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EFFECT OF NUTRIENTS ON CELLULOLYTIC AND PECTOLYTIC ENZYME PRODUCTION OF *ALTERNARIA ALTERNATA* A LEAF SPOT PATHOGEN OF TEAK (*TECTONA GRANDIS L.F.*)

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ABSTRACT

Alternaria alternata is a potential pathogen of *Tectona grandis* L.f., was isolated from diseased *Tectona grandis* L.f. leaves from Nashik and used for the present study. Pathogen was grown on the Czapek-Dox liquid medium substituting or adding different carbon, nitrogen to study cellulolytic and pectolytic enzyme production and total phenol production. The activity of enzyme was observed on the 8th day of incubation period.

A great extent of growth variation was observed on different carbon, nitrogen. Among the carbon source) the maximum loss in percentage viscosity maximum in lactose and fructose. While minimum in glucose and dextrose as compared to other nitrogen source. From nitrogen source the cellulolytic enzyme activity was maximum in control and cobalt nitrate followed by similar activity in potassium nitrate and nickel nitrate. While minimum cellulolytic enzyme activity was seen in barium nitrate.. Variation was also observe in pectolytic enzyme activity. the cellulolytic enzyme activity was maximum in glucose while minimum in dextrose as compared to lactose, control and fructose.. From nitrogen source the cellulose activity was maximum in potasium nitrate and minimum in cobalt nitrate as compared to nickel nitrate, barium nitrate and control.

Keywords **:** *Alternaria alternata,* cellulolytic and pectolytic enzyme, pathogen.

INTRODUCTION

Teak**,** tall deciduous tree (*Tectona grandis*) of the family Verbenaceae, native to India and Malaysia but now widely cultivated in other tropical areas. Teakwood is moderately hard, easily worked, and extremely durable; beams said to be over 1,000 years old are still functional. The wood contains an essential oil that resists the action of water and prevents the rusting of iron. Teak is superior to all other woods for shipbuilding and is also used for furniture, flooring, and general construction. Several other similar woods from unrelated trees are sometimes also called teak.

This plant affected by many microorganism. Some of them are *Olivea tectonae* (rust stem and leaf); *Phyllactinia guttata* and *Uncinula tectonae* (mildew); *Cercopora tectonae* (leaf spot); *Nectria haematococca* (stem canker); *Corticium salmonicolor* (pink disease); *Pseudomonas tectonae* and *P. tectonae* (wilt); *Armillariella mellea* (root rot); *Pellinus noxius*, *P. lamaoensis*, *Ustulina deusta*, *Polyporus rubidus*, *Ganoderma applanatum* and *R. zonalis* (heart rots); *Cossus cadambae* (trunk borer).

This plant affected by many fungi as leaf spot. symptoms progress from lower to upper leaves. Leaf spots begin as small brown areas. These areas enlarge and are surrounded by a border of yellow host tissue. As the spots mature, concentric rings of raised and depressed brown tissue are evident. Heavily infected plants often become defoliated.

The disease is caused by *Alternaria alternata*. The pathogen has worldwide distribution and infects many types of plants, both cultivated and weeds. Disease development is favored by mild (24 - 29^{o}C), rainy weather although it can develop at higher temperature. Conidia develop lesions on the host. Profuse sporulation occurs when heavy dews or rain are present. These conidia serve as secondary inoculums and are disseminated by wind, running water, insects, field workers and implements. The disease progresses most rapidly when alternating periods of dry and wet weather occur. Many worker reviewed physiology and biochemistry of fungi (Stall, 1958; Rajderkar, 1966; Sharma *et al*., 1985; Sankaran *et al*., 1986; Nair and Sumaridi, 2000; Bhanumathi, 2007; Mantri, 1969; Jayraj and Ramabadran, 1998).

MATERIAL AND METHOD

The material used and methods followed during the present investigations were as follows:

The Czapek-Dox solid and liquid medium was used as a common medium for the studies. The composition of media was $NaNO_3$-2.00g, K_2HPO_4-1.00g, $MgSO_4$,$7H_2O$-0.50g, $FeSO_4$, $7H_2O$-0.01g, Sucrose-30g, Distilled water-1000ml.

