INDIAN JOURNAL OF ARID HORTICULTURE
2015, Vol. 10 (1-2):13-17

Physiological and biochemical changes in ber (*Zizyphus mauritiana* Lamk.) due to *Pestalotiopsis* fruit rot

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(Received: 27.03.2015; Accepted: 15.02.2016)

Abstract

In the present experiment, different physiological and biochemical parameters of ber infected with *Pestalotiopsis* fruit rot were studied. The sites of inoculation of ber fruits were tested and the inoculation at epicarp surface was found most sensitive and showing maximum severity of *Pestalotiopsis* rot. The mature and ripe stage inoculated fruits exhibited maximum severity. Changes in biochemical activity indicated that the level of total soluble solids, acidity, ascorbic acid and phenols in healthy ripe and semi-ripe ber fruits were higher as compared to infected ripe and semi-ripe fruits. The total soluble solids in inoculated semi-ripe fruits was significantly lower than uninoculated semi-ripe fruits and inoculated ripe fruits, it was lower than uninoculated ripe fruits. The level of acidity in the inoculated ripe and semi-ripe ber fruits was significantly reduced than uninoculated ripe and semi-ripe fruits. The level of ascorbic acid in inoculated ripe and semi-ripe fruits was significantly low over control. The healthy fruits (semi-ripe stage) of ber had much larger amount of ascorbic acid as compared to the diseased fruits. The activity of total phenols was higher in healthy and diseased semi-ripe fruits (0.62 and 0.48 mg/100 gm fruits) as compared to healthy ripe and diseased ripe fruits (0.52 and 0.19 mg/100 gm fruits), respectively.

Key words: *Pestalotiopsis palmarum*, ber, total soluble solids, acidity, ascorbic acid and phenols.

Introduction

Ber (*Zizyphus mauritiana* Lamk.) is one of the most ancient and common fruits consumed by people of India. It is often called the poor man's fruit. Fruit spoilage at post-harvest stage has gained the greatest significance in view of the loss incurred. Understanding on the mechanism of fruit-rot will help to prevent fruit losses caused by pathogenic fungi. A large number of fungi cause rotting of ber fruits in the market. The most frequently occurring fungi are *Pestalotiopsis palmarum, Alternaria alternata, Cladosporium oxysporum, Rhizoctonia solani, Fusarium solani* and *Aspergillus niger*. Average incidence of various fruit rotting diseases ranged from 2.0 to 15.3 per cent (Jat *et al.*, 1997). Nevertheless, fruit rots are also important problem of orchards and markets in arid and semi-arid regions. Pestalotiopsis rot of ber fruits, caused by *Pestalotiopsis palmarum* (Cooke) Steyart is a major post-harvest fruit rot. Ber fruit rots have been identified as major problem next to powdery mildew. Endemic forms of fruit rot diseases are persisting in Western parts of Rajasthan (Nallathambi *et al.*, 2000). The injuries on three sites of fruit surface *viz.*, stem-end, epicarp and stylar-end can occur during harvest or handling. To understand the impact of different types of site of infection on disease incidence and severity of Pestalotiopsis rot in ber fruits, the present study was conducted and attempts were made to analyze the site of infection, the activity of total soluble solids, acidity, ascorbic acid and phenols in ripe and semi-ripe stages of ber fruits un inoculated and inoculated with *Pestalotiopsis palmarum*.

Materials and Methods

Effect of inoculation sites on disease development

The semi-ripe fruits collected from Bikaner fruit market as well as Horticulture farm (College of Agriculture, Bikaner) were brought to the laboratory in paper bags. The fruits were surface sterilized with 0.1 per cent mercuric chloride and injured in the following manner: (1) Pricked with sterilized pins at stem-end (2) Pricked in epicarp (3) Pricked at the stylar-end (4) Control (uninjured and inoculated) (5) Control (uninjured and un-inoculated).

