

KEYWORDS: Pectinase production, tomato fungi, culture media.

INTRODUCTION:

Tomato (*Lycopersicon esculentum* Mill.) is one of the common vegetables grown all over the country extensively almost the year round. The crop is reported to be affected by about twenty diseases of microbial origin. Among them, the fungal pathogens have been found to affect and damage severely the tomato fruits both in field at different developmental stages as well as in the market during storage. This may result in the qualitative and quantitative loss of tomato fruits.

It is the well known fact that the fungi produce different hydrolytic enzymes during pathogenesis. The hydrolytic enzymes produced by the fungi like cellulases, pectinases, amylases, lipases and proteases are known to degrade food contents. Sreekantiah et al. (1971) found that, Alternaria alternata, Fusarium solani f.sp. minus, Pleospora infectoria and Alternaria solani were capable of producing all the four kinds of hydrolytic enzymes, viz., pectinase, cellulase, amylase and proteinase. Balsubramanian (1972) reported that, protease along with cellulase and pectinase was found to be effective in infection by Rhizopus stolonifer within the tissue. Mehta et al. (1974) found that, during pathogenesis of tomato fruits, Alternaria sp. produce pectolytic and celluloytic enzymes. They also reported that, polygalacturonase and pectin methyl galacturonase are found to play important role in pathogenesis due to Alternaria solani and in A. tenuis (A. alternata) infection. Ramasami and Shanmugam (1976) studied pectolytic and celluloytic enzymes of Rhizoctonia bataticola in vitro and in vivo. Hasija and Batra (1981) recorded that, Phoma destructiva produced pectin transliminase and polygalacturonase in diseased tomato fruits, while pectin methyl esterase, pectin methyl galacturonase occurred in both healthy and diseased tissue. Hasija and Batra (1984) found that, Phoma destructiva causing fruit rot of tomato fruits produced all types of pectic enzymes (PME, PMG, PG and PGTE in vitro).

In the present investigation, pectinase production on different culture media was studied in the fungi isolated from tomato fruits.

MATERIALAND METHODS:

a) Production of pectinase:

For the production of pectinase i.e. pectin methyl galacturonase (PMG), the fungi were grown in different liquid culture media viz. Glucose nitrate, Pectin peptone, Pectin nitrate and Pectin ammonium nitrate. Twenty five ml of each medium was taken separately in 100 ml conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml standard spore suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25 °C. On 7th day, the flasks were harvested by filtering the contents through Whatmann No. 1 filter paper. The filtrates were collected in pre-sterilized bottles and termed as crude enzyme preparations.

b) Enzyme assay (Viscometry):

The Ostwald's viscometer was thoroughly cleaned with distilled water and dried before use. Six ml of 1% pectin in 2 ml of 0.2 M acetate buffer pH 5.2 and 4 ml of enzyme source were taken in viscometer and were thoroughly mixed and incubated at 25° C temperature. The efflux time of the mixture at 0, 5,10,20,30,40,50 and 60 minutes was recorded with the help of stop watch. The percent loss of viscosity was calculated by using the formula:

Per cent loss of viscosity
$$= \frac{To - Tx}{To - Tw} \times 100$$

Where	То	=Flow time in seconds at zero time
	Tx	= Flow time of the reaction mixture at time 'T'
	Tw	= Flow time of distilled water.

RESULTS AND DISCUSSION:

Table 1: Production of pectinase in tomato fungi on different media

Fungi		Media				
	Non-substrate	substrate Substrate				
	Glucose nitrate	Pectin peptone	Pectin nitrate	Pectin ammonium nitrate		
		% Viscosity loss after 40 minutes				
Alternaria solani	66.6	70.5	72.2	76.8		
Geotrichum candidum	15.0	54.7	83.3	85.2		
Fusarium roseum	40.2	65.7	58.7	67.5		
Fusarium oxysporum	45.5	71.2	66.6	73.5		
Phoma destructiva	23.3	62.5	44.5	72.9		
Rhizoctonia solani	25.7	65.2	64.7	61.5		
Phytophthora sp.	11.9	87.5	83.3	84.7		
Cladosporium fulvum	21.4	68.5	66.6	62.3		
Curvularia lunata	30.1	58.3	61.5	67.3		
Aspergillus niger	10.0	79.2	86.6	88.5		
Aspergillus flavus	14.2	81.8	80.2	69.5		
Penicillium expansum	00.00	82.3	80.0	71.3		
Rhizopus stolonifer	17.4	59.7	55.7	68.9		

