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Cellulolytic activity of *Sclerotium rolfsii* and the influence of KH_2PO_4 and $MgSO_4$

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Abstract

'Cellulolysis rate' of a pathogenic fungus, *Sclerotium rolfsii,* was studied on filter paper discs. There was positive correlation between the growth of the fungus and weight loss of the filter paper. The weight loss caused by the fungus was influenced by phosphorus and magnesium content of the nutrient solution. It increased with increasing concentration of phosphorus and magnesium of the nutrient solution.

Keywords: cellulolysis rate, *Sclerotium rolfsii,* KH_2PO_4, $MgSO_4$

INTRODUCTION

The pathogens survive in soil in the absence of host either as dormant propagules or as saprophytes in the dead host tissues/crop residues or as parasites in non-crop host or as competitive colonizers of the organic substrates. The fact that the host tissue infected by the pathogen during its parasitic phase serves as its main source of survival was emphasized by many (Baker and Cook, 1974; Cook and Baker, 1983 and Duczek *et al.*, 1999). Survival as saprophytes is influenced by the intrinsic factors such as cellulolytic activity of the organism and physico-chemical factors of the soil. Cellulolysis rate, as expressed by the loss in dry weight of the filter paper, has been recognized as an attribute of cellulolytic activity (Garrett, 1970, 1975; Panneerselvam and Saravanamuthu, 2003). In the present study cellulolysis rate of *Sclerotium rolfsii*, a soil-borne pathogen that causes diseases in groundnut, was studied under the influence of phosphorus and magnesium content of the nutrient solution.

MATERIALS AND METHODS

Determination of cellulolysis rate

The test organism, *S. rolfsii* was isolated from the infected groundnut plants, purified and maintained in PDA medium.

The `cellulolysis rate' of the fungus was studied by determining the percentage loss in dry weight of the filter paper discs as described by Garrett (1980). Whatman No. 1 filter paper discs (90 mm) were placed in each Petri plate and sterilized. For sterilization the circles were saturated with 2 or 3 ml of mineral solution containing $NaNO_3$ (660 mg), KH_2PO_4 (70 mg), $MgSO_4$ (35 mg) and $FeSO_4$ (0.07 mg) per litre of distilled water. Circles (Discs) saturated with 2 ml of mineral water were designated as "Sterilized 1" and the circles (Discs) saturated with 3 ml of mineral solution were designated as "Sterilized 2" Another set was maintained without sterilization. They were then inoculated with four agar blocks (5 mm diameter) cut from the actively growing margin of the fungus. Three replicates were maintained. The plates were incubated at 25 ± 2°C. Mean loss and per cent loss in dry weight of each paper disc were calculated after 21 days.

Effect of magnesium sulphate and phosphorus

The magnesium and phosphorus content of the nutrient solution was altered by adding in the following proportions S/4, S/2, $S_{3/4}$, S, $S_{1.5}$ and $S_{2.0}$. Magnesium content (35 mg/l) and phosphorus (70 mg/l) in the nutrient solution was taken as standards (Garrett, 1983). These nutrient solutions were added separately in Petri plates with sterilized filter paper discs. The fungus was inoculated as described previously, and the growth rate of fungus and loss in dry weight of the filter paper discs were determined.

RESULTS AND DISCUSSION

The cellulolysis rate of the organism varied in relation to phosphorus and magnesium content of the nutrient solution. *Sclerotium rolfsii* showed maximum weight loss of the filter paper discs at $S_{2.0}$ of phosphorus and magnesium (Table 1). Though the pathogen varied in its response to the available phosphorus and magnesium in *in vitro* conditions, the activity in natural soil could be limited by the complex interactions of environmental factors and the microbial antagonism in space and time.

