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Analysis of biochemical basis of resistance mechanism of aonla (*Emblica officinalis*) varieties against blue mould rot disease (*Penicillium islandicum*)

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ABSTRACT

Experiment was carried out at Chaudhary Charan Singh Haryana Agricultural University, Hisar to understand biochemical basis of resistance under *in vitro* conditions in aonla (*Emblica officinalis* Gaertn.). In biochemical basis of resistance, it was observed that TSS (total soluble solids), total phenol, peroxidase (PO) and polyphenol oxidase activity (PPO) was higher in diseased fruits in comparison to healthy fruits. TSS and total phenol increased in healthy and diseased fruits over time interval (5 and 10 days after inoculation). The titrable acidity was significantly lower in susceptible varieties, viz. Chakaiya (healthy fruit, 1.22 % and diseased fruit, 0.98 %) and Banarasi (healthy fruit, 1.40 % and diseased fruit, 1.32 %) as compared to resistant varieties Desi (healthy fruit, 1.50 % and diseased fruit, 1.42 %) and Kanchan (healthy fruit, 1.52 % and diseased fruit, 1.51 %). Ascorbic acid in resistant varieties Desi and Kanchan was 482.03 and 500.93 mg/100 g m aonla pulp in healthy fruits while 465.59 and 476.18 mg/100 g aonla pulp in diseased fruits after 10 days of storage. PO and PPO was found to be significantly higher in diseased fruits of resistant varieties, i.e. Desi (145, 20.49 OD/min/g) and Kanchan (139, 19.50 OD min/g) as compared to diseased fruits of susceptible Chakaiya (120, 16.19 OD/min/g) and Banarasi (118, 15.14 OD/min/g) at 5 DAI. However, the activity of PO and PPO decreased after 5 days of inoculation, while acidity and ascorbic acid was low in diseased fruits when compared to healthy fruits and further decreased over time interval. Ascorbic acid and acidity was decreased over time in both healthy and diseased fruits but at faster rate in diseased fruits as compared to healthy fruits.

Key words: Acidity, Ascorbic acid, Indian gooseberry, *Penicillium islandicum*, PO, PPO, Total phenol, TSS

Aonla or Indian gooseberry (*Emblica officinalis* Gaertn, syn. *Phyllanthus emblica* L.) is one of the most important indigenous fruit of Indian sub continent (Baghel *et al.* 2007). It grows in tropical and subtropical parts of India, China, Indonesia and the Malay Peninsula (Golechha *et al.* 2012 and Srivasuki 2012). The area under aonla cultivation in India is about 103.55 thousand ha and production 1225.21 thousand MT (Anonymous 2015). In India, nearly 20-35% of perishables fruits and vegetables are lost due to post harvest diseases (Rawal and Saxena 2005). Among post harvest diseases in aonla, fruit rot caused by *Penicillium islandicum* Sopp. is the most important as it affects the fruit quality and quantity in relation to the market value. The open wounds, created during harvesting, handling and packaging are the major sites of invasion by post harvest wound pathogens. Anola has been reported to be attacked by a number of pathogens during post harvest stage. Resistance and susceptibility reaction towards a pathogen is determined by the biochemical constituents present in the fruit. Degree and rate of change in the biochemical properties of fruits depend on the genotypic constituents of the variety that ultimately determines the outcomes of host-pathogen interactions. Phenolic compounds present in plant poses strong antioxidant activity that may help to protect cells against oxidative damage caused by free radicals (Kahkonen *et al.* 1999) which may be due to existence of flavonoids (Sabu and Kuttan 2002). Attack of post-harvest fungi is responsible for changes in biochemical contents of fruits (Pawar 2012, Rajmane and Korekar 2014). Plant enzymes are involved in defense reactions against pathogens by catalyzing the formation of lignins and other oxidative phenols which contribute to the formation of defense barriers for reinforcing the cell structure (Liang *et al.* 2011). Keeping in mind, therefore, the study was conducted to know biochemical resistance in aonla varieties.

MATERIALS AND METHODS

Aonla fruits of two resistant (Kanchan and Desi) and susceptible varieties (Chakaiya and Banarsi) each were inoculated with 7 days old culture of *Penicillium islandicum* by pin prick method (Granger and Horne 1924) to study the biochemical changes in the anola fruits at 5 and 10

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days of inoculation.

