry weight of mycelium (mg)			
83.3	109	249	136	226	277
166.7	149	303	142	457	372
250.0	199	231	200	651	413
333.3	217	154	166	648	445
416.7	203	220	197	Not tested	554

presence of gallic acid induced profuse growth at 35 mM but higher concentrations reduced it (Table IV).

With tannic acid, the optimum concentration of sodium nitrate for maximum growth was 35 mM. It was inhibitory at higher concentration. Urea at 70 mM with tannic acid caused 5 fold increase in growth However, urea and sodium nitrate together inhibited growth except at 35 mM.

Growth of *C. cupreum* increased markedly with increasing concn of sodium nitrate and 0.3% wattle tannin. Urea at 35 mM promoted growth but was inhibitory at higher concentrations. Urea and sodium nitrate at 35 mM in presence of wattle tannin increased the growth of *C. cupreum* and higher concentration was toxic to growth. In catechin medium containing sodium nitrate of 35 mM, growth of the fungus was maximum and in higher concentration of nitrate, it decreased. Growth improved by 56% in 35 mM urea and catechin medium. Similarly, in 35 mM urea and sodium

TABLE IV

EFFECT OF SODIUM NITRATE, UREA AND TANNINS ON *C. cupreum*

Concn (mM)	Treatment	Control	Gallic acid (15 mM)	Tannic acid (1 mM)	Wattle tannin (0.3%)	Catechin (12 mM)
		Dry weight of mycelium (mg)				
35	$NaNO_3$	149	228	236	559	326
	Urea	128	80	299	145	218
	$NaNO_3$ + Urea	149	294	293	246	192
70	$NaNO_3$	155	222	189	516	290
	Urea	88	77	513	78	86
	$NaNO_3$ + Urea	126	216	146	81	120
100	$NaNO_3$	134	154	141	531	178
	Urea	93	34	175	92	68
	$NaNO_3$ + Urea	106	161	146	57	91
150	$NaNO_3$	195	419	128	591	181
	Urea	88	109	166	67	89
	$NaNO_3$ + Urea	70	86	123	42	85
200	$NaNO_3$	157	322	94	—	169
	Urea	80	72	71	53	86
	$NaNO_3$ + Urea	77	103	100	81	110

TABLE V

MYCELIAL PROTEIN OF *C. CUPREUM*

Treatment	Control	Test
	(mg. dry tissue^{-1})	
A. WATTLE TANNIN (0.3%)		
Medium	2.10	11.40
Medium + Sodium nitrate, 150 mM	4.40	8.10
Medium — Sodium nitrate + Urea, 35 mM	0.30	6.10
Medium + Urea + Sodium nitrate	—	0.93
Medium + Glucose (4.5%)	—	16.90
B. CATECHIN (12 mM)		
Medium	12.60	1.50
Medium — Sodium nitrate + Urea	0.38	9.90
Medium — Urea Sodium nitrate	0.32	6.74

Protein estimated in 15 day old mycelium

nitrate and catechin, growth of the fungus was more than in higher concentrations.

Mycelial protein of C. cupreum

C. cupreum accumulated protein in medium containing wattle tannin and 4.5% of glucose ; the increase was 48% (Table V). But addition of sodium nitrate and urea substantially reduced the protein level. In contrast, protein level declined when grown in catechin medium. Growing *C. cupreum* in urea and catechin medium markedly increased the protein level in mycelium by 4.5 fold.

DISCUSSION

A variety of microorganisms occur in rice field soils which utilize tannins as carbon sources (Sivaswamy, 1982).

C. cupreum isolated from such a soil utilized tannins. Concentration is, however, critical. Myrobalan and wattle tannins reduced the growth of *C. cupreum* at concentrations higher than 0.4%. Generally, phenols differ in their penetrating capacity and structure of the molecule profoundly alters the penetrating capacity of the molecule (Mahadevan, 1982). At reduced concentration, tannins may enter the cellular system and are metabolized. But at higher concentrations they exert a toxic action, primarily by forming stable complexes with proteins. By tanning action several enzymes are inhibited by tannins (Mahadevan, 1982). Further, tannins exert a non-specific denaturing action on the cell walls.

pH influences the activity of tannins. At pH 3, growth of *C. cupreum* improved, but at this pH, tannins were toxic. Toxicity of catechin, wattle tannin, myrobalan tannin and gallic acid declined with an increase in pH. pH of the medium significantly influences the oxidation-reduction potential of the molecule. Tannins penetrate cells as neutral molecules (Mahadevan, 1982).

