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Biochemical Changes in Tomato (*Lycopersicon esculentum* Mill.) Fruits against *Fusarium pallidoroseum*

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The activities of Polygalaturonase (PG), Polymethylgalacturonase (PMG) and Cellulolytic enzymes (CX) were higher in ripe fruits (36.47, 34.69 & 30.75%) inoculated with *F. pallidoroseum*, respectively. The enzymatic activity of PG, PMG and CX were found increased with time. Tomato fruits inoculated with *F. pallidoroseum* showed decrease in total soluble sugar (2.04 %), ascorbic acid content (12.36 mg/100g), acidity (0.48 %) content and physiological weight loss (35.15 g) (50.21%) as compared to uninoculated fruits.

Key words: *Fusarium pallidoroseum,* Tomato, Biochemical changes.

Tomato (*Lycopersicon esculentum* Mill.) is an important and most widely grown vegetable crop of both tropics and sub tropics of the world, belonging to the family Solanaceae and ranks second in importance among vegetables.

The incidence of fungal rot in tomato fruits from vegetable markets and stores was 0.5-19.7% and 81.3% of the total spoilage was due to *Fusarium* spp. (*F. equiseti* and *F. pallidoroseum*), *Geotrichum candidum, Didymella lycopersici, Alternaria alternata* and *Phytophthora nicotianae* var. *parasitica* (Sharma, 1994)[1]. Decay caused by pathogens has been reported to constitute up to about60% of losses in fresh tomatoes produced in Nigeria (Kutama *et al*., 2007)[2]. Fresh tomato fruits are vulnerable to post-harvest losses. They are highly perishable with a short shelf life and high susceptibility to fungal diseases. They are difficult to store for long periods without incurring losses and as the fruits ripen, they become more susceptible to microbial infections (Mujib ur Rehman *et al.*, 2007)[3].

Tomato is a good source of vitamin A and C (Verkerke *et al.*, 1998)[4]. Composition data varies due to the wide range of species, stage of ripeness, year of growth, climatic conditions, light, temperature, soil, fertilization, irrigation, and other conditions of cultivation, and handling, storage and post- harvest diseases.

MATERIALS AND METHODS

Fresh naturally infected diseased tomato fruits showing typical characteristic symptoms of Fusarium fruit rot were collected from the Sardar Patel vegetable market, Anand(Gujarat) and brought to the laboratory for isolation of the pathogen. Well-isolated single spores were marked and then transferred on PDA slants separately

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under aseptic conditions and incubated at 25 ± 1^0C in BOD incubator for seven days. Identification of the pathogen was carried out by studying the cultural and morphological characters. The microphotographs of mycelium and spore structure were taken with the help of digital camera. The pure culture was sent to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi – 110 012 for identification and identified as *Fusarium pallidoroseum* (7060/2012).

Cell wall degrading enzymes

Semi-ripe and ripe fruits were surface sterilized and separately inoculated with *Fusarium pallidoroseum* by stem- end pin-prick method. The inoculated fruits were incubated at ambient temperature. On 1st, 2nd, 3rd and 4th day, extracts from semi-ripe and ripe fruits were obtained according to the procedure described by Bell *et al.* (1955)[5].

Five gram of the rotted and healthy fruit tissues were macerated separately with the help of a pestle and mortar in distilled water (15 ml) and 0.5 N NaCl (15 ml). The ground tissues extract were strained through several layers of muslin cloth. The filtrates from semi-ripe and ripe fruits were separately centrifuged at 4000 rpm for 20 min. The supernatant were used for cell wall degrading enzyme study.

The compositions of the reaction mixtures for 2 ml of enzyme sample for the different enzymes are as follows:

Polymethylgalacturonase (PMG)

Five ml of one per cent pectin dissolved in buffer solution (pH 5.0), 1.8 ml of 0.1 M phosphate citrate buffer (pH 5.0) and 1.5 ml of distilled water.

Polygalacturonase (PG)

Five ml of one per cent sodium polypectate dissolved in buffer solution (pH 5.0), 1.8 ml of 0.1 M phosphate citrate buffer (pH 5.0) and 1.5 ml of distilled water.

Cellulolytic enzymes (CX)

Five ml of 1.2 per cent carboxymethyl cellulose (CMC) dissolved in 1.8 ml of 0.1 M phosphate citrate buffer solution (pH 5.0) and 1.8 ml of distilled water.

The enzyme activity was assessed by determining the loss in viscosity of the reaction mixture immediately at intervals of 10, 30 and 120 minutes at 30^{0}C. Each treatment was replicated for four times.