The pathogens survive as saprophytes in soil in the absence of their hosts. The saprophytic behaviour of the pathogen varies depending on the environmental condition and the available nutrients. Besides, cellulolytic ability of the fungus is an important factor

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that influences the prolonged survival in the soil. Cellulolysis rate is one of the important attributes that determines the cellulolytic activity of the organism (Garrett, 1980). Loss in dry weight of the filter paper has often been correlated with the cellulolytic activity of the organism. In the present study it was found that the radial growth rate of the pathogen and the weight loss caused by it were greater in the sterilized filter paper than in the unsterilized ones (Table 2).

Garrett (1975, 1978) suggested that the intrinsic growth rate (growth rate over PD agar) has no apparent effect

Table 1. Colonization of filter paper by *Sclerotium rolfsii* and the influence of phosphorus and magnesium content on the weight losses of discs (Sterilized 1 = treated with 2 ml of mineral solution and Sterilized 2 = 3 ml of mineral solution)

Filter paper	Nutrient solution (ml)	Mean radial growth (mm) after days			Mean loss in dry wt. by filter paper (mg)	Radial growth (mm) rate over PDA 24 h (dia)	% loss in dry wt. of the filter paper	
		2	4	6			(mg)	CAI[1]
Unsterilized	2.0	13.5	16	18.5	18.0	1.5	4.0	0.07
Sterilized 1	2.0	14.0	18	19.0	19.5	1.7	4.6	0.32
Sterilized 2	3.0	15.0	19	20.0	20.0	1.9	5.0	0.34
Effect of phosphorus content								
S/4	2.0	10	14	19	14.5	9.0	4.0	3.30
S/2	2	12	17	20	18.6	11.0	4.6	3.42
$S_{3/4}$	2	15	19	23	20.0	14.2	5.0	4.01
S	2	17	22	26	24.0	17.0	5.7	4.32
$S_{1.5}$	2	20	25	30	25.2	18.0	6.3	4.50
$S_{2.0}$	2	22	26	34	25.9	19.0	7.0	4.90
Effect of magnesium content								
S/4	2.0	8	14	21	27.5	1.55	7.7	4.28
S/2	2.0	11	17	28	30.1	1.60	8.4	4.64
$S_{3/4}$	2.0	13	22	31	45.2	1.65	9.1	4.99
S	2.0	15	26	37	64.3	1.70	9.8	5.30
$S_{1.5}$	2.0	17	30	40	72.6	1.75	1.7	5.75
$S_{2.0}$	2.0	20	36	61	80.0	1.82	1.11	6.04

Table 2 . Growth of *S. rolfsii* over the filter paper discs and the influence of phosphorus and magnesium content on the weight losses of the discs (Sterilized 1 = treated with 2 ml of mineral solution and Sterilized 2 = 3 ml of mineral solution)

Filter paper	Wt. loss by filter paper (mg)	√ WL	Growth rate over PD (cm) 24 h^{-1}	√ WL x growth rate over PD agar	Growth rate of fungus over filter paper
Unsterilized	18.0	4.2	1.5	6.3	14.0
Sterilized 1	19.5	4.4	1.7	7.4	14.5
Sterilized 2	20.0	4.47	1.9	8.3	14.5
Effect of magnesium content					
S/4	27.5	5.24	1.55	8.12	1.58
S/2	80.1	5.48	1.60	8.76	1.62
$S/_{3/4}$	45.2	6.72	1.65	11.08	1.69
S	64.3	8.01	1.70	13.61	1.73
$S_{1.5}$	72.6	8.52	1.75	14.91	1.77
$S_{2.0}$	86.0	8.94	1.80	16.09	1.84
Effect of phosphorus content					
S/4	14.5	3.80	9.0	3.42	10.0
S/2	18.6	4.31	11.0	4.74	10.8
$S/_{3/4}$	20.0	4.47	14.2	6.25	11.2
S	24.0	4.89	17.0	8.31	11.6
$S_{1.5}$	25.2	5.01	18.0	9.01	12.0
$S_{2.0}$	25.9	5.08	19.0	9.6	12.8