Sambandam (1983) reported that catechin 1, 2-dioxygenase secreted by *C. cupreum* was active in a pH range of 2 to 10 ; optima being 2 to 3 and 7 to 8. Admittedly, at a particular pH, tannase is induced rapidly and the fungus effectively degrades and utilizes tannin component, which is not readily cleaved at other pH.

At reduced pH, tannins polymerize and form quinones, since several phenols form polymers in acid solution (Mahadevan, 1982). The protein-tannins complexes formed at lower pH ranges are broken readily by the changes in pH leading to regeneration of enzymes. This aspect has an interesting corollary. In an acid soil, the tannins will be more toxic to the microorganisms that degrade them compared in soils with neutral pH. Furthermore, with phenolic acids, it is the undissociated acid form that is most effective (Simon, 1950). Since the pH of many water-logged soils is neutral, these would be fully dissociated and become largely ineffective.

Carbon sources favour rapid production of tannase (Nishira, 1962 ; Ganga *et al.*, 1977) which cleaves tannin, culminating in enhanced growth of the fungus. Addition of glucose to the medium in presence of tannins improved the growth of *C. cupreum*. Certain tannin molecules have no mobility in water. With the introduction of sugar in the 3-position, the mobility of the molecules increases. Removal of the carbon source from the medium and inactivation of extracellular enzymes by tannins may inactivate the organism, which may metabolize the substances present in the milieu.

Nitrogen sources also profoundly influence growth and tannase production (Nishira, 1962). According to Suseela *et al.* (1978), urea and glutamic acid favoured growth and tannase activity. We found that in presence of sodium nitrate, both wattle tannin and catechin markedly increased the growth of *C. cupreum*. In presence of tannins, urea at lower concentration promoted growth but higher concentrations were toxic. Urea forms precipitates with tannins (Simoncini 1961) and increases the binding of tannins with proteins (Gustavson, 1962) reducing the toxicity of tannins to microrganisms. The protein precipitating power of tannins is affected by the structure of molecule. Introduction of hydroxyl groups decreased the toxicity but nitrogroup increased the antimicrobial and protein-binding power of tannins.

REFERENCES

GANGA, P. S., G. SUSEELA, S. C. NANDY AND M. SANTAPPA 1977. Effect of environmental factors on the production of fungal tannase. Pt. II. *Leather Sci.* **25** : 203-209.

GUSTAVSON, K. H. 1962. A note on the effect of addition of urea to the vegetable tanning bath. In : Rajaduari, S. and K. V. Bhanu (eds.) *Seminar on vegetable tannins*. pp. 119-126. Central Leather Research Institute, Madras.

HASLAM, E. 1981. Vegetable tannins. *In :* Preiss, J. (ed.) *The biochemistry of plants*. **7,** Academic Press, New York, pp. 527-556.

LOWRY, P. H., N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193** : 265-275.

MAHADEVAN, A. 1982. *Biochemical aspects of disease resistance I-The prohibitins*. Today & Tomorrow, New Delhi.

MAHADEVAN, A., T. SAMBANDAM AND N. SIVASWAMY 1982a. Microbial degradation of phenolic substances. *Indian Rev. Life Sci.* **2** : 1-18.

MAHADEVAN, A., N. SIVASWAMY AND T. SAMBANDAM 1982b. On the relevance of cometabolism of phenolic substances by plant parasites. *Curr. Trends Life Sci.* **9** : 45-53.

NISHIRA, H. 1962. Some aspects on the metabolic fate of tannic acid. Studies on the tannic acid decomposing enzyme of molds. *Sci. Rep. Hyogo Univ. Agr.* **5** : 117-123.

SAMBANDAM, T. 1983. *Biochemical aspects of microbial degradation of tannin*. Ph.D. Thesis, Univ. of Madras, Madras.

SIMON, E. W. 1950. Effect of pH on the biological activity of weak acids and bases. *Nature* **166** : 343-344.

SIMONCINI, A. 1961. Precipitation of tannin by dialdehyde starch-urea. *J. Am. Leather Chem. Ass.* **57** : 279-280.

SIVASWAMY, S. N. 1982. *Effect of tanliquor and tannins on micro-organisms*. Ph. D. Thesis, Univ. of Madras, Madras.

SUSEELA, G., S. C. NANDY AND M. SANTAPPA 1978. Influence of various carbon, nitrogen and mineral sources on growth and production of fungal tannase by *Aspergillus flavus* and *A. oryzae*. *Leather Sci.* **25** : 227-233.