The per cent enzyme activity was calculated by the following formula:

$$\frac{V_o - V_t}{V_o - V_w} \times 100$$

Where,

V_o = The flow time at 0 min

V_t = The flow time after 10/30/120 min

V_w = The flow time of distilled water.

Total soluble sugar content

Total soluble sugar from the semi-ripe tomato pulp both inoculated and uninoculated was determined by phenol sulphuric acid method as described by Dubois (1956)[6].

The pulp of 100 mg. was macerated in 5 ml 80% alcohol and taken in 30 ml test tubes and total volume was made to 10 ml with 80 % alcohol. The test tubes were kept overnight. Next day 1.0 ml supernatant were taken from each test tube and was evaporated to dryness in water bath. After evaporation the volume was made to 25 ml with distilled water in beaker. From this 25 ml, one ml test solution was used for assay in which freshly prepared 1 ml of 5 % phenol solution was added followed by immediate direct addition of 5 ml concentrated sulphuric acid solution. The tubes were kept for 10 min. at room temperature for colour development. After mixing the solution it was kept for further 15 min. in cold water bath. The intensity of stable yellow colour developed was recorded at O.D. 490 nm in spectrophotometer.

In a similar way 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard glucose solution having 10 to 50 µg glucose was taken and pipetted out into a series of test tubes. The volume of each test tube was made up to 1ml. with distilled water. For blank, one ml of distilled water was taken. Sugar content was determined by using the following formula:

$$\text{Total soluble sugar } (g/100g) = \frac{\text{Sample O.D}}{\text{Graph factor } (\text{mg sugar})} \times 250/1000$$

Physiological weight loss

Semi-ripe and ripe fruits of Gujarat Tomato – 2 variety were surface sterilized with $HgCl_2$ (0.1%) and finally washed with distilled sterile water and separately inoculated with *F. pallidoroseum* by pin-prick method. The inoculated fruits were incubated at ambient temperature. Physiological losses in weight of

infected fruits were assessed on 2nd, 4th, 6th and 8th day and losses in weight were calculated by the following formula:

$$\frac{W_1 - W_2}{W_1} \times 100$$

Where,

W_1= The weight of fruit recorded at the time of inoculation

W_2= The weight of fruit recorded after 2nd, 4th, 6th and 8th day after inoculation

Ascorbic acid content

The titrimatic method described by Ranganna (1979)[7] was adopted to estimate the ascorbic acid content. Ten grams of diseased/ infected as well as healthy homogenized pulp from four treatments was taken and transferred to 100 ml volumetric flask. Volume was made up with 3 per cent metaphosphoric acid solution. After 30 minutes, the solution was filtered through Whatman filter paper No.1. The dye (2,6 dichlorophenol indophenol) was standardized by titrating against standard ascorbic acid and the dye factor was calculated. Ten ml. of supernatant aliquot was taken in 100 ml. conical flask and titrated against standard dye solution through a burette. Titration was continued till the light pink colour persisted for more than 15 seconds. The ascorbic acid content was calculated by using following formula :

$$\text{Ascorbic acid } (\text{mg}/100 \text{ g pulp}) = \frac{\text{Titrate x Dye Factor x Volume made up}}{V \times W_1} \times 100$$

Where

V = Aliquot of filtrate taken for estimation

W = Weight or volume of sample taken for estimation

Acidity content

The method described by Ranganna (1979)[7] was adopted to estimate the acidity. Ten grams of infected as well as healthy homogenized pulp was taken and transferred to 100 ml volumetric flask and volume was made up with distilled water. The suspension was filtered through Whatman filter paper No. 1 and the filtrate was used for titration. Five milliliter of aliquot was taken from the filtrate and titrated against standard sodium hydroxide using phenolphthalein as an indicator. The titrable acidity was expressed as percentage of ascorbic acid equivalents adopting the following formula :

$$\text{Acidity } (\%) = \frac{\text{T x N x Q x E}}{\text{V x W x 1000}} \times 100$$

Where,

T = Titrate volume

N = Normality of alkali

Q = Volume made up

E = Equivalent weight of citric acid

V = Volume of sample taken for estimation

W = Weight of sample

RESULTS AND DISCUSSION

Studies on cell wall degrading enzymes.

The activity of Polygalaturonase (PG), Polymethylgalacturonase (PMG) and Cellulolytic enzymes (CX) were studied of ripe and semi ripe healthy and inoculated (*F. pallidoroseum*) fruits. The enzymatic activities of PG, PMG and CX were higher in ripe fruits (36.47, 34.69 & 30.75%) than in semi ripe (28.37, 16.19 & 13.96%), respectively. The enzymatic activity of PG, PMG and CX were found increased with time. Highest reduction in viscosity was observed in PG at 120 min with 28.37, 36.47 and 12.04 per cent, respectively (Table 1). The interaction effect between stage of fruit ripeness and period found significant. The enzymatic activity (PG, PMG & CX) was lowest in healthy fruits as compared to inoculated fruits.

