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Studies on blue mould rot of kinnow (*Citrus reticulata*) due to *Penicillium italicum* Wehmer in relation to temperature, relative humidity and biochemical parameter

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Abstract

Five different post-harvest rots i.e. blue mould rot, green mould rot, sour rot, core rot and stem-end rot were prevalent in kinnow fruits during the survey period *viz.*, December, 2009 to March, 2010 in fruit markets of Bikaner, Rajasthan. The blue mould rot was most predominant during February 1, 2010 to March 1, 2010. The average incidence of all the five rots studied ranged from 5.17 to 5.32 per cent during first fortnight of March, 2010. The cork-borer injury method of inoculation proved to be most effective in developing blue mould rot in kinnow fruits. The severity of blue mould rot was maximum at 25°C closely followed by 20°C. While, it was lowest at 30°C. The disease severity increased with the increase in relative humidity. It was highest at 100 per cent RH and lowest at 50 per cent RH. The levels of total soluble solids, acidity, ascorbic acid, total soluble sugar, reducing sugar and non-reducing sugar decreased in kinnow fruits with the increase in the severity of blue mould rot. Maximum reduction in total soluble solids, acidity, ascorbic acid, total soluble sugar, reducing and non-reducing sugars was observed in fruits having more than 75 per cent disease severity.

Key words: Kinnow, blue mould rot, *Penicillium italicum*, temperature, relative humidity,TSS, acidity, ascorbic acid, total soluble sugar,

Introduction

Kinnow is a hybrid of mandarin group of citrus, which belongs to order Geraniales, family *Rutaceae* and sub-family Aurantoideae. It is a cross between the King sweet orange (*Citrus nobilis* Lour) and Willow leaf mandarin (*Citrus deliciosa* Ten.) made in 1915 at Citrus Research Centre, Riverside, University of California (Frost and Krug, 1942; Thind *et al.*, 2006). It was first introduced in India during early 1940 at the fruit experiment station of Punjab Agricultural College and Research Institute, Lyallpur (Singh *et al.*, 1978). An healthy kinnow plant may yield about 600-700 fruits as compared to single sweet orange plant which yield about 200 fruits. India is the leading producer of citrus fruit with an area of 9.23 lakh hectares with production of 86.08 lakh tones annually (Anonymous, 2009). Kinnow is grown mainly in the state of Punjab, Rajasthan, Haryana, Himachal Pradesh, Jammu & Kashmir and Uttar Pradesh. In Sriganganagar district of Rajasthan, it is grown in an area of 8650 hectares having production of 25000 M.T. (Anonymous, 2008).

Infection of these pathogens leads to quantitative and qualitative losses in kinnow fruits. The fruits may also be infected in orchard right from the setting to harvesting stage leading to fruit dropping due to pre-harvest stemend rot and incipient pre-harvest infection causing subsequent post- harvest rotting during storage and transit (Naqvi, 1993; Pathak, 1980; Sharma and Alam, 1998). The optimum period of harvesting of kinnow fruits is mid-January to mid-February when the fruits attain TSS/acid ratio of 12:1 to 14:1. The conservative estimation on post-harvest losses of kinnow fruits has been reported to be around 25-30 per cent (Singh *et al.*, 2002). Out of the total post-harvest losses of kinnow fruits, 80-90 per cent losses are due to the fungal diseases (Dris *et al.*, 2001).

The present paper reports the effect of temperature and relative humidity on disease severity of blue mould rot caused by *Penicillium italicum* Wehmer and biochemical changes in blue mould infected kinnow (*Citrus reticulata*) fruits.

Materials and methods

Methods of inoculation

Four methods of inoculation were compared in this experiment. Healthy mature fruits were used in all the experiments. The kinnow fruits were surface sterilized by dipping in sodium hypochlorite solution (1%) for 1-2 minutes followed by three washings with sterile distilled water and inoculated with the test fungus. The inoculum consisted of a 2 mm diameter disc cut away from the periphery of 7 day-old culture of the fungus grown in Petridishes. Each treatment was replicated for four times having three kinnow fruits in each replication.