TSS in aonla fruits were estimated by using Abbe's hand refractometer (0-32^0 Brix). Ten fruits were selected from three replications of each cultivar for the analysis. One g of pulp of aonla fruit from resistant and susceptible varieties was macerated in pestle and mortar and was squeezed by putting it on muslin cloth. A drop of juice was taken and was put on Abbe's hand refractometer. Readings were taken in terms of ^{0} Brix. Mean values of ten fruit samples were expressed as TSS.

The total titratable acidity was determined by the standard method (AOAC 1990). One g of fruit pulp was macerated in a pestle and mortar by adding water. The extract was titrated against 0.1 N sodium hydroxide using 1% phenolphthalein as an indicator. The appearance of light pink color which persists for one minute was taken as end point. The acidity of fruit was expressed on percent basis.

$$\text{Total acidity (\%)} = \frac{\text{Titer value} \times \text{Normality of alkali} \times \text{Volume made} \times \text{Equivalent weight of acid}}{\text{Volume of sample taken} \times \text{weight of sample} \times 1000} \times 100$$

The aonla fruits of four different varieties were selected for this study. Ascorbic acid was determined by titration method of AOAC (1990). To a 1 g of fruit pulp sample, 25 ml of 3% metaphosphoric acid solution was added. The sample was macerated in pestle and mortar and the volume was made to 25 ml with metaphosphoric acid solution. It was filtered rapidly through Whatman No.1 filter paper. One g of aonla fruit pulp was mixed with 25 ml distilled water and it was filtered rapidly through Whatman No.1. Two ml of above aliquot was taken in 50 ml conical flask in duplicate and titrated against 2, 6-dichlorophenol indicator dye till the appearance of light pink color, which persisted at least for 5 sec. Similarly, 1 ml of standard ascorbic acid solution was titrated against 2, 6-dichloro indophenols dye. The results were calculated and expressed as mg of ascorbic acid per 100g of fruit pulp.

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titer value} \times \text{Total value made up}}{\text{Standard reading} \times \text{Volume taken} \times \text{Weigjht of sample taken}} \times 100$$

The samples (fruit pulp) were collected at after 5 and 10 days of after inoculation and also from the healthy (uninocualted fruit). Extraction and estimation of total phenols was carried out by standard methods (Mahadevan and Sridhar 1982). One g of the sample was taken and ground in previously chilled pestle and mortar in 10 time volume of 80 % ethanol. The extract passed through two layers of chease cloth. The homogenate was centrifuged at 10000 rpm for 20 min in a refrigerated centrifuge. The supernatant was saved in refrigerator. The residue was re-extracted with five times the volume of 80 % ethanol, centrifuged and the final volume of the supernatant was made to 10 ml with 80 per cent Ethanol. The supernatant was evaporated in vacuum at 40ºC to dryness. The residue was dissolved in a known volume of distilled water (5 ml). One ml of extract was pipetted into a graduated test tube, to which 1 ml of folin-ciocalteau reagent was added. After 3 min, add 2ml of Na_2CO_3 (20 %) for alkaline medium. The tube was shunken and heated in a boiling water bath for exactly 1 min and then cooled under running tap water. Absorbance of resulting blue solution was measured at 650 nm in spectrophotometer against a reagent blank. For comparison a blank containing all the reagents minus folin reagent was used to adjust the absorbance to zero. Total phenol was determined as catechol equivalent after comparing with the standard curve prepared from distilled catechol (1-5 mg/ml) obtained by using same reagents. Total phenol was expressed as mg/g fresh weight of tissue.

Peroxidase activity was measured following the method of Mahadevan and Sridhar (1982). Samples were collected from uninoculated and inoculated fruits at 5 and 10 days post-inoculation. Sample required for enzymatic studies were collected in plastic bags and deep frozen until used.