The fruits were pricked to a depth of 2 mm on fruit surface. The injured and uninjured fruits were separately inoculated with spore suspension (10^6 spores/ml) of *Pestalotiopsis palmarum*. The injured and control fruits were surface sterilized (dipping in $HgCl_2$ 0.1 per cent for 2 minutes followed by three washings with sterilized water) and then separately inoculated with *Pestalotiopsis palmarum* by dipping them in spore suspension for 2 minutes. The fruits were then air-dried for 15-20 minutes. The inoculated as well

as un-inoculated fruits were placed in sterilized polythene bags and four fruits were accommodated in each bag and incubated at 25 2C.

Effect of fruit ripeness on disease severity

Fruits of uniform size at following stages of ripeness were inoculated by the pin-pricking method. (1) Unripe fruits (green in colour) (2) Semi-ripe fruits (light pale colour) (3) Ripe fruits (fruit surface tone yellow). Fruits at these three stages were inoculated with uniform amount of inoculum. The inoculated fruits were placed in polythene bags and incubated at 25 2C. The experiment was arranged in completely randomized design. The fruits were examined daily till the appearance of the symptoms to record incubation period. The disease incidence and disease severity were recorded on 4th, 8th and 12th day of inoculation.

Effect on biochemical changes

Total Soluble Solids (T.S.S.) : Pulp from five randomly selected infected as well as healthy fruits were taken and macerated for juice extraction and total soluble solids of the juice was determined by using a hand refractometer of 0-30 per cent range. The values were corrected at 20C and expressed as per cent total soluble solids of the fruit juice (A.O.A.C., 1990).

Acidity: The acidity was determined by diluting the known volume of clean juice, filtered through muslin cloth and diluted with distilled water. The dilute juice titrated against standard N/10 sodium hydroxide solution using phenolphthalein as an indicator. The appearance of light pink colour was marked as the end point. The result was expressed in terms of per cent acidity of the fruit juice (A.O.A.C. 1990).

Ascorbic acid : 10 ml of sample was taken and made up to 100 ml with 3% metaphosphoric acid and filtered. Pipetted 10 ml of filtrate into a conical flask and titrated with the standard dye to a pink end-point.

Phenols: Total phenols were estimated by the method described by Bray and Thorpe (1954). One gram of sample was ground with a pastle and mortar in 10 time volume of 80 per cent ethanol. The homogenate was centrifuged at 1000 rpm for 20 minutes. The supernatant was filtered and the residue was re-extracted with five time volume of 80 per cent ethanol, supernatant was cooled and evaporated to dryness in water bath. The residue was then dissolved in 5 ml of distilled water. Different aliquots (0.2 to 2 ml) were then pipetted out into test tubes and the volume in each tube was made to 3 ml with distilled water. Then 5 ml of Folin-ciocalteau reagent was added in each sample and mixed thoroughly. After 3 minutes, 2 ml of 20 per cent sodium carbonate solution was poured in each tube and thoroughly mixed. The tubes were then placed in a boiling water for one minute. After cooling the absorbance was measured at 650 nm against a reagent blank. The corresponding phenol content was plotted on a standard curve, which was prepared by taking different concentrations of catechol ranging from 10 to 50 g/ml in water.

Total sugar content: Total sugar content was determined by colorimetric method using anthrone reagent. One ml of diluted fruit juice (100 times) 4 ml of anthrone reagent was added, heated, for 20 minutes in a water bath, cooled to room temperature and absorbance was measured at 630 nm on Spectronic-20. The amount of sugars present in the juice was plotted against standard curve prepared from glucose. The content was expressed on percentage basis (Dubois *et al.*, 1951). Reducing Sugar content was measured by following "Nelson's modification of "Somogyi's method" (Somogyi, 1952).

