Int.J.Curr.Microbiol.App.Sci (2017) *6*(7): 1953-1961

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 7 (2017) pp. 1953-1961**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.607.232

Studies on Biochemical Changes and Changes in Cell Wall Degrading Enzymes in Papaya Fruit Inoculated with *Colletotrichum demetium*

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Keywords

Colletotrichum demetium, Poly ethylene glycol, *C. gloeosporioides*

Article Info

Accepted:
21 June 2017
Available Online:
10 July 2017

A B S T R A C T

Studies on biochemical changes (soluble sugar, phenol, protein, ash, phosphorus, calcium) and changes in cell wall degrading enzymes (PG, PMG and CX) in papaya fruit inoculated with *Colletotrichum demetium* was carried out. Total protein content of inoculated papaya fruits was decreased by 0.63, 0.58 and 0.36 per cent at 6th, 7th and 8th day after inoculation, respectively, while in control fruit protein content was 1.80 mg/g. The TSS content in inoculated papaya fruits is progressively decreased by 3.93, 3.71 and 3.42 per cent at 6th, 7th and 8th day after inoculation. The total phenol content in papaya fruits inoculated with *C. dematium* were appreciably increased up to 3rd day and after that there was gradual decline in phenol content observed. Ash content in inoculated fruits was decreased progressively as the duration after inoculation increased after 6th, 7th and 8th day of inoculation, respectively. Calcium content was gradually reduced after inoculation as there was an increase in incubation period. Significantly lowest calcium content was recorded on 8th day after inoculation (12.17 mg/100ml). The phosphorous content decreased by 7.73, 5.17 and 3.53 mg/100ml at 6th, 7th and 8th day after inoculation, respectively. The enzymatic activities of PG, PMG and CX were higher in ripe fruits as compare to semi ripe fruits, respectively.

Introduction

The papaya is the fruit of the plant *Carica papaya* L., the sole species in the genus *Carica* of the family *Caricaceae*. It is native of tropical America. It was first cultivated in Mexico. Papaya is called as "common mans" fruit which is due to reasonable price and has a high nutritive value. It is low in calories and rich in natural vitamins and minerals. Papaya place first among the fruits for vitamin C and A, riboflavin, folate calcium, thiamine, iron, pantothenic acid, niacin, potassium and fibre. The comparative low calories content (32 Kcal/100 gm of ripe fruits) make this favourite fruit of obese people who are into weight reducing regime (Krishna *et al.*, 2008; Thamaraikannan and Sengottuvel, 2012).

Papaya is prone to many diseases incited by fungi, bacteria, nematodes and viruses leading to enormous loss in yield. Among all, papaya anthracnose incited by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. appears to be more severe causing substantial losses to papaya fruits during transit and storage.

Materials and Methods

Estimation of total protein, total sugar and total phenol, calcium and phosphorous from infected and healthy tissues

Total protein content

Protein content was determined by the method developed by Lowry *et al.*, (1951). Take 1 g of sample and homogenized in five ml 0.1 N NaOH and filtered through Whatman No.1 filter paper. The sample extracts 1 ml was taken and made to 10 ml volume with distilled water. 5 ml of alkaline copper solution (50 ml 2% Na_2CO_3 in 0.1 N NaOH + 1 ml 0.5% $CuSO_4$ in 10% Sodium Potassium tartrate) was added. The content was allowed to stand for 10 minutes at room temperature followed by addition of 0.5 ml solution Folin Ciocalteu reagent (1:1 v/v). The content was kept for 30 minutes at room temperature and the absorbance was measured at 750 nm. The protein content was calculated using bovine serum albumin as standard range from 50 - 300 µg.

$$\text{True protein (\%)} = \text{Graph factor} \times \frac{\text{Sample reading}}{\text{weight of sample}} \times \frac{\text{Total volume}}{\text{Taken volume}} \times 10^{-4}$$

Total soluble sugar content

Total soluble sugar from the semi-ripe papaya pulp both inoculated with pathogen and uninoculated was determined by phenol sulphuric acid method as described by Dubois *et al.*, (1956). One gram of pulp sample was macerated in 5 ml 80% alcohol and taken in 30 ml sugar test tubes and total volume was made to 10 ml with 80 % alcohol. The test tubes were kept overnight. Next day take one ml supernatant from each test tube and was evaporated to dryness in water bath. After evaporation make the volume 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 5 per cent 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 take 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 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:

Total soluble sugar (g/100g) = Sample O.D x Graph factor (mg sugar) x 250/1000

Total phenol content

Total phenol content from the semi-ripe papaya pulp both inoculated and uninoculated was estimated by Folin ciocalteau method as described by Bray and Thorpe (1954) with some modification. Take 1g sample of papaya tissue and homogenized in 80% methanol using mortar and pestle and final volume was made to 10 ml. The content was refluxed for two hour on boiling water bath at 65°C. Supernatant was collected and residue was re-extracted twice with 80% methanol. All supernatant were combined and final volume was made to 25 ml. The extract was used for the assay of total phenol. Total phenol was estimated by following the method of Bray and Thorpe (1954) with some modifications. Aliquot 0.5 ml was taken and made the final volume 1.0 ml with distilled water. To this add 0.5 ml of Folin–Ciocalteu reagent and after 3 min 2 ml of 20% Na_2CO_3 was added and the tubes were incubated in boiling water

bath for 1 min., cooled it, and made total volume 10 ml with distilled water. The absorbance was measured at 650 nm. Phenol content was calculated from the standard curve prepared from Catechol.

$$\text{Total Phenols (\%)} = \text{Graph factor} \times \frac{\text{Sample reading}}{\text{weight of sample}} \times \frac{\text{Total volume}}{\text{Taken volume}} \times 10^{-4}$$

Estimation of calcium

Estimation of ash

2 gm of fruit pulp was placed in a previously weighed crucible and it was subjected for heating on hot plate till the sample was sufficiently turned black about 30 minutes. Then it was placed in muffle furnace, pre-heated to 600°C for 2 hours with automatic control. Crucible were transferred directly to desiccators, cooled and weighed immediately. Weigh of ash was obtained per 2 gm of sample and further calculated the ash content. An aliquot (25 ml) of the acid solution ash portion was diluted to about 150 ml with distilled water. Few drops of methyl red are added and the mixture is neutralized with ammonia (NH_3) solution till the pink colour changes to yellow. The solution was heated to boiling and the 10 ml ammonium oxalate solution was added.

The mixture was allowed to boil for a few minutes. Glacial acetic acid was then added till distinctly pink colour reappeared. The mixture was then kept aside for 12 to 24 hours at room temperature. When the precipitate at calcium oxalate settles down, it was filtered through Whatman's filter paper No.42. The precipitate was washed several times with distilled water, to make it free from acid. It was then transferred in a small beaker by piercing a hole in the filter paper and by pouring over it about 15 ml 2N H_2SO_4. This is heated to above 40°C and titrated against 0.01N $KMNO_4$ solution until the first drop which gives the solution a pink colouration persisting for at least 30 second. The amount of calcium was calculated using an equation. 1ml of $KMNO_4$=0.2004 mg of Ca. The percent Ca on dry Matter basis was then calculated on the basis of the amount of sample used for ashing, the volume to which acid solution of ash is diluted and the volume of the aliquot taken for the precipitation of calcium.

Estimation of phosphorous

0.5 ml of acid soluble portion of ash was taken in a test tube and diluted it to a volume of 10 ml with distilled water. Simultaneously a blank containing only 10 ml distilled water was taken and 1 ml molybdate solution was added to each test tube and mix, and then 0.4 ml ANSA reagent (In a 100-mL volumetric flask dissolve 2.0 g sodium sulphite (Na_2SO_3) in approximately 80 mL of reagent water. Add 0.25 g of 1-amino-2-napthol-4-sulfonic acid) was added and again mixes. Allowed to stand for 5 minutes and noted/observed the optical density (O.D.) at 660 nm using colorimeter by setting it to a zero with the blank.

Estimation of cell wall degrading enzymes on disease development

Semi-ripe were surface sterilized and separately inoculated with *Colletotrichum dematium* by 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). Five grams 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 cheese 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 30^0C. Each treatment was replicated for three times.

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

$$\frac{Vo - Vt}{Vo - Vw} X100$$

Where,

Vo = The flow time at 0 min
Vt = The flow time after 10/30/120 min
Vw = The flow time of distilled water.

Results and Discussion

Estimation of total protein, total sugar and total phenol, calcium and phosphorous from infected and healthy tissues

Total protein, total sugar and phenol

The results of total protein content in fruits inoculated with *Colletotrichum dematium* and control fruits at different periods are given in table 1.