One g of fresh fruit pulp was extracted in 10 ml of 0.1 M phosphate buffer pH 7 by grinding with a pre-cooled mortar and pestle. The homogenate was centrifuged at 10000 rpm for 5 min. The supernatant thus obtained was used as enzyme source (within 2-4 hr). The extract was stored in a refrigerator. In preliminary studies, it was first established that under the assay conditions employed, the rate of enzyme catalyze reaction was proportional both to the amount of enzyme as well as reaction time. In a clean dry cuvette 3 ml buffer was taken. Added 0.1 ml enzyme extract and 0.1 ml freshly prepared 0-dianisidine solution. The cuvette was placed in the spectrophotometer set at 430 nm then after adding 0.2 ml H_2O_2 and mixed the contents. The initial absorbance and at every 30 sec intervals upto 3 min was read. The time required was noted in min (Δt) to increase the absorbance by 0.1 OD. Enzyme activity was expressed in terms of units. A blank was also used in assay. Blank did not contain H_2O_2.The increase in absorbance was plotted against time. From the linear phase or average value, the change in absorbance per 30 sec was recorded. Enzyme activity was expressed in terms of rate of increased absorbance/min/g tissue weight.

Activity of polyphenol oxidase was assayed following the method of Mahadevan and Sridhar (1982). Root samples were collected from uninoculated and inoculated fruits at 5 and 10th days post-inoculation. Sample required for enzymatic studies were collected in plastic bags and deep frozen until used.

One g of fresh fruit pulp was extracted in a pre-cooled glass mortar with 5 ml of 0.05 M Tris-HCl buffer (pH 7.2). The homogenate was centrifuged at 20000 rpm for 10 min in refrigerated centrifuge and used as a source of enzyme. In a dry cuvette 2.5 ml of 0.1 M phosphate buffer and 0.3 ml of catechol solution (0.01 M) were taken. Cuvette was placed in spectrophotometer at 495 nm to set the absorbance at zero. Enzyme extract was added and the change in absorbance for every 30 seconds up to 5 min was recorded. A suitable blank was also used in assay. The

Table 1 Total soluble solids of aonla due to *Penicillium islandicum* inoculation in different varieties

Variety	TSS (°/Brix)			
	5 DAI**		10 DAI	
	Healthy Fruits	Diseased fruits	Healthy fruits	Diseased fruits
Chakaiya	9.60 ± 0.070 *	11.87 ± 0.050	10.83 ± 0.036	13.66 ± 0.043
Banarsi	9.93 ± 0.043	12.66 ± 0.042	11.27± 0.041	14.32 ± 0.060
Desi	8.52 ± 0.043	10.32 ± 0.034	9.43 ± 0.047	11.27 ± 0.041
Kanchan	9.00 ± 0.040	11.15 ± 0.043	9.50 ± 0.042	11.93 ± 0.034
CD (P=0.05) DAI		0.023		
Healthy × Diseased fruits		0.023		
Varieties		0.032		

*Average of four replications, **Days after inoculation

enzyme activity was expressed as change in absorbance (□ 0.1 OD)/min/g fresh tissue. The increase in absorbance was plotted against time. From the linear phase or average value, the change in absorbance per 30 sec was read. Express enzyme activity in terms of rate of increased absorbance/ min/g/tissue weight.

RESULTS AND DISCUSSION

Total soluble solids (TSS)

Data depicted in Table 1 indicated the changes in the level of TSS in inoculated and healthy fruits at 5 and 10 days after inoculation. The data in the table revealed significant reduction in TSS due to pathogenesis. TSS content was higher in healthy and diseased fruits of susceptible varieties then resistant ones. Susceptible var. Chakaiya fruits showed 10.83^0 and 13.66^0 Brix TSS content in healthy and diseased fruits, respectively. Another susceptible cv Banarsi had 11.27º and 14.32º Brix TSS in the two categories respectively. The TSS content in cultivar Desi was 9.43º and 11.27º Brix in healthy and diseased fruits. The second resistant cultivar contained Kanchan 9.50º Brix in healthy fruit and 11.93º Brix in diseased fruits after 10 days of inoculation. The increase in TSS was more in diseased fruits as compared to healthy fruits and increased with the time of storage.

Acidity

A perusal of data in Table 2 showed a significant reduction during incubation/storage in the levels of titrable acidity both in healthy and inoculated (disease) fruits. The titrable acidity decreased significantly in susceptible varieties, viz. Chakaiya (healthy fruit, 1.22 % and diseased fruit, 0.98 %) and Banarasi (healthy fruit, 1.40 % and diseased fruit, 1.32 %) as compared to resistant varieties Desi (healthy fruit, 1.50 % and diseased fruit, 1.42 %) and Kanchan (healthy fruit, 1.52 % and diseased fruit, 1.51 %). A gradual decline in titrable acidity was observed during passage of incubation time however, the rate was more pronounced in diseased than healthy tissues. The balance between TSS and acidity is fundamentally responsible for the flavor in the fruits and later forms an important source of respiratory energy in the plant cell.