(a) Cork-borer injury method

A shallow injury of 2 mm depth was made with the help of a sterilized cork-borer. The inoculum was inserted in the injured portion and the host tissue was replaced.

(b) Rubbing method

Two fruits were rubbed with each other for a while and the inoculum was placed on the rubbed area of fruit

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surface.

(c) Scrapping method

The fruit skin was injured by scrapping with a sterilized razor blade and the fungal inoculum was placed on the injured surface.

(d) Pin-pricking method

Fruit skin was pierced 6-7 times with the help of sterilized needle. The inoculum was placed on the pierced area with the fungal growth facing the fruit surface.

(e) Inoculation on uninjured surface

The inoculum was placed on uninjured fruit surface with the fungal growth facing the fruit surface.

Effect of temperature on blue mould severity

Cork-borer injury method was followed in this experiment. The fruits were incubated at 15, 20, 25, 30, and 35° C. The experiment was conducted following Completely Randomized Design (CRD) with three replications. The incidence and severity of fruit rots were calculated as follows: Fruit rot incidence (%) = (Number of infected fruits / Total number of fruits observed) X 100. The disease severity was calculated on the basis of per cent infected area (Mckinney's, 1923). Disease severity (%) = (Sum of all numerical ratings / No. of fruits observed X Maximum grade) X 100

Effect of relative humidity (RH) on blue mould severity

The effect of six different levels of RH *viz.*, 50, 60, 70, 80 and 90 per cent on severity of blue mould rot of kinnow was studied. Cork-borer injury method was followed for rot development. Solution of different concentrations of sulphuric acid (H_2SO_4) was used to maintain different levels of RH according the procedure described by Waller *et al.* (2002). The inoculated fruits were placed in desiccators containing sulphuric acid solution to provide a particular level of relative humidity. The desiccators were then sealed with greese and kept at 25±1°C. This experiment was conducted following CRD keeping three replications. The severity was calculated as described earlier.

Determination of total soluble solids

TSS was determined as °brix by placing a drop of juice on the prism of refractometer. The values were corrected to 20° C with the help of a temperature correction chart (Ranganna, 2000).

Determination of acidity

The acidity of juice samples was determined by titration method (A.O.A.C., 1980). For this purpose, 10 ml filtered juice from each of rotted as well as healthy fruits was taken and diluted to 100 ml by adding distilled water. Ten milliliter aliquot of diluted juice was taken and titrated with 0.1 N NaOH using 1 per cent phenolphthalein as indicator. The appearance of faint pink colour was considered as the end point. The acidity of juice was expressed as percentage of citric acid, which was calculated as follows: Acidity (%) = (Titre value X 0.1 N NaOH X Volume made up of diluted juice X 0.06404 / Volume of sample taken for estimation X Volume of aliquot taken for determination) X 100

Determination of ascorbic acid

The following reagents and procedure were used for the estimation of ascorbic acid in rotted as well as healthy fruits (A.O.A.C., 1980).

Standardization of dye factor

Diluted 5 ml of standard ascorbic acid solution with 5 ml of 3% HPO, solution. Titrated with the 2,6-dichlorophenol-indophenol dye solution till faint pink color persisted for at least 10-15 seconds. Calculated the dye factor (mg of ascorbic acid per ml of the dye) by using the following formula (A.O.A.C., 1980): Dye factor = (0.5 / Titre value) X 100

Preparation of sample and titration

.Ten milliliter of filtered juice from rotted as well as healthy fruits each was taken and it was diluted with 90 ml 3% Metaphosphoric acid (HPO3) solution to make the final volume of 100 ml and then filtered. Ten milliliter of this aliquot was taken in a beaker and then titrated with the standard dye till faint pink colour persisted for at least 10-15 seconds which was considered as end point. The results were expressed as mg of ascorbic acid per 100 ml of juice and calculated as follows (A.O.A.C., 1980): Ascorbic acid (mg 100 ml?') = (Titre value X Dye factor X Volume made up of diluted juice / Volume of sample taken X Volume of aliquot taken for determination) X 100.