Results and Discussion

Effect of site of inoculation on severity of Pestalotiopsis rot of ber fruits

On 4th day of inoculation, the semi-ripe ber fruits showed incidence of the rot irrespective of the site of inoculation (Table 1). The fruits inoculated at epicarp (60%) and stylar-end (60%) showed higher incidence of Pestalotiopsis rot than in fruits inoculated at stem-end (40%) fruit surface. The severity (9.56%) of the rot in fruits inoculated at epicarp was more than in fruits inoculated at stem-end (7.69%) and stylar-end (6.13%). The inoculation at epicarp rendered significantly higher severity than the fruits inoculated at stem-end, stylar-end and controls (uninjured inoculated and uninjured uninoculated). It can be concluded that all the sites of inoculation differed significantly from uninjured controls.

On 8th day of inoculation, the fruits inoculated at epicarp, stem-end and stylar-end showed higher incidence (100%) of Pestalotiopsis rot on fruit surface than uninjured inoculated (25%) fruits. The severity (19.06%) of the rot inoculated at epicarp was more than in fruits inoculated at stem-end (15.31%) and stylar-end (13.56%) fruit surface (Table-1). The inoculation at epicarp rendered significantly higher severity (19.06%). The disease severity of the rot was at par in fruits inoculated at stylarend (13.56%) and stem-end (15.31%) and rendered significantly higher than control.

On 12th day of inoculation, the fruits showed higher incidence (100%) of the rot on the different sites of inoculation than control (40%). The severity (46.88%) of the rot in fruits inoculated at epicarp differed significantly from stylar-end (28.75%) and stem-end (34.38%) surface of the fruits. The lowest severity (8.00%) of the rot was recorded in fruits uninjured inoculated (control). The uninjured uninoculated fruits (control) remained rot free on 4th, 8th and 12th days of inoculation.

Effect of fruit ripeness on disease severity of Pestalotiopsis rot of ber fruits

On 4th day of inoculation, the ber fruits inoculated at

ripe stage showed higher disease incidence (60%) of Pestalotiopsis rot than in fruits inoculated at semi-ripe (40%) and unripe (20%) stages. The unripe stage of the fruits showed lowest disease incidence than semi-ripe and ripe fruit stages of ber, after 4th day of inoculation. The disease severity of the rot in fruits inoculated at ripe (12.13%) stage was more than in fruits inoculated at semi-ripe (8.63%) and unripe (7.06%) stages (Table 2). The inoculation at unripe stage rendered significantly lower disease severity than the fruits inoculated at semi-ripe and ripe stage of the fruit.

On 8th day of inoculation, the fruits inoculated at unripe, semi-ripe and ripe stages showed 100 per cent disease incidence. The disease severity (19.38%) of the rot inoculated at ripe stage was more than in fruits inoculated at semi-ripe (16.56%) and unripe (14.38%) stages of the fruit (Table-2). The disease inoculated at ripe stage rendered significantly higher severity than the fruits inoculated at semi-ripe and unripe stages.

On the 12th day of inoculation, at all stages of fruit maturity 100 per cent incidence of the fruit rot was recorded. The severity of the rot in fruits inoculated at ripe stage (36.25%) was more than in fruits inoculated at semi-ripe (26.25%) and unripe (23.13%) stages (Table 2). It can be concluded that among the three stages, the ripe stage was found to be significantly most susceptible for the fungal infection. The inoculation at ripe stage rendered significantly higher disease severity than the fruits inoculated at semi-ripe and unripe stage.

Biochemical changes in ber fruits due to *Pestalotiopsis* infection

Effect on total soluble solids: The level of total soluble solids decreased in the fruits inoculated with Pestalotiopsis over healthy fruits in control. The total soluble solids in inoculated semi-ripe fruits (10.00° Brix) was significantly lower than uninoculated semi-ripe fruits (12.09° Brix) and in inoculated ripe fruits (9.00° Brix) it was lower than uninoculated ripe fruits (10.78° Brix).

Effect on acidity: The level of acidity of both healthy and infected fruits decrease as fruits are stored for longer period. The level of acidity in the ripe (0.32%) and semi-ripe (0.45%) ber fruits inoculated with the Pestalotiopsis was significantly reduced than uninoculated ripe (0.58%) and uninoculated semi-ripe (0.77%) fruits.