Azadirachta indica L. leaves affected with different diseases were collected from different locations of Nashik district. Isolation from these affected leaves was carried out on Czapek-Dox agar medium by usual tissue incubation technique. The Petri plates were incubated at room temperature (22-28^{0}C) until good growth of organism was observed. The colonies free from contamination were transferred on Czapek-Dox agar slant and maintained for further studies. Eight days old culture of organism was used for biochemical studies.

Due to the action of enzymes, the polymer cellulosic substrate is broken down into small molecular compounds which results into the loss in the viscosity. The loss in viscosity is measured by Oswald viscometer. The substrate enzyme mixture is used in the following composition:

1. 0.5% CMC solution 5 ml
2. 0.2M Citrate phosphate buffer at optimum pH 2 ml
3. Culture filtrate 3 ml

10ml substrate-enzyme mixture is taken in clean viscometer and viscosity was measured at different interval of time (0, 15, 30 min.). During the incubation period, the viscometer kept at constant temperature 25^{0}C.

To measure pectolytic activity, Cylindrical plugs, 8 mm in diameter, are cut from healthy potato tubers with a No. 4 cork borer. The plugs are injected with distilled water under vacuum for one hour. Disc of 0.4 mm thickness are cut with sliding hand microtome from these plugs. They are washed quickly with distilled water and stored in a petridish.

Ten discs are placed in five ml of an enzyme solution (culture filtrate) in a watch glass. At interval of 5 min. they are subjected to slight tension by hand or pulled apart. As soon as the first disc has lost coherence, the mean time for loss of coherence in all discs is noted and taken as the reaction time (R.T.) in minute.

$$\text{Mean time} = \frac{\text{Sum of time when discs macerated}}{\text{No of attempts at which disc macerated}}$$

Macerating activity (ME) is expressed as

$$\text{ME} = \frac{1000}{\text{R.T.}}$$

The test are carried out at room temperature and optimum pH.

RESULTS AND DISCUSSION

Alternaria alternate was grown on Czapek-Dox liquid medium and cellulolytic and pectolytic enzyme was recorded.

Loss in percentage viscosity of culture filtrate was calculated after different time interval. After 30 min. there was considerable variation in viscosity which ultimately shows effect on production of cellulolytic enzyme.

The results shows (Table-1) The maximum and similar macerating activity was seen in lactose and fructose followed by control. There was very little difference was found in between glucose and dextrose.

Data in the table-2 indicates that there was large variation in the production of cellulolytic enzyme of *Alternaria alternata* on different nitrogen compounds. The maximum macerating activity is seen in cobalt nitrate followed by potassium nitrate, barium nitrate and control. The minimum macerating activity is seen in nickel nitrate.

The pectolytic enzyme activity was measure on different carbon and nitrogen The result shows (Table-3) Loss of coherence in cotton fiber is tested by calculating macerating activity. The maximum macerating activity in glucose which were followed by lactose, control and fructose. The minimum macerating activity was seen in dextrose.

The five nitrogen compounds studied, Data in the table - 4 indicates that maximum macerating activity was seen in potasium nitrate followed by nickel nitrate, barium nitrate and control while cobalt nitrate shows minimum macerating activity.

CONCLUSION

A great extent of growth variation was observed on carbon, nitrogen. Among the carbon source) the maximum loss in percentage viscosity maximum in lactose and fructose. While minimum in glucose and dextrose as compared to other nitrogen source. From nitrogen source the cellulolytic enzyme activity was maximum in control and cobalt nitrate followed by similar activity in potassium nitrate and nickel nitrate. While minimum cellulolytic enzyme activity was seen in barium nitrate.. Variation was also observe in pectolytic enzyme activity. The cellulolytic enzyme activity was maximum in glucose while minimum in dextrose as compared to lactose, control and fructose. From nitrogen source the cellulose activity was maximum in potasium nitrate and minimum in cobalt nitrate as compared to nickel nitrate, barium nitrate and control.

