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Effect of temperature and pH on production of cellulolytic enzyme by *Alternaria solani cuasing* early blight of tomato

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ABSTRACT

The studay was carried out the production of the cellulolytic enzyme and effect of physical factors like temperature and pH on enzyme production. Maximum enzyme production was recorded at temperature 26^{0}C and pH 7.8. However, minimum enzyme production was recorded at 10^{0}C and pH 4.0. The cellulolytic enzyme activity was increased with decrease in temperature. The rate of enzyme production was high when temperature is optimum which is 30 to 40^{0}C. From this it's clear that, the optimum cellulolytic enzyme activity was at 26^{0}C Temperature and pH 7.8.This investigation supported by the other investigators.

Key words : : Enzyme production, Cellulolytic enzyme, *Alternaria solani*, Optimum, Temperature, pH.

INTRODUCTION

Botanically, tomato belongs to the family Solanaceae and the genus *Lycopersicon*. The genus comprises of a few species of annual or short lived perennial herbaceous plants. It has become one of the common vegetables allover the country and grown extensively in many parts, almost thought out the year.

Like other crops, tomato is also most severely affected by various diseases of microbial origin. There are over two dozen diseases of tomato reported from the different parts of the country. Three fungal foliar diseases (late blight, early blight, and Septoria leaf spot) are common and especially troublesome to tomato growers for its successful cultivation. Early blight is a common and serious problem *Alternaria solani* and the symptoms usually appear leaves as circular or irregular, dark brown to black dead spots with concentric rings. It results the defoliation of leaves and reduction in yield. In order the pathogenicity, celluolytic enzyme activity was studied celluloses are found is cellulolytic enzyme. It is polymer of P-glucose unit joined by B-14 glucosidic linkages about 280 to 800 chains of cellulose which bound laterally by hydrogen bound and other cellulolytic enzyme are classified on the basis of their group as cellulose-1, Cx, and Cellobia.

MATERIALS AND METHODS

Collection of samples:

Diseased plants parts like leaves of tomato plants were collected in polyethine and used for isolation of pathogen (Mukadam and Gangawane, 1982).

Potato dextrose agar (PDA)

Compostion of media:

Peeled potato	-	250.0 g
Glucose	-	20.0g
Agar	-	12.0g
pH	-	6.0 to 6.5
Water	-	

Carboxyl methyl cellulose (CMC):-

CMC	-	10 g
KNO_3	-	2.5 g
KH_2PO_4	-	1.0g
$MGSO_4$	-	0.5g
Distilled water	-	1000ml.
pH	-	4.0

All the compounds were weighed. 100ml distilled water was taken in a flask and then the compounds were dissolved one after the other by heating slightly about pH was maintained.

The media sterilized at pressure of 15 lbs for 20 minutes in autoclave. 15ml of media was poured in each culture tube for slant preparation.50ml of liquid media was poured in each 250ml conical flask for enzyme production.

Glasswares were washed with potassium dichromate solution, followed by sterile distilled water, dried and kept in oven at 160^{o}C temperature for 1 hour.

Culture vessels :

Carboxyl methyl cellulose medium (50ml) was distributed in Erlenmeyer flasks (250 ml) and was sterilized in an autoclave before use.

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Inoculum :

Spore suspension was prepared by adding 10ml of sterile water to five day old PDA slant culture and 1ml of it was used as inoculum in all experiments unless and other wise stated. In every, case spore suspension was standardized with 0.8 optical densities (0.1), unless other wise stated all experiments were conducted in triplicate and results have been presented.

Incubation :

Cultures were incubated at $26^0C \pm 4^0C$ in laboratory and variation in temperature was recorded during period of study in all experiments. Cultures were incubated for eight days.

Method for assessing pH :

The P^H value of culture filtrate (CF) in liquid media was determined directly with P^H meter after every 14 hours.

Enzyme assay :

In all experiments crude C.F as used as enzyme source.

Effect of temperature on cellilytic enzyme production:

Alternaria solani was grown on carboxyl methyl cellulose (CMC).8 days old culture medium was filtered through whatman no.1 filter paper and centrifuged at 2000 rpm for 20 minutes. The culture filtrate was used as crude enzymes. For the study of effect of temperature, method given Lentz and Vandenberg (1971) and Arinze and Yabedee (2000) was used. The enzyme activity was measured by viscosity method and the results are presented in Table 1.

Effect of pH on celloulytic enzyme production:

The effect of pH was studied as per the procedure described by Deshpande (1981) and Mukadam and Gangawane (1982). The observations are recorded in Table 2.

RESULTS AND DISCUSSION

It is clear from the results presented in Table 1 that % viscosity loss was found to be increased with increase in time period. The maximum loss of viscosity was 74 % after 40 minutes and it was minimum 7% after 10 minutes at 10^0C. At 26^0C, the % viscosity loss was found to be increased with increase in time period. The maximum % loss of viscosity (74 %) after 40 minutes and it was minimum 20 % after 10 minutes the % viscosity loss was found to be increased with increase in time period. The maximum % loss of viscosity (70%) after 40 min and it was minimum 16 per cent after 10 minutes, for 37^0C temperature.

The enzyme activity was measured by viscosity method and the results are presented in following Table 2 *i.e.*, tested at different pH *i.e.*4.0, 4.5, 6.8, 7.8. It is clear from the results presented in Table 2 that the % viscosity loss was found to be increased with increase in time period. The maximum % loss of viscosity (0%)after 40 minutes and it was minimum o% after 10 minutes at pH 4.0. at 4.5 pH % viscosity loss was found to be increased with increase in time period .The maximum % loss of viscosity (0%) after 40 minutes and it was minimum 0% after 10 minutes.The % viscosity loss was found to be increased with increase in time period the maximum % loss of viscosity (100%) after 40 minutes and it was minimum 25 % after 10 minutes at pH 6.8.At pH 7.8 % viscosity loss were also found to be increased with increase in time period. The maximum % loss of viscosity (100 %) after 40 minutes and it was minimum 40 % after 10 minutes.