[1]Cellulolysis Adequacy Index

S/4 = 165 mgl^{-1}, S/2 = 330 mgl^{-1}, $S_{3/4}$ = 495 mgl^{-1},
S = 660 mgl^{-1}, $S_{1.5}$ = 990 mgl^{-1}, $S_{2.0}$ = 1320 mgl^{-1}.

on cellulolysis rate. He also pointed out that the radial growth of the fungal colony across the paper circle need not be directly correlated with weight loss (WL) of the paper, because the weight must vary with area of the fungal colony and not with its radius (Table 2). Further he suggested that the growth rate over filter paper is controlled by 'cellulolysis` rate as well as the intrinsic radial growth rate of the fungus. The present study suggests that not only growth rate and `cellulolysis rate' of the organism over the substrate but also the available mineral nutrients such as phosphorus, magnesium, etc., could influence their prolonged survival through efficient continued utilization of the substrates such as crop residues.

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Meliola exacigena sp. nov. from Kodaikanal, Tamil Nadu, India

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Abstract

During a survey of the leaf infecting microfungi in Kodaikanal shola forests, we located a black mildew fungus on *Exacum* sp. Microscopic examination of the fungus revealed that it is a hitherto undescribed species of the genus *Meliola*.

Keywords: black mildew, foliicolous fungus, *Meliola,* new species

The leaves of *Exacum* sp. collected by the third author from Mathikettan Shola, Kodaikanal, Tamil Nadu, India, were examined for the occurrence of black mildew fungus, *Meliola* sp. The perusal of the literature proved that *Meliola exaci* Hosag. is the only species reported from *Exacum tetragonum* (Hosagoudar, 1996; Hosagoudar *et al.*, 1997; Hu *et al.*, 1996, 1999; Mibey and Hawkswoth, 1997; Mibey and Cannon, 1999). The present species differs from *M. exaci* by having straight mycelium, entire to angular head cells of the appressoria, longer mycelial setae and larger ascospores :

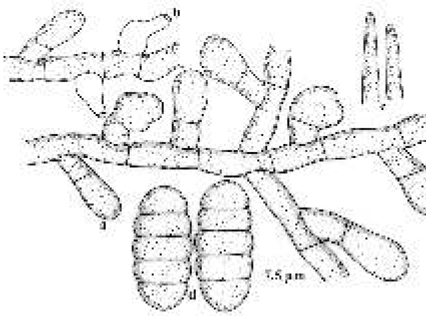

Figure 1. *Meliola exacigena* sp. nov. a, Appressorium; b, Phialide; c, Apical portion of mycelium; d, Ascospores.

Meliola exacigena sp. nov.

Coloniae hypophyllae, densae, ad 2 mm diameter, confluentes. Hyphae rectae vel subrectae, plerumque opposite, saepe alternate acuteque vel laxe ramosae, laxe vel arte reticulatae, cellulae 12-26 x 6-10 µm. Appressoria alternata vel unilateralis, antrorsa, subantrorsa vel retrorsa, recta vel curvula, 16-29 µm longa; cellulae basilares cylindraceae vel cuneatae, 6-10 µm longae; cellulae apicales ovatae, oblongae, integrae vel angularis, 9-19 x 9-16 µm. Phialides appressoriis intermixtae, alternatae, ampulliformes, 12-19 x 6 - 10 µm. Setae myceliales simplices, rectae, ad apicem acutae, ad 530 µm longae. Perithecia dispersa vel aggregata, globosa, ad 235 µm in diameter; ascosporae obovoideae, 4-septae, constrictae ad septatae, 38-42 x 14-18 µm.

