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"ESTIMATION OF PRODUCTION OF EXTRACELLULAR PHENOL BY CERCOSPORA TECTONAE A LEAF SPOT PATHOGEN OF TEAK (TECTONA GRANDIS L.F.)."

Dr. Abhijeet Bajirao Pawar*
Head of Botany Department and Assistant Professor, *K. K. W Arts, Science and Commerce College, Pimpalgaon B., Dist-Nashik, State - Maharashtra, India.
Email: abhipawar77@rediffmail.com

Abstract: *Cercospora Tectonae* causes leaf spot disease to the teak plant is a potential pathogen of *Teak (Tectona Grandis L.F.)*, was isolated from diseased *Tectona Grandis* leaves from Nashik district 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 Considerable variation in the production of total phenol was observed. When different carbon compounds were incorporated in the medium dextrose shows maximum production of phenol. Nitrogen source compound were favorable for production of phenol. Highest phenol was detected on potassium nitrate followed by nickel nitrate, barium nitrate and control.

Key Words: *Cercospora Tectonae*, Phenol, Teak, Carbon source, Nitrogen source.

1. INTRODUCTION:

Phenolic compounds are widely distributed amongst living organisms, particularly in plants where they play an important role in response to pathogenic agents, having antibacterial and antiparasitic activity, sometimes being highly specific (Del Signore *et al.*, 1997). Their presence has been reported in many species of fungi these compounds have been little studied in the genus *Ganoderma*. It has been reported that polyphenols are the principal antioxidant components in methanol extracts of *G. lucidum* (16.5 to 27.9 mg) and of *G. tsugae* (24.0 to 35.6 mg/g) (Mau *et al.*, 2005).

Teak is easily worked and has natural oils that make it suitable for use in exposed locations, where it is durable even when not treated with oil or varnish. Teak cut from old trees grow slowly in natural forests is more durable and harder, teak from young trees grown in plantations is more prone to splitting and water damage, however drying allows for sustainable, plantation-grown teak to nearly on par with old-growth teak. Leaf spot diseases are caused by *Cercospora* on teak. The symptoms are brown to grayish brown, which develop near the tip and along the margin of the leaves. The disease spreads laterally in the nursery through overlapping foliage of the adjoining seedlings often resulting in group blighting of seedlings. In each case of severe infection, defoliation is high. *Cercospora* leaf spot can, reduce overall plant vigor by repeated defoliation. For big leaf-type, *Cercospora* leaf spot tends to be less severe under shady conditions, but in nursery environments under shady conditions, frequent overhead irrigations can intensify disease activity and subsequent defoliation and loss of vigor.

2. LITERATURE REVIEW:

Efforts have thus been directed towards producing bioactive substances in submerged fermentation, mainly by studying culture conditions and media formulated for producing biomass and metabolites such as polysaccharides and ganoderic acid (Yang and Liau, 1998; Yang *et al.*, 2000; Fang *et al.*, 2002; Tang and Zhong., 2002; Chang *et al.*, 2006; Tang *et al.*, 2010; Zhu *et al.*, 2010). However, there are no reports of studies for producing phenolic compounds in submerged culture.

3. MATERIALS: *Tectona grandis* leaves affected with different diseases were collected from different locations of Nashik district.

4. METHOD: 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.

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.

The culture filtrate was treated with folin-ciocaltean reagent. The blue colour obtained is measured calorimetrically and compared with that of standard obtained by the treatment with catecoal.

Pipette 1ml of culture filtrate into a graduated (25ml) test tube; add 1 ml of folin-ciocaltean reagent followed by 2 ml 20% Na_2CO_3 solution. Shake the tube and heat in boiling water-bath for exactly 1 min. cool in a running tab. Dilute the blue solution to 25 ml with water and measured the O. D. at 650 nm.

Prepare the standard curve with different concentrations of catecoal. Read the unknowns from known curve and calculate the amount of total phenol. (Sadashivan S et.al; 1996)

5. RESULT AND DISCUSSION: The phenolic compounds act as hydrogen donars/acceptors in host parasite interaction during host pathogen interaction (Parihar, 2012).

Production of polyphenols by *Cercospora tectonae*:-

a) **Effect of carbon sources:-** Considerable variation in the production of total phenol was observed (Table-1) when different carbon compounds were incorporated in the medium. Dextrose shows maximum production of phenol, followed by control, lactose and fructose. While glucose show minimum phenol production.

Table1
Production of total phenol (mg/l) in culture filtrate by *Cercospora tectonae* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period.

Carbon sources	Phenol (mg/ l)
Control	2.333
Dextrose	2.599
Glucose	1.399
Lactose	2.199
Fructose	1.865

b) Effect of nitrogen sources:- It was observed (Table - 2) that nitrogen compound were favorable for production of phenol. Highest phenol were detected on potassium nitrate control followed by nickel nitrate, barium nitrate and control. The interesting thing was that compared to other nitrogen sources lowest phenol were detected on cobalt nitrate.

Table – 2
Production of total phenol (mg/l) in culture filtrate by *Cercospora tectonae* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period.

Nitrogen Sources	Phenol (mg/ l)
Control	3.133
KNO_3	4.865
$Ni(No_3)_2$	4.133
$Co(NO_3)_2$	2.466
$Ba(NO_3)_2$	3.399

6. CONCLUSION: Considerable variation in the production of total phenol was observed when different carbon compounds were incorporated in the medium. For *Cercospora tectonae* phenol production the dextrose shows maximum production of phenol, followed by control, lactose and fructose. While glucose show minimum phenol production. When different nitrogen compound were incorporated in the medium. For *Cercospora tectonae* phenol production Highest phenol were detected on potassium nitrate followed by nickel nitrate, barium nitrate and control. The interesting thing was that compared to other nitrogen sources lowest phenol were detected on cobalt nitrate.

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