Determination of sugars

Total soluble sugar

Total sugar content was determined by colorimetric method using anthrone reagent (Dubois et al., 1951). One ml of diluted fruit juice sample (100 times) and 4 ml of anthrone reagent was transferred to test tube and then 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 sugar present in the sample was determined from standard curve and expressed as percentage basis.

Reducing sugar

Reducing sugar content was measured by following Nelson's modification of 'Somogyi's method' (Somogyi, 1952) using arseno-molybdate colour forming reagent and two copper reagent 'A' and 'B'. One ml of juice sample (100 times diluted) was added to 1 ml of mixture of copper reagents, prepared from 24 part of copper reagent 'A' and 1 part of copper reagent 'B' solutions. This mixture in test tubes was heated in boiling water bath, cooled and than 1 ml colour-forming reagent was added. The absorbance was measured at 620 nm on Spectronic-20. The value was plotted against a standard curve prepared from glucose solutions. The figures were expressed on percentage basis.

Non- reducing sugar

Subtraction of reducing sugars from the total soluble sugars in the juice and after multiplying the values with the factor (0.95) gave the amount of the non-reducing sugars present in samples.

Non-reducing sugar (%) = (%Total soluble sugar - %Reducing sugar) x 0.95

Results and Discussion

Effect of inoculation methods on severity of blue mould rot

The data presented in Table 1showed that cork-borer injury method of inoculation was most effective in developing blue mould rot in kinnow fruits as compared to other methods. The disease severity was less after four days of inoculation in all the four methods tested. After eleven days of inoculation, the maximum disease severity (76.67%) was recorded in cork-borer injury method followed by pin-pricking method (52.22%) and it was minimum (19.44%) in rubbing method of inoculation. The inoculation without injury remained rot after on 4, 7 and 11 days of incubation.

The present study showed that cork-borer injury method was more effective in developing blue mould rot in kinnow fruits in comparison to other methods of inoculation. Similar observations were also recorded by Sharma (2002) in case of green mould of kinnow caused by P. digitatum and Choudhary (2009) in fruits rot of lime caused by Colletotrichum gloeosporioides.

Effect of temperature on severity of blue mould rot

The severity of blue mould rot was maximum at 250C closely followed by 200C and it was lowest at 300C. Symptoms of the disease did not appear at 35oC temperature. There was no significant difference in disease severity recorded at 20 and 250C after 4 and 7 days of inoculation. However, the severity was significantly higher at 250C as compared to 200C after 11 days of inoculation (Table 2).

Table 1. Effect of different inoculation methods on severity of blue mould rot of kinnow fruits after 4, 7 and 11 days of inoculation

Method of inoculation	Disease severity (%) after (days)		
	4	7	11
Cork-borer injury	31.67 (34.24)*	58.33 (49.80)	76.67 (61.13)
Pin-pricking	18.89 (25.74)	43.33 (41.17)	52.22 (46.26)
Scrapping	11.67 (19.94)	19.44 (26.15)	31.67 (34.24)
Rubbing	7.22 (15.58)	10.56 (18.95)	19.44 (29.48)
Inoculation on uninjured surface	0.00	0.00	0.00
S.Em. ±	0.72	0.64	1.72
CD (P=0.05)	2.25	2.01	5.42
CV (%)	6.48	4.06	8.71

*Figures in parentheses are angular transformed values.