Colonies hypophyllous, dense, up to 2 mm in diameter, confluent. Hyphae straight to substraight, branching mostly opposite, alternate at acute to wide angles, loosely to closely reticulate, cells 12-26 x 6-10 µm. Appressoria alternate to unilateral, antrorse, subantrorse to retrorse, straight to curved, 16-29 x 6-10 µm long; stalk cells cylindrical to cuneate, 6-10 µm long; head cells ovate, oblong, entire to angular 9-19 x 9-16 µm. Phialides mixed with appressoria, alternate, ampulliform 12-19 x 6-10 µm. Mycelial setae simple, straight, acute at the tip, up to 530 µm long. Perithecia scattered to grouped, globose, up to 235 µm in diameter; ascospores obovoidal, 4-septate, constricted at the septa, 38-42 x 14-18 µm.

MATERIAL EXAMINED

On leaves of *Exacum* sp. (Gentianaceae), Mathikettan Shola, Kodaikanal, Tamil Nadu, India, July 18, 2006, K. Kandavel and all, HCIO (type), TBGT 2721 (Isotype).

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Industrial pollution on domestic water and the consequent impact on human health

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Abstract

Human health is one of the most important factors in economic development. A healthy workforce is essential for the development of an economy. A healthy workforce requires a healthy environment with clean air and water, and wilderness.Water is being very rapidly depleted, jeopardizing healthy life of local communities.Unsafe water consumption is hazardous to human health. Our survey has found the prevalence of water related health problems due to tannery pollution in a village in Tamil Nadu, India and describes the economic costs of tannery polution on human health.

Keywords: drinking water, human health, per capita, economic cost, pollution

INTRODUCTION

The most pressing environmental problems being faced by the developing countries like India include water and air pollution and soil degradation. Of these, water pollution poses a serious challenge as it has direct impact on a large number of economic activities. "It is estimated that 75 per cent of the world population mostly in developing countries does not have access to safe drinking water. The problems of water pollution acquire greater relevance in the context of an agrarian economy like that of India. Sewage is not the only cause of water pollution; industrial wastes are also significant polluters"(Behera and Reddy, 2002).

Domestic water constitutes a substantial proportion of natural resource endowment. Saving this resource from further depletion is the most formidable challenge of the developing world. Ironically, notwithstanding, water is being very rapidly depleted, jeopardizing the healthy life of local communities as the consumption of scarce and unsafe water has proved to be a factor which deteriorates the very survival of poor.

Water quality and human health are directly related issues. Improved quality and increased quantities of water would bring forth health benefits. Safe water eliminates the infective agents associated with water-borne diseases. Availability of greater quantity of water can improve health by allowing improved personal hygiene. Thus improved water supply shall mitigate the incidence of many water-borne diseases. It is worth to quote the words of Halfdan Mahler, former Director General of the World Health Organization (WHO) "The number of water taps per 1000 persons is better indicator of health than the number of hospital beds" (Sachidananda, 1999).

Human health is one of the most important factors in economic development. A healthy workforce is essential for the development of an economy. A healthy workforce requires a healthy environment, that is, clean air and water and wilderness. Pearce and Warford (1993) have argued that the most important and immediate consequences of environmental degradation in the developing world take the form of damage to human health.

This paper is an attempt in the direction of facing up this challenge. Its principal aim is to understand the problems faced by the local communities in India due to industrial pollution and to suggest certain sustainable measures for the mitigation of these problems. It deals with the status and consumption of unsafe water and its impact on health related problems faced by people in and around leather industries of Erode district, Tamil Nadu, India, in particular to assess the socio-economic and environmental impact on society by the tannery industrial pollution by estimating the loss of opportunity cost. In addition to that, this paper estimates the loss of man-days and health damage cost due to water quality contaminations. Both primary and secondary data were used for this purpose.

MATERIALS AND METHODS

Problems faced by villagers with respect to loss of productivity, employment, income, health and social damage cost vary across villages related to proximity to pollution. A micro level investigation on a case study basis will help to understand the socio- economic and environmental constraints existing in a village.