Table 1 : Determination of cellulolytic enzyme activity by loss of coherence test tissue in filter paper disc of *Alternaria alternata* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period.

No of Disc / Carbon sources	1	2	3	4	5	Total Time	Mean Reaction Time	M. A
Control	14	16	16	16	16	78	15.6	64.10
Dextrose	16	16	18	18	18	86	17.2	58.14
Glucose	16	16	16	18	18	84	16.8	59.52
Lactose	14	14	14	16	16	74	14.8	67.57
Fructose	14	14	14	16	16	74	14.8	67.57

Table 2: Determination of cellulolytic activity by loss of coherence test tissue in filter paper disc of *Alternaria alternata* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period.

No of Disc / Nitrogen Sources	1	2	3	4	5	Total Time	Mean Reaction Time	M. A
Control	14	14	14	16	16	74	14.8	67.57
KNO_3	14	14	16	16	16	76	15.2	65.79
$Ni(No_3)_2$	14	14	16	16	16	76	15.2	65.79
$Co(NO_3)_2$	14	14	14	16	16	74	14.8	67.57
$Ba(NO_3)_2$	14	16	16	16	16	78	15.6	64.10

Table 3: Determination of cellulolytic activity by loss of coherence test tissue in cotton fiber of *Alternaria alternata* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period.

No of fiber / Carbon sources	1	2	3	4	5	Total Time	Mean Reaction Time	M. A
Control	8	10	10	10	12	50	10	100
Dextrose	10	10	10	12	12	54	10.8	92.59
Glucose	8	8	8	8	10	42	8.4	119.05
Lactose	8	8	10	10	10	46	9.2	108.70
Fructose	8	8	10	12	12	50	10	100

Table 4: Determination of cellulolytic activity by loss of coherence test tissue in cotton fiber of *Alternaria alternata* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period.

No of fiber / Nitrogen source	1	2	3	4	5	Total Time	Mean Reaction Time	M. A
Control	10	10	10	12	12	54	10.8	92.59
KNO_3	8	8	8	10	12	46	9.2	108.70
$Ni(No_3)_2$	8	8	10	12	12	50	10	100
$Co(NO_3)_2$	10	10	12	12	12	56	11.2	89.29
$Ba(NO_3)_2$	8	10	10	12	12	52	10.4	96.15

REFERENCES

Stall, R.E. (1958). An Investigation of Nuclear Number in *Alternaria alternata, American Journal of Botany*, *Botanical Society of America*, 45(9): 657-659.

Rajderkar, N.R. (1966). The influence of nitrogen nutrition on growth and sporulation of *Alternaria alternata* (Ell. & Mart.) *Jones & grout, Springer Netherlands*, 29(1-2): 55-58.

Mantri, J.M. (1969). Studies on physiology of *Phytopthora* spp. Ph.D. thesis Dr. B.A. Marathwada university, Aurangabad.

Sharma, J.K.; Mohanan, C. and Maria Florence, E.J. (1985). Disease survey in nurseries and plantations of forest tree species grown in Kerala. Kerala Forest Research Institute, KFRI Research Report No. 36.

Sankaran, K.V.; Balasundaran, M. and Sharma, J.K. (1986). Seedling diseases of Azadirachta indica in Kerala, India, *Forest Pathology*, 16(5-6): 324-328.

Agrios, G.N. (1988). Plant Pathology, 3rd. ed. Academic Press, Inc.: New York. pp.803.

Jayraj, J. and Ramabadran, R. (1998). Effect of certain nitrogenous sources on the *in vitro* growth, sporulation and production of antifungal substances by *Trichoderma haezianum*. *J. Mycology of plant pathology*, 28(1): 23-25.

Nair, K.S.S. and Sumardi (2000). Insect pests and diseases of major plantation species. pp. 15–38 in CIFOR, Bogor.

Bhanumathi, A. and Ravishankar Rai, V. (2007). Leaf blight of *Azadirachta indica* and its management in *African Journal of Agricultural Research,* 2(10): 538 -543.