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Effect of nutrients on cellulolytic and pectolytic enzyme production of *Phomopsis viticola* a leaf spot pathogen of grapes (*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 grate extent of growth variation was observed on different carbon, nitrogen. Among the carbon source, maximum growth on Glucose followed by Control, lactose, fructose and dextrose while in absence of carbon source there was no growth. From nitrogen source maximum growth on nickel nitrate. While barium nitrate, cobalt nitrate and control had more or less similar effect on the growth. The least growth was observed in potassium nitrate. In absence of nitrogen source in the medium resulted in lowest growth as compared to other compound.
Nitrogen sources shows much variation in cellulolytic enzyme activity. The cellulose activity was maximum in nickel nitrate and minimum in barium nitrate and potassium nitrate as compare to cobalt nitrate and control.

Keywords: *Phomopsis viticola*, cellulolytic and pectolytic enzyme, pathogen

Introduction
Vitis vinifera L is a 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 causes 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).

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 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

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

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

Results and Discussion

Phomopsis viticola 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 loss in percentage viscosity in fructose followed by dextrose, there was not much variation in loss of percentage viscosity in dextrose and control, as compared to this the minimum loss in percentage viscosity in lactose.

Table 1: Viscosity of culture filtrate of *Phomopsis viticola* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period

Carbon Sources	Loss in viscosity of culture filtrate at		
	0 min.	15 min. (%)	30 min. (%)
Control	0.018567	6.295	19.788
Dextrose	0.018312	4.746	20.527
Glucose	0.018095	4.324	16.686
Lactose	0.019148	5.576	10.054
Fructose	0.019293	2.176	20.282

Data in the table - 2 shows maximum viscosity loss followed by cobalt nitrate and potassium nitrate. There is not much variation is seen in loss of viscosity of nickel nitrate and control which shows minimum viscocity loss.

Table 2: Viscosity of culture filtrate of *Phomopsis viticola* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period

Nitrogen Sources	Loss in viscosity of culture filtrate at		
	0 min.	15 min. (%)	30 min. (%)
Control	0.018077	2.335	11.041
KNO_3	0.018158	4.296	14.720
$Ni(No_3)_2$	0.019640	1.977	11.344
$Co(NO_3)_2$	0.019652	5.323	16.322
$Ba(NO_3)_2$	0.018351	9.102	17.525

The pectolytic enzyme activity was measure on different carbon and nitrogen the result shows (Table-3) that the maximum macerating activity in dextrose, followed by similar activity in glucose and fructose as a carbon source. Very little differences were found in between lactose and control which shows minimum macerating activity.

Table 3: Determination of pectolytic activity by macerating enzyme method of *Phomopsis viticola* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period

Time / Carbon source	2	4	6	8	10	12	14	No of time Discs Macerated	Total time	Mean time	M. E.
Control	-	1	3	3	2	1	-	5	40	8	125
Dextrose	2	1	2	3	2	-	-	5	30	8	166.66
Glucose	1	2	1	1	3	2	-	6	42	7	142.85
Lactose	2	1	2	1	-	3	1	6	46	7.6	130.43
Fructose	3	2	1	1	2	1	-	6	42	7	142.85

The five nitrogen compounds studied, Data in the table - 4 indicates large variation in the pectolytic enzyme of *Phomopsis viticola* on different nitrogen compounds. The result shows that maximum macerating activity were seen in nickel nitrate and potassium nitrate. The minimum macerating activity was seen in barium nitrate as compaired to control and cobalt nitrate.

Table 4: Determination of pectolytic activity by macerating enzyme method of *Phomopsis viticola* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period

Time / Nitrogen source	2	4	6	8	10	12	14	No of time discs Macerated	Total Time	Mean time	M.E.
Control	1	2	1	2	3	1	-	6	42	7	142.85
KNO_3	2	3	1	2	2	-	-	5	30	6	166.66
$Ni(No_3)_2$	2	2	2	3	1	-	-	5	30	6	166.66
$Co(NO_3)_2$	-	3	1	3	2	1	-	5	40	8	125
$Ba(NO_3)_2$	1	3	2	-	2	1	1	6	40	8	125

Summary

A grate extent of growth variation was observed on different carbon, nitrogen. Among the carbon source, cellulose activity of *Phomopsis viticola* was maximum in fructose followed by dextrose, control, glucose and minimum cellulolytic enzyme activity in lactose. From nitrogen source cellulolytic enzyme activity of *Phomopsis viticola* was maximum in barium nitrate followed by cobalt nitrate, potassium nitrate, nickel nitrate and control. Variation was also observe in pectolytic enzyme activity. Dextrose shows maximum pectolytic enzyme activity, while control with minimum cellulolytic enzyme activity. From nitrogen source the pectolytic activity was maximum in nickel nitrate and potassium nitrate. The minimum pectolytic activity was in barium nitrate as compared to cobalt nitrate and control.

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