Table 2. Effect of temperature on severity of blue mould rot of kinnow fruits after 4, 7 and 11 days of inoculation

Temperature (±1 °C)	Disease severity (%) after (days)		
	4	7	11
15	21.11 (27.36)*	41.11 (39.87)	52.22 (46.28)
20	29.44 (32.86)	53.89 (47.24)	71.67 (57.85)
25	31.67 (34.24)	58.33 (49.80)	76.67 (61.13)
30	8.33 (16.72)	12.22 (20.41)	13.89 (21.84)
35	0.00	0.00	0.00
S.Em. ±	0.68	0.82	0.77
CD (P=0.05)	2.16	2.60	2.42
CV (%)	5.33	4.54	3.56

*Figures in parentheses are angular transformed values.

Effect of relative humidity (RH) on severity of blue mould rot

The results presented in Table 3 clearly indicate that the disease severity increased with the increase in RH in all the three observation days i.e. 4, 7 and 11 days after inoculation. The disease severity was highest at 100 per cent (76.67%) followed by 90 per cent RH (65.56%). While, it was lowest (33.33%) at 50 per cent RH (Table 3).

The results revealed that severity of blue mould rot was higher at 20 to 25°C temperature. While, the severity was less at higher temperature. It was also observed that the severity of blue mould was higher at relative humidity level of 90 to 100 per cent. Maximum decay of different fruits by various pathogens was recorded at 15 to 30°C (Bhargava, 1972 and Pathak, 1980). The incidence of blue mould rot of kinnow has been also reported to be greater at 25°C and 100 per cent relative humidity (Godara, 1994). The present study also indicated that a drastic reduction of the severity occurred at higher temperature or low humidity. More studies arerequired to ascertain the effect of temperature and relative humidity alone and in combinations on pathogenesis of *P. italicum* in kinnow fruits.

Effect on total soluble solids (TSS)

A perusal of data presented in Table 4 indicated that the levels of total soluble solids decreased significantly in the kinnow fruits with the increase in the severity of blue mould rot. TSS was significantly less in fruits having more than 50 per cent disease severity. It was quite less at 50-75 per cent disease severity. While, it was highest (12%) in control (healthy) fruits. The TSS content was quite higher at lower level of rot i.e. at disease severity below 25 per cent (Table 4).

Effect on acidity

The data revealed that the level of acidity significantly decreased in infected kinnow fruits with the increase in severity of blue mould rot. The maximum acidity (1.11%) was recorded in control fruits. The acidity was quite high at low level of disease severity i.e. 1-10 per cent. It was lowest (0.41%) in fruits having more than 75 per cent disease severity (Table 4).

Effect on ascorbic acid

The content of ascorbic acid in kinnow fruits decreased with the increase in severity of blue mould. The results presented in Table 4 showed that the ascorbic acid content was highest in control i.e. uninoculated healthy fruits. Significant decrease in ascorbic acid content was recorded at disease severity range 10-25 per cent and above. While minimum ascorbic acid content was observed at more than 75 per cent disease severity level (Table 4).

Effect on total soluble sugar

Gradual reduction of total soluble sugar was recorded with the increase in severity of blue mould rot. Initially it was highest in control and slightly decreased at low level of disease severity i.e. 1-10 per cent. It was lowest (5.58%) at discase severity level more than 75 per cent (Table 5).

Effect on reducing sugar

The data presented in Table 5 clearly revealed that the reducing sugar content was significantly less in rotted fruits as compared to control. Like total soluble sugar, the amount of reducing sugar decreased with the increase in level of disease severity. It was significantly lower in fruits having disease severity range 50-75 per cent and above (Table 5).

Effect on non-reducing sugar

The results showed that non-reducing sugar content was less in blue mould infected fruits as compared to healthy fruits. Again, it was significantly less in infected fruits having different severity grades. It was lowest in fruits which exhibited more than 75 per cent severity. Further, the non-reducing sugar content was statistically at par in infected fruits having disease severity ranged from 1-10 to 50-75 per cent (Table 5).