Results similar to the present investigation were reported by Patil and Pathak (1994)[8]. They found that activity of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulolytic enzymes (CX) were highest at all intervals (10, 30 and 120 min.) in ripe mango fruits compared to semi-ripe fruits infected with *Botryodiplodia theobromae* and *Rhizopus arrhizus*.

Al-Hindi *et al.* (2011)[9] reported that cell wall degrading enzymes as pectinases, xylanases, cellulases and amylases were always greater in healthy fruit and diminished as the disease progressed in the fruit infected with *Aspergillus, Fusarium, Rhizopus* causing rots in many fruits.

Virk and Gemavat (1982)[10] studied the production of pectinolytic and cellulolytic enzymes by *Fusarium oxysporum* f. sp. *sesami* with 3 isolates (Isolate 1, 3 and 5). He observed that the activity of pectinolytic enzymes was more in plants with low wilt intensity as compared to plants with severe infection.

Salami and Akintokun (2008)[11] inoculated the cassava fruits with *Lasiodiplodia theobromae*, *Macrophomina phaseolina*, *Rhizopus stolonifer* and *F. pallidoroseum* and assessed the cell wall degrading enzyme activity. The enzyme activities were found to increase with the incubation period between 6 and 8 days of inoculation and declined after 10th day. Singh (2011)[12] studied the activities of Polygalaturonase (PG), Polymethylgalacturonase (PMG) and Cellulolytic enzymes (CX) on banana fruits inoculated with *F. moniliforme*. The result revealed that the activity of PG, PMG and CX enzymes were higher in ripe fruits which were inoculated with *F. moniliforme* as compared to semi- ripe ones.

Table 1. Effect of fruit ripeness on synthesis of cell wall degrading enzymes by *Fusarium pallidoroseum*

Stage	Per cent reduction in viscosity											
	Polygalacturonase				Polymethylgalacturonase				Cellulolytic enzymes			
	Minutes			Mean	Minutes			Mean	Minutes			Mean
	10	30	120		10	30	120		10	30	120	
Semi-ripe	13.15	31.22	40.75	28.37	8.96	14.06	25.55	16.19	9.02	11.85	21.02	13.96
Ripe	21.75	33.55	54.11	36.47	17.22	38.16	48.71	34.69	18.17	31.65	42.45	30.75
Healthy	5.42	10.30	20.40	12.04	4.99	5.85	10.65	7.16	4.53	6.80	13.75	8.36
Mean	13.44	25.03	38.42		10.39	19.35	28.30		10.57	16.76	25.74	
Source	S. Em.+	C. D.			S. Em.+	C. D.			S. Em.+	C. D.		
Stage (S)	2.41	4.95			1.79	3.68			1.80	3.67		
Period (P)	2.41	4.95			1.79	3.68			1.80	3.67		
S X P	Sig.	Sig.			Sig.	Sig.			Sig.	Sig.		
C.V. %	13.33		13.13		14.38							

Table 2. Total Soluble Sugar content in tomato fruits inoculated with *Fusarium pallidoroseum*

S. No.	Treatments	TSS(%)
1	1st day	3.08
2	2nd day	2.81
3	3rd day	2.67
4	4th day	2.41
5	5th day	2.35
6	6th day	2.04
7	Control	3.83
	S.Em. ±	0.002
	C.D. at 5%	0.006
	C.V. %	2.63

Total soluble sugar content

The results of Total soluble sugar content in fruits inoculated with *Fusarium pallidoroseum* and control fruits (without pathogen) after different periods of incubation are given in Table 2. The results revealed that TSS in inoculated tomato fruits with *F. pallidoroseum* progressively decreased (3.08, 2.81, 2.67, 2.41, 2.35 & 2.04 %) as the incubation period increased (1st, 2nd, 3rd, 4th, 5th

Table 3. Change in physiological weight loss, ascorbic acid content and acidity of tomato fruits inoculated with *Fusarium pallidoroseum*

S. No.	Treatments	Fruit weight (g)	Physiological weight loss (g)	Percent Physiological weight loss (g)	Ascorbic acid (mg/100g)	Acidity (%)
1	2nd day	64.20	5.80	08.28	21.13	00.78
2	4th day	52.85	17.15	24.50	18.23	00.66
3	6th day	41.27	28.73	41.04	14.53	00.55
4	8th day	34.85	35.15	50.21	12.36	00.48
5	Control	70.00	00.00	00.00	24.03	00.87
	S.Em. ±				0.57	0.02
	C.D. at 5 %				1.82	0.04
	C.V. %				5.46	3.38

& 6th day), while in control fruit TSS was 3.83 per cent. Least TSS content (2.04 %) was found after 6th day of inoculation followed by 2.35 and 2.41 per cent on 5th and 4th day after inoculation, respectively. It was observed that TSS of tomato pulp decreased when inoculated with *F. pallidoroseum* as compared to control fruits (3.83 %).