From Table-1, it becomes clear that, all the fungi except *Penicillium* expansum were able to produce pectinase in Glucose nitrate (nonsubstrate) medium. However, maximum pectinase production was observed on the substrate media. Among the substrate media, maximum pectinase production was found on Pectin ammonium nitrate by all fungi except *Rhizoctona solani, Phytophthora sp.*, *Cladosporium fulvum, Aspergillus flavus* and *Penicillium expansum* which showed maximum pectinase production on Pectin peptone. As

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compared to Pectin peptone and Pectin ammonium nitrate, Pectin nitrate was found to be poor medium for pectinase production. Constitutive nature of pectinase production was also reported by Brown (1915) and Tribe (1955). Pectic substances were found to be inhibitory for pectic enzyme production in Pythium debaryanum (Ashour, 1955 and Gupta, 1956) and Cladosporium cucurmerianum (Husain and Rich, 1958). On the other hand, adaptive nature of pectinase production was reported in Fusarium moniliforme (Singh and Wood, 1956), Rhizoctonia solani (Deshpande, 1960), Helminthosporium apattarnae (Deshpande and Deshpande, 1968) and Penicillium chrysogenum (Phaff, 1947) and Fusarium lycopersici (Waggoner and Diamond, 1955).

REFERENCES:

- Ashour, W.E. (1955). Pectinase production by Botrytis cinera and Pythium debaryanum. 1. Trans. Brit. Mycol. Soc. 37: 343-352. Balsubramanian, K.A. (1972). The possible role of proteolytic enzymes in the
- 2. pathogenicity of Rhizopus stolonifer (Ehreneb Ex. Fr.) Lind. Indian Phytopath. 25(2): 475-476
- Brown, W. (1915). Studies on physiology of parasitism I. The action of Botrytis cinera. 3. Ann. Bot. Lond. 29: 313-348
- Deshpande, K.B. (1960). Studies on the pectolytic enzyme system of Rhizoctonia solani 4 Kuhn. I. Production of protopectinase. J. Biol. Sci. 3:1-8.
- Deshpande, K.S. and K. B. Deshpande (1968). Pectic enzyme production and morphogenesis in Helminthosporium atypicum. Biologia Plantarum 101: 245-250. 5. 6.
- 7.
- Morphogenesis in Heiminnosportum atypicum. Biologia Plantarum 101:245-250. Gupta, S.C. (1956). Studies on physiology of parasitism XIII. The production of pectic enzymes by Pythium debaryanum Hess. Ann. Bot. Lond. N. S. 20: 179-190. Hasija, S.K. and Batra, S. (1981). In Vitro production of pectic enzyme by Phoma destructiva. Indian Phytopath. 34 (2): 384-387. Hasija, S.K. and S. Batra (1984). In Vitro production of pectic enzymes by Phoma 8.
- destructiva. Indian Phytopath. 37(1):413-145. Husain, A. and S. Rich (1958). Extracellular pectic and cellulolytic enzymes of 9.
- Cladosporium cucurmerianum. Phytopathology 48: 318-320. Mehta, P., K.M. Vvas and S.B. Saxena (1974). Production of pectolytic and cellulolytic 10.
- enzymes by Alternaria sp. during pathogenesis of tomato fruits 11
- Hindustan Antibiotics bulletin 16 (4): 210-214. Phaff, H. J. (1947). The production of extracellular pectic enzymes by Penicillium 12. chrysogenum-I. On the formation and adaptive nature of polygalacturonase and pectinesterase. Arch. Biochem. 13: 67-81.
- 13. Ramasami, R. and N. Shanmugam (1976). Studies on pectolytic and celluloytic enzymes of Rhizoctonia bataticola in Vitro and in Vivo. Indian Phytopath. 29 (4) 385-388
- Singh, R. K. and R. K. S. wood (1956). Studies on physiology of parasitism XXI. The production and properties of pectic enzymes secreted by Fusarium moniliforme Shdon.. 14.
- Ann. Bot. Lond. N. S. 20: 89-103. Sreekantaiah, K.R., K.S. Nagaraja and T.N. Ramchandra Rao (1971). The production of 15. certain extracellular hydrolytic enzymes by four species of plant pathogenic fungi. Proceedings of Second International Symposium of Indian Phytopathological Society, 75-76
- pp. 75-76. Tribe, H. T. (1955). Studies on physiology of parasitism XIV. On the killing of plant cellsby enzymes from Botrytis cinera. Ann. Bot. Lond. N. S. 19: 351-369. 16.
- 17. Waggoner, P. E. and A. E. Diamond (1955). Production and role of extracellular pectic enzymes of Fusarium oxysporum f.sp. lycopersici. Phytopathology 45: 79-87.

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