Ascorbic acid

The comparative effect of *Penicillium islandicum* in utilization of ascorbic acid in inoculated (diseased) aonla fruits at different intervals of incubation was mentioned in Table 3. From the data, it is evident that ascorbic acid content gradually declined during pathogenesis, storage but the decline was significantly more in diseased fruits. All the varieties varied in their capability to affect reduction in ascorbic acid content. The fall in ascorbic acid was more

Table 2 Changes in acidity of aonla due to *Penicillium islandicum* inoculation in different varieties

Variety	Acidity (%)			
	5 DAI**		10 DAI	
	Healthy Fruits	Diseased fruits	Healthy fruits	Diseased fruits
Chakiya	1.60 ± 0.085*	1.32 ± 0.033	1.22 ± 0.029	0.98 ± 0.033
Banarsi	1.70 ± 0.037	1.47 ± 0.036	1.40 ± 0.051	1.32 ± 0.033
Desi	1.84 ± 0.047	1.79 ± 0.029	1.50 ± 0.057	1.42 ± 0.039
Kanchan	1.90 ± 0.051	1.88 ± 0.045	1.52 ± 0.051	1.51 ± 0.051
CD (P=0.05) DAI		0.045		
Healthy × Diseased fruits		0.045		
Varieties		0.054		

*Average of four replications, **Days after inoculation.

Table 3 Ascorbic acid content of aonla after inoculation of *Penicillium islandicum* inoculation in different varieties

Variety	Ascorbic acid (mg/100 g fruit pulp)			
	5 DAI**		10 DAI	
	Healthy fruits	Diseased fruits	Healthy fruits	Diseased fruits
Chakiya	535.90 ± 4.05*	250.99 ± 9.41	513.48 ± 4.65	141.19 ± 5.75
Banarsi	514.53 ± 5.81	246.87 ± 8.18	498.92 ± 7.37	132.52 ± 5.75
Desi	495.18 ± 6.74	476.81 ± 8.66	482.03 ± 4.60	465.59 ± 6.60
Kanchan	510.01 ± 4.13	491.56 ± 8.30	500.93 ± 5.28	476.18 ± 6.90
CD (P=0.05) DAI		3.313		
Healthy × Diseased fruits		3.313		
Varieties		4.685		

*Average of four replications, **Days after inoculation.

apparent in susceptible varieties then resistant ones. The susceptible varieties Chakaiya and Banarsi possessed 513.48 and 498.92 mg/100g aonla pulp in healthy fruits, whereas 141.19 and 132.52 mg/100 g aonla pulp in diseased fruits. The figures for resistant varieties Desi and Kanchan were 482.03 and 500.93 mg/100 g aonla pulp in healthy fruits and 465.59 and 476.18 mg/100 g aonla pulp in diseased fruits after 10 days of storage.

Total phenol

Phenolic compounds play a predominant role in determining the colour and flavor of fruits. Phenolic make a vast class of compounds comprising anthocyanins, leucoanthocynins, glycosides, sugars etc. The pattern of changes in phenols in the pulp of healthy and diseased fruits of aonla are depicted in Table 4. The statistical analysis for total phenols revealed that all the varieties differed in causing reduction in phenol content of fruits at every stage of pathogenesis. The healthy fruits of susceptible varieties Banarsi possessed 1.58 total phenols (mg/g fresh pulp weight) followed by Chakaiya, where it was 1.53 mg/g fresh pulp weight after five days of storage. A gradual fall in the content was recorded in diseased fruits pulp at values were 1.42 and 1.41 mg/g fresh pulp weight in the two varieties, respectively. After 10 days decreasing trend in content was recorded in both the cases. The resistant varieties had more phenols content than susceptible ones at both the intervals of incubation/storage.

Peroxidase activity

The relative activity of healthy and diseased tissue in susceptible Chakaiya and Banarsi and resistant (Desi and Kanchan) at five and 10 days of incubation/storage is indicated in Table 5. There was gradual decline in the activity of peroxidase in the tissues of susceptible and resistant varieties after 10 days of incubation, however, an increase in enzyme activity was recorded after five days of incubation/storage. This increase was more prominent in diseased tissues of resistant varieties. The activity in Desi (seedling) was 112/min/g pulp of healthy fruits and 110/min/g in cv Kanchan, whereas in diseased tissue it was 145/min/g in the former and 139/min/g in the latter case. The enzyme activity significantly reduced in both healthy and diseased tissues after 10 days of incubation/storage.