Ghadsingh and Mandge (2012)[13] observed similar trend of results in tomato fruit infected with *Alternaria solani, Colletotrichum* sp., *Aspergillus niger* and *Phytophthora parasitica* revealing 2.7, 2.5, 2.1 and 1.8 per cent decrease in total sugar, respectively as compared to control (3.00%).

Oladiran and Lwu (1992)[14] observed that tomato fruits inoculated with *F. equiseti* and *F. clamydosporium* resulted decline in total soluble sugar and ascorbic acid content as the period was prolonged in storage. Total sugar, reducing and non-reducing sugar and ascorbic acid decreased faster in banana fruits inoculated with *Botryodiplodia theobromae, Fusarium oxysporum* or *Aspergillus niger* than in healthy fruits, showing maximum decrease in fruit infected by *B. theobromae* (Singh *et al*., 1991)[15].

Physiological weight loss

The result revealed that tomato fruits infected with *Fusarium pallidoroseum* showed loss in weight compared to uninoculated healthy fruits. Highest physiological weight loss (35.15 g) (50.21 %) was recorded after 8th day of inoculation followed by (28.73 g) (41.04 %) and (17.15 g) (24.50 %) on 6th and 4th day after inoculation, respectively. It was observed that physiological weight of tomato fruits decreased when inoculated with *F. pallidoroseum* as compared to control fruits (70.00g) (Table 3).

The results of present investigation were corroborate with the results obtained by Bashyal *et al* (2009)[16]. They reported higher physiological loss in weight in sweet orange and guava fruits infected with *Pestalotia psidii* (33%), and *Penicillium* sp. (36%), respectively as compared to uninoculated healthy fruits (48.78%). According to Chundawat *et al* (1976)[17], infected guava fruits had more enzymatic activities of the pathogen which resulted in physiological weight loss as compared to uninoculated fruits.

Ascorbic acid content

Changes in ascorbic acid content in tomato fruits inoculated with *F. pallidoroseum* were compared with that of control (uninoculated) fruits at different inoculation periods. The data presented in Table 3 revealed that there was appreciable decrease in ascorbic acid content in tomato fruits when inoculated with *F. pallidoroseum*. Significantly lowest amount of ascorbic acid content was (12.36 mg/100g) observed on 8th day after inoculation followed by 14.53, 18.23 and 21.13 per cent on 6th, 4th and 2nd day after inoculation, respectively. Thus, it was observed that ascorbic acid content was progressively decreased as the inoculation period is increased in inoculated tomato fruits as compared to control fruits (24.03 mg/100g). Similar to the present investigations, the depletion in ascorbic acid content was observed in tomato fruits inoculated with *F. equiseti, F. chlamydosporium, Geotrichum candidum* and *Aspergillus* spp., when compared with control fruits (Oladiran and Lwu, 1992)[14].

Ogaraku *et al*. (2010)[18] carried out studies on storage decay of tomato fruits and vitamin C content in fruits inoculated with *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Alternaria solani and Fusarium oxysporium.* The results revealed that the infected fruits contains 2.2 mg/100g vitamin C. While the healthy tomato fruits contains 2.51 mg/100g vitamin C.

Acidity content

The results of acidity content in fruits inoculated with *Fusarium pallidoroseum* and control fruits (without pathogen) after different periods of incubation are given in Table 3. The results revealed that acidity content of inoculated tomato fruits with *F. pallidoroseum* progressively decreased (0.78, 0.66, 0.55 & 0.48 %) as the incubation period is increased (2nd, 4th, 6th & 8th day) while in control fruit acidity was 0.87 per cent.

Sharma *et al.* (2011)[19] reported the post-infectional changes pertaining to physical, biochemical and nutritional aspects caused due to major post-harvest mycobial rot pathogens *viz. Alternaria alternata, Botryodiplodia theobromae, G. candidum, Penicillium digitatum and P. italicum* in kinnow fruits. The results revealed significant decrease in fruit acidity and ascorbic acid content as compare to uninoculated healthy fruits.

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