The results revealed that total protein content of inoculated papaya fruits with pathogen (*C. dematium*) was decreased by 1.40, 1.25, 1.01, 0.91, 0.70, 0.63, 0.58 and 0.36 per cent at 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th day after inoculation, respectively while in control fruit protein content was 1.80 mg/g.

Least protein content (0.36 mg/g) was found after 8th day of inoculation which was at par with 7th day (0.58 mg/g) after inoculation. It was observed that protein content of papaya pulp decreased progressively as there is an increase in duration after inoculation with *C. dematium* as compared to control fruits (1.80 mg/g).

The results of total soluble sugar content in fruits inoculated with *Colletotrichum dematium* at different periods. The results revealed that TSS in inoculated papaya fruits with *C. dematium* progressively decreased by 10.08, 9.21, 7.59, 6.20, 5.63, 3.93, 3.71 and 3.42 per cent at 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th day after inoculation, respectively while in control fruit TSS was 11.07 per cent. Lowest TSS content was found after 8th day (3.42 %) of inoculation which was at par with 7th day after inoculation (3.71%).

It was observed that TSS of papaya pulp progressively decreased as there is an increase in duration after inoculation with *C. dematium* as compared to control fruits (11.07 %).

The results of total phenol content in papaya fruits inoculated with *C. dematium* were compared with that of control (uninoculated) fruits at different periods. The data revealed that there was appreciable increase in phenol content up to 3rd day after inoculation due to rapid accumulation and then there was gradual decrease in total phenol content in papaya fruits when inoculated with *C. dematium* at different periods.

Highest amount of total phenol content (544mg/g) was observed on 3rd day after inoculation followed by which was at par with 0.522, 0.544 and 0.495 mg/g on 2nd (0.522 mg/g) and 3rd day (0.516 mg/g) of inoculation, respectively. After 4th day of inoculation, there was gradual decrease in phenol content observed. Significantly lowest phenol content was recorded on 8th day (0.274 mg/g).

Phenol accumulated in the fruits infected by pathogen which might be inactivate the enzymes of parasite or pathogens by forming poly phenol oxydase which in turn prevented further advancement of pathogen by limiting its source of nutrients. The significance of phenols in papaya fruit is to trigger the resistance.

Ash, calcium and phosphorous content

Ash, calcium and phosphorous content of papaya fruits inoculated with *C. dematium* were assayed and compared with that of control (uninoculated) fruits at different periods. The data presented in table 2 revealed that there was decrease in Ash, Calcium and phosphorous content in papaya fruits when inoculated with *C. dematium* at different period as compared to control.

Ash Content in fruits inoculated with *Colletotrichum dematium* at different periods are given in table 2. The results revealed that Ash content of inoculated papaya fruits with *C. dematium* decreased progressively (393.33, 386.67, 377.33, 355.67, 337.67, 304.33, 273.00 and 263.33 mg/100g) as the duration after inoculation increased at 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th day after inoculation, respectively while in control fruit ash content was 403.67 mg/100g.

The calcium content from papaya tissues inoculated with *C. dematium* was progressively reduced as per the duration. The results indicated that the calcium content was reduced at inoculation and it was gradually reduced at subsequent periods of incubation. The lowest calcium content was recorded on 8th day of inoculation (12.17 mg/100ml) followed by 7th day (13.80 mg/100ml).

The results revealed that calcium content of inoculated papaya fruits with *C. dematium* decreased by 18.75, 18.02, 17.42, 16.43, 15.53, 14.90, 13.80 and 12.17 mg/100g at 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th day after inoculation, respectively while in control fruit calcium content was 19.53 mg/100 g.

The same trend of results was observed in phosphorous content. Changes in phosphorous content in papaya fruits inoculated with *C. dematium* were compared with that of control (uninoculated) fruits at different periods. The results revealed that phosphorous content of inoculated papaya fruits with *C. dematium* decreased by 14.07, 12.80, 11.60, 9.37, 9.27, 7.73, 5.17 and 3.53 mg/100ml at 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th day after inoculation, respectively.

While in control fruit phosphorous was 15.20 mg/100ml. Least phosphorous content (3.53 mg/100ml) was noted in 8th day of inoculation followed by 5.17 mg/100ml on 7th day after inoculation. Pathogens are responsible for reduction in biochemical content of papaya fruit which revealed that the fungi have utilized it as a substrate for development.