For the present study, a typical problem village,

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Suriyapalayam, in the Erode District of Tamil Nadu, India, where the impact of pollution is acute due to tannery pollution, was chosen. The multistage sampling method *viz.*, purposive and proportionate random sampling methods, were adopted to select the study village and the households. 75 sample households were chosen. Detailed information regarding the damage costs due to industrial pollution were collected. Information were gathered with the help of a structured questionnaire and also through participatory rural appraisal methods such as informal group discussions with the senior citizens of the village.

The secondary data related to water quality have been collected from the Department of Environmental Science, Bharathiyar University, Coimbatore, India. To estimate the negative externalities on human health due to industrial pollution was evaluated through analyzing cause and effect variables such as water quality parameters, water borne diseases, loss of man-days and health damage cost due to loss of opportunity cost.

RESULTS AND DISCUSSION

The results of the present study clearly indicated that the concentration of salinity both in well water and stand pump water in the study area were high. Furthermore, they had high levels of total dissolved solids, electrical conductivity , Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) (Table 1).

Our survey carried questions on the prevalence of water related health problems due to industrial pollution. Respondents reported to have suffered from water-related diseases like, diarrhea, cholera, typhoid, gastro-enteritis and jaundice. Among the landless households, 46 per cent of adult and 33 per cent of adult females in the study population were found to be affected by water borne diseases (Table 2). It was because of consumption of unsafe water during working hours.

Table 3 describes man-days loss due to waterborne diseases in the study village. This table shows that the per capita man-days loss by the landless is higher (57.87) followed by medium and small farmers, i.e., 57.8 percent, 24.6 percent ,respectively . In the case of

Table 1. Concentrations of various water quality parameters in the water samples of the selected village (Values are compared with ICMR water quality standards)

Parameters[1]	ICMR[2] standard	Well Water				Stand Pump Water	
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
pH (ppm)	7.0-8.5	6.8	6.8	6.6	6.2	7.1	7.0
EC (Mho)	750	4120	3220	4112	5114	3420	4100
TDS (mg/1)	500	3120	2640	2780	3640	3660	2116
TSS (ppm)	100	4240	5632	3660	4240	3660	2127
Alkanity (mg/1)	75	2540	2540	3640	3920	2720	3684
Chloride (mg/1)	200	322	480	536	292	418	492
Sulphate (mg/1)	200	440	450	412	331	452	302
COD (ppm)	250	412	444	962	416	319	411
BOD (ppm)	30	262	314	562	491	490	416

Source: Environmental Science Laboratory Bharathiar University, Coimbatore.

[1]TDS = Total Dissolved Solids;TSS= Total Suspended Salids;

EC = Electrical Conductivity; COD = Chemical Oxygen Demand;

BOD= Biological Oxygen Demand.

[2]Indian Council of Medical Research

Table 2. Percent occurrence of Water-borne diseases in the study area

Farm size	Male Adult	Male Child	Female Adult	Female Child
Landlesslabourers	46.1	0	33.3	0
Marginalfarmers	26.9	100	38.8	50
Small Farmers	23.0	0	22.2	50
Large Farmers	3.8	0	5.5	0
Total	100	100	100	100

Source: Primary survey - 2006.

Table 3. Loss of Man-days owing to Water- borne diseases in the study area

Farm size	No.of man-days lost/year (Male)	No.of man-days lost/year (Female)	Total loss of man - days	Loss of per-capita man-days
Landless Labourers	77.0 (50.3)	170 (75.8)	247 (65.5)	99.25 (57.8)
Marginal Farmers	46.0 (30.0)	23.0 (10.3)	69.00 (18.3)	42.25 (24.6)
Small Farmers	30.0 (19.6)	1.0 (0.4)	31.00 (8.2)	42.25 (24.6)
Large Farmers	0.0 (0)	30.00 (13.4)	30.00 (8.0)	15.0 (8.7)
Total	153 (100)	224.00 (100)	377.00 (100)	171.50 (100)

Source: Primary survey 2006. Values in Parentheses are percentages large farmers only 8.7 per cent were affected. It clearly indicated that those who have contributed as a labourer in agriculture sector are the worst affected. This is in accordance with the general notion that the economically poor people are affected by any environmental disturbance or problem.

more, had reviewed some of the studies conducted in the US to estimate national health costs of polluted water. It was estimated that the unit social cost per case gastroenteritis was $100 and that there were approximately one million cases of gastroenteritis each year in the US.