Polyphenol oxidase activity

Analysis of data on polyphenol oxidase activity in healthy and diseased aonla fruits (Table 6) revealed that the enzyme activity in healthy and diseased fruits was reduced during incubation, however considerably stimulated upon inoculation. The enzyme activity was found to be higher in resistant cv Desi (healthy fruit 15.02 OD min/g and

Table 4 Changes in total phenol of aonla due to *Penicillium islandicum* inoculation in different varieties

Variety	Total phenol (mg/g fresh pulp weight)			
	5 DAI**		10 DAI	
	Healthy fruits	Diseased fruits	Healthy fruits	Diseased fruits
Chakaiya	1.41 ± 0.017*	1.48 ± 0.034	1.53 ± 0.029	1.61 ± 0.025
Banarsi	1.42 ± 0.022	1.53 ± 0.034	1.58 ± 0.021	1.63 ± 0.034
Desi	1.59 ± 0.026	1.72 ± 0.034	1.62 ± 0.033	1.81 ± 0.059
Kanchan	1.45 ± 0.017	1.64 ± 0.039	1.81± 0.026	2.01 ± 0.064
CD (P=0.05) DAI		0.017		
Healthy × Diseased fruits		0.017		
Varieties		0.025		

*Average of four replications, **Days after inoculation.

Table 5 Changes in peroxidase activity in aonla fruits due to *Pencillium islandicum* inoculation in different varieties

Variety	Peroxidase activity (changes in OD/ min/g of sample)			
	5 DAI**		10 DAI	
	Healthy Fruits	Diseased fruits	Healthy Fruits	Diseased fruits
Chakiya	108 ± 2.16	120 ± 3.56	106 ± 2.16*	118 ± 2.58
Banarsi	107 ± 1.63	118 ± 2.94	105 ± 2.16	116 ± 2.94
Desi	112 ± 0.82	145 ± 1.63	109 ± 2.58	128 ± 2.58
Kanchan	110 ± 1.83	139 ± 2.16	107 ± 2.58	123 ± 3.37
CD (P=0.05) DAI		1.232		
Healthy × Diseased fruits		1.232		
Varieties		1.742		

*Average of four replications, **Days after inoculation.

Table 6 Changes in polyphenol oxidase activity in aonla fruits due to *Pencillium islandicum* inoculation in different varieties

Varieties	Polyphenol oxidase activity (changes in OD/min/g of sample)			
	5 DAI**		10 DAI	
	Healthy Fruits	Diseased fruits	Healthy Fruits	Diseased fruits
Chakaiya	13.35 ± 0.67	16.19 ± 1.85	12.34 ± 0.56*	15.36 ± 1.41
Banarsi	13.28 ± 1.31	15.14 ± 1.28	11.59 ± 1.01	14.58 ± 1.05
Desi	15.02 ± 0.85	20.49 ± 0.51	13.52 ± 0.41	18.19 ± 1.39
Kanchan	13.95 ± 0.35	19.50 ± 0.83	13.14 ± 1.07	16.22 ± 1.23
CD (P=0.05) DAI		0.54		
Healthy × Diseased fruits		0.54		
Varieties		0.76		

*Average of four replications, **Days after inoculation.

diseased fruit 20.49 OD min/g) and Kanchan (healthy fruit 13.95 OD min/g and diseased fruit 19.50 OD min/g) as compared to susceptible cv Chakaiya (healthy fruit 13.35 OD min/g and diseased fruit 16.19 OD min/g) and Banarsi (healthy fruit 13.28 OD min/g and diseased fruit 15.14 OD min/g) after 5 days of inoculation. The polyphenol oxidase activity declined significantly after 10 days inoculation in both healthy and diseased fruits.