Effect on ascorbic acid: The ascorbic acid content of both healthy and infected fruits declines as fruits are stored but the reduction in ascorbic acid content is far more pronounced in diseased fruits. The level of ascorbic acid in ripe and semi-ripe fruits infected with Pestalotiopsis rot declined significantly over control. The healthy fruits (semi-ripe stage) of ber have much larger amount of ascorbic acid as compared to the diseased fruits. The ascorbic acid content in ripe fruits (63.00 mg/100 gm fruits) and semi-ripe fruits (79.80 mg/100 gm fruits) inoculated with Pestalotiopsis decreased than in uninoculated ripe (84.00 mg/100 gm fruit) and uninoculated semi-ripe fruits (113.40 mg/100 gm fruits).

Effect on phenols: The phenolic compounds are mostly considered as one of the important biochemical parameters for disease resistance and also the accumulation of total phenols is usually higher in semi-ripe fruits as compared to mature or ripe fruits. The level of total phenols was higher in healthy and diseased semi-ripe fruits (0.62 and 0.48 mg/100 gm fruits) as compared to healthy and diseased ripe fruits (0.52 and 0.19 mg/100 gm fruits), respectively.

Effect on sugar contents: The total sugar contents (8.95%) was increased in uninoculated ripe stage of healthy ber fruits compared with inoculated ripe fruits (4.20%) (Table-4). The total sugar content (5.74%) in uninoculated semi-ripe fruits increased significantly than inoculated semi-ripe fruits (2.66%), which was lowest.

The reducing sugar content (5.53%) increased in uninoculated ripe stage of healthy fruits compared with inoculated ripe fruits (1.89%). The reducing sugar content (3.50%) in uninoculated semi-ripe fruits increased significantly than inoculated semi-ripe fruits (1.33%). The lowest reducing sugar content (1.33%) was recorded in semi-ripe fruits inoculated with Pestalotiopsist.

The non-reducing sugar content (1.23%) of semi-ripe fruits inoculated with Pestalotiopsis decreased than in uninoculated semi-ripe fruits (2.13%). Further, the non-reducing sugar content (2.19%) in ripe fruits inoculated with Pestalotiopsis decreased significantly over uninoculated ripe (3.25%) fruits. The present findings indicated that the level of total soluble solids, acidity, ascorbic acid and phenols in healthy ripe and semi-ripe ber fruits was higher as compared to infected ripe and semi-ripe fruits. The present investigations are in conformity with those of Rai (1982) who reported that healthy fruits of ber have much large amounts of vitamin C as compared to the diseases ones. The level of total soluble solids, acidity, ascorbic acid and total sugars content in ripe and semi-ripe stages of guava fruits inoculated with Pestalotiopsis were less as compared to healthy fruits (Meena, 2006). Goel and Siddiqui (1999) reported the changes in total soluble solids (TSS), acidity, ascorbic acid, phenols, and total sugars in the ber fruits of different maturity stages. Total soluble solids, acidity and total sugars increased whereas, ascorbic acid and phenolic contents decreased with the ripening process. Similarly, Fourie and Holz (1998) reported the nutrient changes during the advanced development or ripening of the nectorines and plums fruit. A direct relationship was found between the sugar content in nectarines and plums and the late season susceptibility of these fruits to *B. cinerea* infection. The present experiments conducted on these aspects revealed that the total sugar, reducing sugar and non-reducing sugar contents increased in un-inoculated ripe and semi-ripe healthy ber fruits in comparison to Pestalotiopsis rot infected fruits.