It was recorded during present investigation, the biochemical constituents of kinnow fruits like TSS, acidity and ascorbic acid were significantly reduced in rotted fruits as compared to healthy ones. Further, the magnitude of reduction of these constituents was considerably higher in fruits having more than 50 per cent disease severity. These results are in conformity with the previous reports of reduction in the amount of TSS, acidity and ascorbic acid in rotted as compared to healthy fruits of sweet orange (Kapur *et al.*, 1992 and Sharma, 2007), mosambi (Singh and Sinha, 1982) and mandarin orange (Dutta *et al.*, 2009). The reduction in the amount of these constituents in the rotted fruits might be attributed to their utilization by the fungus or their degradation by the enzymes. The amount of total soluble sugar, reducing and non-reducing sugars in rotted kinnow fruits was also found to be reduced considerably as compared to healthy ones during the present investigations. Similarly, the depletion in sugar content has also been reported in infected citrus fruits like kinnow (Ramanjulu and Reddy, 1989, Kapur *et al.*, 1992 and Sharma, 2007), mosambi (Singh and Sinha, 1982). Sharma, *et al.*, 1992 gave the following Physico-chemical characteristics of kinnow fruits weight 139.0 g, size of fruit 5.0-7.9 x 6.0-9.7 cm, peel thickness 0.61 cm, peel (%) 27.99, rag (%) 20.97, juice (%) 51.04, TSS (%) 11.50, acidity (%) 0.905, TSS/acid ratio 12:1 and vitamin 'C' (mg/100 ml juice) 23.51 of fruits. The decrease in sugar content in infected tissues might be due to enzymatic brake down of soluble carbohydrates and utilization of these sugars by the fungus during the process of pathogenesis (Cheema *et al.*, 1974 and Dutta *et al.*, 2009).

Table 3. Effect of different levels of relative humidity on severity of blue mould rot of kinnow fruits after 4, 7 and 11 days of inoculation

Relative humidity (%)	Disease severity (%) after (days)		
	4	7	11
50	10.56 (18.95)*	18.33 (25.34)	33.33 (35.26)
60	12.78 (20.93)	22.22 (28.11)	40.56 (39.54)
70	13.33 (21.39)	24.44 (29.62)	50.00 (45.00)
80	13.89 (21.86)	26.67 (31.08)	56.11 (48.51)
90	16.67 (23.68)	32.22 (34.58)	65.56 (54.07)
100	31.67 (34.24)	58.33 (49.80)	76.67 (61.13)
S.Em. ±	0.65	0.72	0.74
CD (P=0.05)	2.01	2.23	2.28
CV (%)	4.80	3.79	2.71

*Figures in parentheses are angular transformed values.

Table 4 Effect of blue mould rot on total soluble solids, acidity and ascorbic acid contents in kinnow fruits

Disease severity (%)	Total soluble solids (°Brix)	Acidity (%)	Ascorbic acid (mg/100 g fruit)
1-10	11.17	0.98	17.19
10-25	9.83	0.90	7.81
25-50	8.83	0.77	7.03
50-75	7.33	0.64	4.95
Above 75	7.00	0.41	2.99
Control (Healthy)	12.00	1.11	25.00
	0.18	0.03	0.53
CD (P=0.05)	0.55	0.08	1.65
CV (%)	3.33	5.66	8.54

Table 5. Effect of blue mould rot on total soluble sugar, reducing sugar and non-reducing sugar contents in kinnow fruits

Disease severity (%)	Total soluble sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)
1-10	7.13	3.20	3.73
10-25	6.82	2.95	3.68
25-50	6.44	2.64	3.61
50-75	5.84	2.20	3.46
Above 75	5.58	1.97	3.42
Control (Healthy)	8.69	4.38	4.09
	0.07	0.11	0.13
CD (P=0.05)	0.23	0.33	0.39
CV (%)	1.89	6.35	6.03

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