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Effect of different carbon and nitrogen source on production of extracellular phenol by *Phomopsis viticola* a leaf spot pathogen of grape *(Vitis vinifera)*

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Abstract
Phomopsis viticola causes leaf spot disease to the Grapes plant is a potential pathogen of *Grapes plant (Vitis Vinifera)*, was isolated from diseased *Vitis Vinifera* 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 fructose shows maximum production of phenol, followed by dextrose. Nitrogen source compound were favorable for production of phenol. Highest phenol was detected on nickel nitrate followed by cobalt nitrate, potassium nitrate and barium nitrate.

Keywords: *Phomopsis viticola*, phenol, *Vitis vinifera*, carbon source, nitrogen source

Introduction

Vitis vinifera L is an indigenous to southern Europe and western Asia, and now it is today cultivated worldwide for its high value berries, or grapes. This plant affected by many fungi. Phomopsis cane and leaf spot is a grape fungal disease caused by fungi *Phomopsis viticola*. The Pathogenic fungi cause's spots on old leaves of plant requires cool, wet weather for spore release and infection. The fungus produces flask shaped fruiting bodies called pycnidia. These pycnidia release spores in early spring and are spread by splashing rain droplets to developing shoots, leaves, and clusters. In the presence of free water, the spores germinate and cause infection. The optimum temperature for leaf infections was between 60 and 68 degrees F and at least six hours wetness duration is required at these temperatures for infection to occur. As the wetness duration increases, the opportunity for infection greatly increases. Spot on leaves appear at seven to ten days after infection. Fully expanded leaves become resistant to infection. Lesions on canes require two to four weeks to develop. The fungus does not appear to be active during the warm summer months, but it can become active during cool, wet weather later in the growing season. Pycnidia eventually develop in infected wood and will provide the initial inoculum for infections. 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) [12, 8, 11, 9, 5, 1, 4, 3].

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 ant parasitic activity, sometimes being highly specific (Del Signore *et al.*, 1997) [2]. Phenolic compounds (PCs) are produced as secondary metabolites by most plants, in which they probably act as natural antimicrobial agents, as natural deterrents to grazing animals or as inhibitors of pre-harvest seed germination. This is attributable at least partly to the long history of phenol oxidase investigations, started in the 1890s when a phenol oxidase was discovered in mushrooms, and partly because some microbial sources (eg, Pleurotus ostreatus and Trametes versicolor), produce both types of enzyme (Palmieri G *et al*; 1993) [6].

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.
Vitis vinifera 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.
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) [10].

Results and Discussion

Production of Polyphenols by *Phomopsis viticola*

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. Fructose shows maximum production of phenol, followed by dextrose. Similar production of phenol was seen in glucose and control. While lactose show minimum phenol production.

Table 1: Production of total phenol (mg/l) in culture filtrate by *Phomopsis viticola* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period.

Carbon sources	Phenol (mg/ l)
Control	3.532
Dextrose	3.753
Glucose	3.532
Lactose	3.331
Fructose	3.933

Effect of Nitrogen Sources

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

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

Nitrogen Sources	Phenol (mg/ l)
Control	3.853
KNO_3	4.248
$Ni(No_3)_2$	4.757
$Co(NO_3)_2$	4.425
$Ba(NO_3)_2$	3.933

Summary

Considerable variation in the production of total phenol was observed when different carbon compounds were incorporated in the medium. For *Phomopsis viticola* phenol production the fructose shows maximum production of phenol, followed by dextrose. Similar production of phenol was seen in glucose and control while lactose show minimum phenol production.

For *Phomopsis viticola* phenol production on that nitrogen compound were favorable for production of phenol. Highest phenols were detected on nickel nitrate followed by cobalt nitrate, potassium nitrate and barium nitrate. As compared to other nitrogen sources lowest phenol were detected on control.

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