Phenolic compounds are mostly considered as one of the important biochemical parameters for disease resistance. The accumulation of total phenol is usually higher in resistant

genotypes as compared to susceptible ones. The antifungal properties of phenolic compounds and their derivatives, and the fact that these compounds are frequently found in the young fruit at higher concentrations than in the ripe fruit, led to the hypothesis that these compounds play an important role in the maintenance of latency in the unripe fruit (Barkai-Golan, 2005). Farkas and Kiraly (1962) observed that presence of phenolic compounds in plants or their synthesis in response to infection, have been associated with resistance against fungal pathogens. Similarly, Hudson and Mahgoub (1980) and Takahama (1985) reported that some of the phenolics are known to act as antioxidants and induce resistance. Toxic phenolic compounds, may be exuded from the lesions and also may exist as normal components of the phylloplane. Analysis of the material that reached into water droplets on necrotic lesions showed the presence of significant levels of phenols within one hour. The phenols released into the water that carries conidia to new infection sites may inhibit conidium germination and germtube elongation resulting in suppression of disease spread (Nicholson *et al.*, 1989). The results of the present investigations suggests that the level of total phenols was higher in healthy and diseased semi-ripe fruits as compared to healthy and diseased ripe fruits.

Table 1. Incidence and severity of pestalotiopsis rot of ber fruits inoculated at different location.

Site of inoculation	4th day of inoculation		8th day of inoculation		12th day of inoculation	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Stylar –end	60	6.13 (14.33)*	100	13.56 (21.61)	100	28.75 (32.42)
Epicarp	60	9.56 (18.01)	100	19.06 (25.89)	100	46.88 (43.21)
Stem-end	40	7.69 (16.10)	100	15.31 (23.04)	100	34.38 (35.90)
Control (uninjured inoculated)	20	0.50 (4.05)	25	5.50 (13.56)	40	8.00 (16.43)
Control (uninjured un-inoculated)	00	0.00 (4.05)	00	0.50 (4.05)	00	0.50 (4.05)
SEm ±		**0.44**		**0.51**		**0.73**
CD (P=0.05)		**1.34**		**1.52**		**2.21**

* Figures in parentheses are angular transformed values.
** Fruits were incubated at 25 ± 2°C.

Table 2. Incidence and severity of Pestalotiopsis rot of ber fruits inoculated at different maturity stages of fruits**

Stages of fruit m	4th day of inoculation		8th day of inoculation		12th day of inoculation	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Unripe fruits	20	7.06 (15.41)*	100	14.38 (22.28)	100	23.13 (28.74)
Semi-ripe fruits	40	8.63 (17.08)	100	16.56 (24.01)	100	26.25 (30.82)
Ripe fruits	60	12.13 (20.38)	100	19.38 (26.11)	100	36.25 (37.02)
SEm ±		**0.41**		**0.48**		**0.56**
CD (P=0.05)		**1.30**		**1.54**		**1.80**

* Figures in parentheses are angular transformed values.
** Fruits were incubated at 25 ± 2°C.

Table 3. Effect of Pestalotiopsis rot on total soluble solids, acidity, ascorbic acid and phenol contents of ber fruits*

Stage of fruit ripeness	Total soluble solids (°Brix)	Acidity (%)	Ascorbic acid (mg/100 g fruit)	Phenols (mg/100 g fruit)
Semi-ripe fruit (Inoculated)	10.00	0.45	79.80	0.48
Ripe fruit (Inoculated)	9.00	0.32	63.00	0.19
Control (Uninoculated semi-ripe fruits)	12.09	0.77	113.40	0.62
Control (Uninoculated ripe fruits)	10.78	0.58	84.00	0.52
SEm ±	**0.04**	**0.03**	**1.71**	**0.008**
CD (P=0.05)	**0.12**	**0.08**	**5.28**	**0.02**

*Fruits were incubated at 25 ± 2°C.

Table 4. Effect of Pestalotiopsis rot on sugar contents of ber fruits*

Stage of fruit ripeness	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)
Semi-ripe fruit (Inoculated)	2.66	1.33	1.23
Ripe fruit (Inoculated)	4.20	1.89	2.19
Control (Uninoculated semi-ripe fruits)	5.74	3.50	2.13
Control (Uninoculated ripe fruits)	8.95	5.53	3.25
SEm ±	**0.10**	**0.12**	**0.03**
CD (P=0.05)	**0.32**	**0.36**	**0.10**

* Fruits were incubated at 25 ± 2°C.

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