Table.1 Total protein, sugar and phenol content in papaya fruits inoculated with *Colletotrichum dematium*

Sr. No.	Treatments	Protein (mg/g)	Sugar (%)	Phenol (mg/g)
1	1st day	1.40	10.08	0.516
2	2nd day	1.25	9.21	0.522
3	3rd day	1.01	7.59	0.544
4	4th day	0.91	6.20	0.495
5	5th day	0.70	5.63	0.343
6	6th day	0.63	3.93	0.322
7	7th day	0.58	3.71	0.291
8	8th day	0.36	3.42	0.274
9	Control	1.80	11.07	0.442
	S.Em ±	**0.060**	**0.107**	**0.209**
	C.D. at 5%	**0.180**	**0.319**	**0.800**
	C.V. (%)	**3.829**	**2.749**	**2.11**

Table.2 Calcium and phosphorous content of papaya fruits inoculated with *Colletotrichum dematium*

Sr. No.	Treatments	Ash (mg/100ml)	Calcium (mg/100ml)	Phosphorous (mg/100ml)
1	1st day	393.33	18.75	14.07
2	2nd day	386.67	18.02	12.80
3	3rd day	377.33	17.42	11.60
4	4th day	355.67	16.43	9.37
5	5th day	337.67	15.53	9.27
6	6th day	304.33	14.90	7.73
7	7th day	273.00	13.80	5.17
8	8th day	263.33	12.17	3.53
9	Control	403.67	19.53	15.20
	S.Em ±	2.865	**0.305**	**0.120**
	C.D. at 5%	8.513	**0.907**	**0.357**
	C.V. (%)	1.442	**3.248**	**2.112**

Table.3 Impact of papaya fruit ripening on synthesis of cell wall degrading enzymes by *C. dematium*

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	19.13	23.24	31.85	24.94	14.09	22.09	30.30	22.16	14.81	22.33	30.41	22.52
Ripe	32.24	39.38	45.65	39.09	19.27	27.90	36.48	27.88	19.30	26.10	34.92	26.77
Healthy	9.46	13.58	21.27	14.77	9.88	12.47	17.27	13.20	8.34	11.67	16.80	12.27
Mean	20.28	25.60	32.93		14.41	20.82	28.07		14.15	19.90	27.37	
Source	**S. Em. +**		**C. D.**		**S. Em. +**		**C. D.**		**S. Em. +**		**C. D.**	
Stage (S)	0.47		1.86		1.54		6.17		0.138		0.391	
Period (P)	0.02		1.07		0.17		0.49		1.194		4.686	
S X P	0.04		0.12		0.30		0.86		Sig.		Sig.	
C.V. %	1.56				5.00				3.94			

CD at 5 %

Estimation of cell wall degrading enzymes on disease development

The activities of Polygalaturonase (PG), Polymethylgalacturonase (PMG) and Cellulolytic enzymes (CX) were studied at ripe and semi ripe fruit stage inoculated with *C. dematium* and healthy fruit. The enzymatic activities of PG, PMG and CX were higher in ripe fruits (39.09, 27.88 and 26.77%) than in semi ripe ones (24.94, 22.16, 22.52%), respectively.

The enzymatic activities of PG, PMG and CX were found to be increased with time. Highest reduction in viscosity was observed in PG in ripe fruit at 120 min with 39.09, 27.88 and 26.77 per cent respectively (Table 3).

The interaction effect between stage of fruit ripeness and period were found significant. The enzymatic activity (PG, PMG and CX) was lowest in healthy fruits as compared to inoculated fruits.

Results similar to the present investigation were reported by Patil and Pathak (1994). 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*.

The present results found similar to the result obtained by Agrawal and Agarwal (1982). According to them the phenolic metabolism of unripe papaya fruits inoculated with *C. dematium* was altered.

They reported that total phenol accumulated rapidly and remained in high concentrations for four days in tissues. Thereafter, total phenols markedly decreased in the virulent pathogen-host combinations. They concluded that significance of phenols in the resistance of papaya fruits to *Colletotrichum* spp. Majumdar and Pathak (1989) concluded that content of ascorbic acid, sugars and protein content was declined in the guava fruits infected by *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides* and *Pestalotiopsis versicolor.*

Maximum decrease in protein content was observed due to infection of *Colletotrichum gloeosporioides*. Rathod and Chavan (2012) revealed that Post-harvest fungi depleted and reduced the pectin, sugar, ash, phosphorous, calcium and ascorbic acid content of papaya fruit infected with *Colletotrichum gloeosporioides*.