Table 4. Distribution of Damage cost of tannary pollution in the study area.

Farm size	No.of days taking treatment per year	Per capita Medical Expenditure (Rs./ year)	Per capita Transport Cost (Rs./Year)	per capita loss of opportunity cost for working population (Rs./Year)	Per capita damage cost (Rs./Year)
Landless Labourers					
Mean	16.94	1749.93	68.43	205.83	1974.99
Sum	288.00	29748.81	1163.33	3499.17	33574.82
Marginal Farmers					
Mean	7.03	667.73	138.13	98.62	861.26
Sum	204.00	19364.17	4005.83	2860.00	24976.67
Small Framers					
Mean	5.53	467.82	53.27	16.02	462.18
Sum	144.00	12163.33	1385.00	416.67	12016.67
Large Farmers					
Mean	5.33	174.07	18.52	66.67	314.92
Sum	16.00	522.22	55.56	200.00	944.78
Total					
Mean	8.69	823.98	88.13	93.01	953.51
Sum	652.00	61798.53	6609.72	6975.83	71512.93

Table 4 explains the loss of opportunity cost due to waterborne diseases among the sample respondents.The expenditure on health depends on three factors such as loss of man-days, medical expenses due to water-borne diseases and number of days taken for treatment. The per capita loss of opportunity cost for landless labourers was Rs. 1974 per year followed by medium (Rs. 861/ year), small Rs. (462/year), and large farmers (Rs. 314/ year), respectively. This indicated that loss of income and increase in the expenditure on health care due to the incidence of sickness was higher in the case of landless laborers who involved in the farm activities. This was be cause the landless labourers are prone to sickness caused by the water-borne diseases as they are living in very close proximity to industries.Furthermore,they had a higher number of man-days lost due to sickness and other negative effects of pollution. Similarly, the number of days taken for the treatment for water-borne disease by the land less was the highest (Table 4).

It is obvious from table 5 ,the number of days taken for treatment and loss of opportunity cost are the foremost parameters, which influenced the health damage cost extensively and significantly at the study area.

Similarly (Pearce, 1978) have estimated mortality and morbidity from water borne diseases in Africa, Latin America and Asia and found that waterborne diseases due to water pollution have a definite impact on morbidity and mortality, and ultimately a serious negative impact on economic activities in the form of loss of man-days, death of trained workers, expenditure on hospitalisation, and so on. The above authors, further-

Two million working days are lost in the US each year due to acute gastroenteritis and diarrhoea and based on average wage loss per day, it is estimated that the value of the 1000 deaths due to hepatitis infection per year is around $ 100,000 per life.

Table 5. Regression to predict the health damage cost of tannery industrial pollution at the study area ($R^2 = 0.647 : p < 0.05$)

Name of the variables	Coefficient	t value
Constant	823.04	1.32
No. of days taken for treatment	402.91	5.57*
Per capita loss of opportunity cost(Rs)	2.86	2.3*

* significant at 5% level

CONCLUSION

The definition of pollution in Economics is based not only on the physical effect on the environment but crucially on the human response to the physical effect. For instance, around half of the landless people of the study village are affected due to industrial pollution on domestic water bodies.It is proved through water quality testing, except pH all other parameters were found to be greater than the permissible limits prescribed by ICMR, in the study area. It is evident that the loss of man-days by the landless was significant. In addition to that per capita loss of opportunity cost also was greater for the landless when compared to others. This is because of proximity to industry and also working in agricultural fields where the industrial pollution got mixed.

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