Enzyme substances secreted by pathogen in plant, seems to be involved in the production of disease either directly or indirectly in the diseases, like leaf spot, rot, etc.and with disease, enzyme seems to play the most important role. Therefore, the present study was carried out to study the production of the enzyme and effect of physical factors like temperature and pH on enzyme production. It clear from the results that maximum enzyme production was recorded at temp 26^0C. However, minimum enzyme activity was recorded at temperature 10^0C.

The cellulolytic enzyme activity was found to be more at 26^0C temperature similarly, it was investigated by several works that the reaction rate increases with rising and decreases with following temperature for most enzyme catalyzed reaction. Their rate is higher when temperature is optimum which is usually some were between 30^0C to 40^0C. It is evident from the results presented in Table 1 that the activity of Celluloytic enzyme activity was increased with increase in temperature.

It is evident from the results that *Alternaria solani* produced cellulose in the culture filtrate. Similarly, it was reported early by Airnze and yubedee (2000) that apply pectin and sodium polyepectin supported the production of polygacturonase by *Fusarium moniliforme viz*. like paper and carboxyl methyl cellulose supported the production of either polygalactral or celluloses glucose and starch did not support the production of either polyglatoranse or

Table 1 : Effect of temperature on cellulolytic enzyme production by *Alternaria solani.*

Sr. No.	Temperature	Time mint. 0		10		20		30		40	
		Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity
1.	10^0C	65	0%	63	7%	60	18%	55	37%	48	60%
2.	26^0C	90	0%	75	20%	68	40%	56	61%	49	74%
3.	37^0C	80	0%	72	16%	60	40%	55	50%	45	70%

Table 2 : Effect of pH on cellulolytic enzymes production by *Alternaria solani*.

Sr. No.	pH	Time mint. 0		10		20		30		40	
		Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity	Flow rate of CF+ substrate	%Loss of viscosity
1.	4	0	0%	38	0%	36	0%	35	0%	31	0%
2.	4.5	0	0%	35	0%	35	0%	34	0%	33	0%
3.	6.8	38	0%	36	25%	33	60%	37	80%	30	100%
4.	7.8	35	0%	33	40%	32	60%	31	80%	30	100%

cellulied in higher quantity then form carboxyl methyl cellulose be cellules.

It was clearly observed from results that the optimum temperature for the enzymes production is 26^0C and optimum pH for production of celluloytic enzyme was pH 6.8. Result relieved that two factors had pronounced effects on the rate of diseased progress all the fungi tested failed to show appreciable amount of routings at the temperature of 10^0C to 15^0C, where as they showed Sevier routine between 25^0C and 30^0C. Similarly ward laws in 1935 pointed out that temperature as an important roles to determine the type of cause fungi in storage.

Choudary (1955), Wook (1960), Bhargawa et.al (1965), Prasad and Bilgrami (1973), Tandon (1967) have emphasized the role of temperature and humidity on fruit rots caused by different fungi in chillies, mango, banana, guava, apple, lemons, litchi and pomegranate, receptively.

There observation revels that the low temperature (10^0c) and low pH (4.0) are inhibitory for the cellulolytic enzyme activity. The maximum production of the enzyme occurred at 26^0C and pH 6.8. It is clear from the result that the pathogen not showing a priceable amount of enzyme production at temperature of 10^0C. Results of present investigation clearly adovate the necessity of the favourable physical factor for the more production of emzyme and ultimately development of disease.

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REFERENCES

Arinze, A.E. and Yabedee, A.G. (2000). Digesation of carbon materials by pectolytic and celluloytic enzyme of *Fusarium moniliforme* Sheld, *Global Pure and Applied Sci.*, **6** (4) : 619-622.

Bhargao, S.N, Ghosh, A.K, Grivastava, M.P, Singh, R.H. and Tandon, R.N. (1965). Studies on fungal diseases of tropical fruit VII- effect of temperature on mango, banana, guva caused by some important Pathogen. *Proavat, Acid Sci. India took The Moral High*, **36** : 393-398.

Baig, M.M.V. (1997). Role of cell wall degrading enzyme in induction of resistance in plants Ph.D. Thesis S.R.T.University, Nanded.

Chaudary, S. (1955). Studies on development and control of fruit rot of chillies *Indian Phytopath*, **10** : 55-61.

Deshpande K.B. (1961). Studies on pectolytic enzymes system of *Alternaria solani* pectic esterase and polyglactorange. *Indian Botanical Sci*., **40.**

Lentz, C.P. and Vandenbera, L. (1971). Study of factors affecting (Temp, relative humidity and moisture) in fresh fruit and vegetable storage. *J. IDST. On Techol. Aciment.*, **4** : 146-153.

Mehrotra, R.S. (1961). Tata Mc. Grew Hill Publishing Company Limited, New Delhi. Plant Pathology.

Mukadam, D.S.and Gangawane, L.V. (1982). *Methods in Experimental Plant Pathology*. Marathwada University Publication, Aurangabad.

Prasad, S.S. and Bilgrami, R.S. (1973). Investigation on diseases of litchi II. Influence of temperature and humidity on the tealy of fruit caused by nine virulent pathogens. *Indian Phytopath*., **26** : 517-522.

Tandon, R.N. (1967). Observation of storage diseases of certain fruits. *Indian Phytopath*., **20** :1-10.

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