The fruit flavour is fundamentally due to balance between total soluble solids and acids while the latter form an important source of respiratory energy in the fruit cell. The pattern exhibited by TSS in healthy and diseased fruits in different varieties revealed that TSS content was higher in susceptible varieties (Chakaiya and Banarsi) as compared to resistant varieties (Desi and Kanchan). TSS increased in diseased fruits as compared to healthy fruits and also increased with incubation/storage. Total soluble solid content is an important quality factor for fresh fruits (Lu 2004) because solids include the soluble sugars such as sucrose, glucose and fructose as well as acids. Increase in TSS is probably due to the hydrolysis of starch to soluble sugars such as glucose, sucrose and fructose (Bashir and Abu – Goukh 2003 and Soltani *et al.* 2010). Another possible reason for increase in TSS might be due to the conversion of organic acids to sugars through gluconeogenesis (Echeverria and Ismail 1987) and loss of water and thus increase in concentration. The Navel orange fruits inoculated by *Penicillium digitatum* and *P. italicum* had higher TSS content (Tarabih and Metwally 2014). The present study confirms the same trend for aonla infected with *P. islandicum*. On the contrary, decline in TSS in blue and green mould infected mandarin orange has been advocated by Dutta *et al.* (2009). This may be attributed to the fruit quality of aonla.

The acidity content of aonla fruits decreased both in healthy and diseased/inoculated fruits during incubation/ storage period in all the varieties has been observed. Decrease in acidity during storage has also been reported in aonla fruits (Singh *et al.* 2005), grapes (Shiri *et al.* 2011). Pear (Nath *et al.* 2012) and jujube fruits (Xing *et al.* 2015). Maximum acidity content was recorded in resistant varieties (Desi and Kanchan) and minimum in susceptible varieties (Chakaiya and Banarsi). This might be due to the reason that different varieties have different rate of respiration as well as physiological processes. *Penicillium islandicum* affect acidity content of aonla fall in line with the findings of Dutta *et al*. (2009) in blue and green mould of mandarin orange.

Ascorbic acid is believed to act as one of the biological oxidative –reductive substance. It is known to contribute resistance to host against pathogenic organisms and is decarboxylated with fungal enzyme system. Ascorbic acid declined during incubation/storage in all the varieties.

Gradual decrease in ascorbic acid content as the incubation progressed was observed in amla fruit rot incited by *Phoma exigua* (Reddy and Laxminarayana 1984). The decreasing trend in ascorbic acid content during incubation/storage could be attributed to the conversion of ascorbic acid into dehydroascorbic acid in presence of ascorbic acid oxidase enzyme (Nayak *et al.* 2011). Nayak *et al.* (2011) also observed decrease in ascorbic acid content in aonla during storage. Decrease in ascorbic acid content has also been reported in pear (Nath *et al.* 2012) and ber (Shahi *et al.* 2015) during storage. Among the different varieties of aonla studied, maximum decline in ascorbic acid content was recorded in susceptible varieties (Chakaiya and Banarsi) compared to resistant varieties (Desi and Kanchan). Considerable variation in decreasing trend of ascorbic acid content in different varieties of aonla might be associated with genetic variability among the varieties. The decreasing ascorbic acid content might also be associated with differential activity of ascorbic acid oxidase in the fruits. Singh *et al.* (2005) and Gangwar *et al.* (2012) has also reported similar trend in different varieties. Tandon (1970) found that ascorbic acid of mango pulp was decresed due to *Aspergillus niger*. The infected (*Aspergillus niger* and *A. flavus*) bananas showed a decrease in the quantity of ascorbic acid (Sawant and Gawai 2011).

Phenolic compounds play predominant role in determining colour and flavour of fruits. Phenolics make a vast class of compounds comprising anthocynins, leucoanthocynins, anthoxanthin-hydrobenzoic acid, glycosides, sugars, esters of quinic and shikimic acids, esters of hydroxy cinnamic acids and coumarin derivatives. The biochemical changes in plant-pathogen interactions are accompanied by rapid increase in phenolic compounds (Khan and Ravise, 1985). The phenolic compounds in fruit tissues have been related with disease resistance in number of plant-parasite interaction (Padule *et al.* 1989, Gupta *et al.* 1990, Sawant and Gawai, 2011, Pradeep and Jambhale 2002).The pattern of changes in phenolics in the pulp of control and inoculated fruits of aonla exibited increased total phenols with increasing period of incubation/storage in all the varieties of aonla. The key enzyme in phenol biosynthesis is phenylalanine ammonium lyase, which leads to formation of phenols (Leja *et al.* 2001). Leja *et al.* (2001) reported that an increase in phenol content might also be due to lower activity of polyphenol oxidase, so that oxidation processes were minimized. In present investigation, increase in phenol content might have been associated with either increase in PAL or decrease polyphenol oxidase enzyme activity. Increase in total phenol content has been reported by Singh and Kumar (2000) and Neeraj *et al.* (2002) in aonla and Tehrani *et al.* (2011) in Jambu air fruits during storage. Among the different varieties of aonla studied, total phenol content was shown to be variety dependent. Variation of increase in phenol during incubation/storage among the different varieties of aonla might be due to differential activity of PAL and polyphenoloxidase enzyme. The present results are in close agreement with earlier findings of Pradeep and Jambhale (2002) who observed significantly higher amount of total phenols noticed in unripe ber fruits of resistant genotypes Guli and Vilaiti (0.8 and 0.6) and susceptible types Chhuhara and Illaichi (0.9 and 0.8) as compared to susceptible varieties Umran and Dandan (0.4 and 0.5) to powdery mildew.