They recorded gradual reduction in calcium and phosphorous content (11.5 mg/100ml and 8.4 mg/100ml) as compare to control (16.0 and 9.1 mg/100ml). Rajmane and Korekar (2014) reported that reducing sugar content of mango and papaya were found to be decreased due to some post-harvest fungi (*Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Penicillium chrysogenum Aspergillus niger*, *Aspergillus flavus*) as compare to control.

These differences may be due to deterioration caused by the fungi since fungi require some essential nutrients for growth and survival. Srivastava and Kumar *et al.,* (2010a) reported that the nutrient value of vegetables decreased due to infection of *Colletotrichum* sp. infecting capsicum.

The reduction in protein, reducing sugar and non-reducing sugar was noted in infected capsicum by *Colletotrichum* sp.

Paull and Chen (1983) studied enzyme like pectinmethylesterase (PME), poly galacturonase (PG), xylanase, cellulase and proteinase activity related to respiration, ethylene evolution and changes in skin color of papaya fruit from harvest till to the start of

fruit breakdown. The activity of PG declined to a level one-quarter of peak activity. Hossain *et al.*, (1999) also studied the activities of peroxidase, polyphenol oxidase and catalase. They noted that the activity increased about five, two and three times in infected leaves of mango with *Colletotrichum gloeosporioides* as compared to those in healthy leaves, respectively. A close relationship exists between PG, PMG and CX of inoculated papaya at different stages which rise in respiration, ethylene evolution, and softening of tissues.

Acknowledgement

The authors are highly grateful to the Professor and Head, Department of Plant Pathology and Principal, B.A. College of Agriculture, Anand Agricultural University, Anand, for providing necessary facilities and other faculty members for guidance and moral support.

References

Agrawal, P. and Agarwal, G. P. 1982. Alteration in the phenols of papaya fruits infected by *Colletotrichum* spp. *Proc. Indian Natn. Sci. Acad.* 3: 422-426.

Bell, T. A., Etchells, J. L. and Jones, I. D. 1955. A method for testing cucumber salt stock brine for softening activity. *U. S. Dept. Agr. Res. Serv*. pp. 72-75.

Bray, H. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. *Methods in Biochemistry Analysis*. 1: 27-52.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances**.** *Anal. Chem.* 28 (3): 350–356.

Hossain, M. T., Alam, M. Z. and Absar, N. 1999. Changes in different nutrients and enzyme contents in mango leaves infected with *Colletotrichum gloeosporioides. Indian Phytopath.* 52(1): 75-76.

Krishna, K. L., Paridhavi, M. and Patel, J. A. 2008. Review on nutritional, medicinal and pharmacological properties of Papaya (*Carica papaya*), *Natural product radiance*. **7** (4): 364-373.

Majumdar, V. L. and Pathak, V. N. 1989. Bio-control of postharvest diseases of Guava fruits by *Trichoderma* spp. *Acta Bot. Indica*. 23: 263-267.

Lowry, O. H., N. J. Rosebrough, Farr, A. L. and Randall, R. J. 1951. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem*. 193:265-275.

Paull, R. E. and Chen, N. J. 1983. Postharvest variation in cell wall-degrading enzymes of papaya (*Carica papaya* L.) during fruit ripening. *Plant Physiol*. 72:382-385.

Rajmane S. D. and Korekar S. L. 2014. Biochemical changes (Reducing Sugar) in different mango and papaya fruits varieties. *International Journal of Scientific and Research Publications*. 4(3): 1-3.

Rathod, G. and Chavan, A. M. 2012. Status of biochemical content in papaya (*Carica papaya* L.) after post-harvest pathogenesis by fungi. *Current Botany*. 3(3): 28-33.

Srivastava, A. and Kumar, S. 2013. Biochemical Changes in post harvested *Allium cepa* (Onion) and *Capsicum annuum* (Capsicum) under the influence of pathogens. *IOSR Journal of Agriculture and Veterinary Science.* 5(4):18-21.

Thamaraikannan, M. and Sengottuvel, C. 2012. Papaya cultivation and export, *Facts for you*, December-2012, pp. 21-24.

How to cite this article:

Prajapati, H.N., R.K. Patil and Shukla, Y.M. 2017. Studies on Biochemical Changes and Changes in Cell Wall Degrading Enzymes in Papaya Fruit Inoculated with *Colletotrichum demetium*. *Int.J.Curr.Microbiol.App.Sci.* 6(7): 1953-1961.
doi: https://doi.org/10.20546/ijcmas.2017.607.232