The oxidative enzymes, such as peroxidase are known to be associated with the browning of host tissues. They are also capable of oxidizing phenolics and related compounds, thus, increasing their toxicity (Kosuge 1969). The peroxidase enzymes are reported to regulate protein synthesis and phenyl propanoid cycle in host. In our study, gradual decline in the activity of peroxidase in the tissues of susceptible and resistant varieties after 10 days of incubation, however, an increase in enzyme activity was recorded after five days of incubation/storage. This increase was more prominent in diseased tissues of resistant varieties. The activity in Desi (seedling) was 112/min/g pulp of healthy fruits and 110/ min/g in cv Kanchan, whereas in diseased tissue it was 145/min/g in the former and 139/min/g in the latter case. The enzyme activity significantly reduced in both healthy and diseased tissues after 10 days of incubation/storage. Several workers (Padule 1989, Pradeep and Jambhale 2002, Gayaso *et al.* 2004, Ali *et al.* 2005, Sarkar *et al.* 2010) have reported similar changes in the level of oxidative enzymes due to fungal infection. Phenolic compound and related oxidative enzymes are mostly considered as one of the important biochemical parameters for disease resistance and also that the accumulation of total phenol is usually higher in resistant varieties as compared to susceptible ones (Gupta *et al.* 1990).

Polyphenol oxidase (PPO) is a key enzyme of phenylpropanoid metabolism. Kenten (1956) found that rapid initial increase in the activity of PPO in plant tissue after infection results from the accumulation of latent phenolase or from solubilizing phenolase from cellular structure. An increase in polyphenol oxidase activity has been noted in infected tissues, and is believed to block the infection through its oxidation products (Lovrekowich *et al.* 1967, Weber *et al.* 1967). Higher activity of PPO was correlated with resistance of host tissue to various pathogens. This could be due to the conversion of phenols by activity of this enzyme to fungitoxic compounds, quinones, which are more toxic to the pathogens than the phenolics (Retig 1974, Vidhyasekaran 1988). The enzyme activity was found to be higher in resistant cv Desi (healthy fruit 15.02 OD min/g and diseased fruit 20.49 OD min/g) and Kanchan (healthy fruit 13.95 OD min/g and diseased fruit 19.50 OD min/g) as compared to susceptible var. Chakaiya (healthy fruit 13.35 OD min/g and diseased fruit 16.19 OD min/g) and Banarsi (healthy fruit 13.28 OD min/g and diseased fruit 15.14 OD min/g) after 5 days of inoculation. The polyphenol oxidase activity declined significantly after 10 days inoculation in both healthy and diseased fruits. Almost similar trend was observed for powdery mildew of ber (Pradeep and Jambhale 2002). Sarkar *et al.* 2010 also reported that PPO activity increased considerably due to *Macrophomina phaseolina*

causing fruit rot in banana.

In biochemical basis of resistance, TSS (Total soluble solids), total phenol, peroxidase (PO) and polyphenol oxidase activity (PPO) was higher in diseased fruits in comparison to healthy fruits. TSS and total phenol was increased in healthy and diseased fruits over time interval (5 and 10 days after inoculation) also. PO and PPO activity decreased after 5 days of inoculation, While acidity and ascorbic acid was low in diseased fruits and further decreased over time interval (5 and 10 days after inoculation). Ascorbic acid and acidity were also decreased over time in both healthy and diseased aonla fruits but at faster rate in diseased fruits.

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