INNOVATIVE STRATEGIES FOR ECO-FRIENDLY MANAGEMENT OF CITRUS BLUE MOLD DISEASE CAUSED BY *PENICILLIUM ITALICUM* WHEMER

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Abstract

Postharvest losses during handling, shipping, storage and supply are the most important problems in agriculture based economy, especially in perishable fruits. Blue mould (Penicillium italicum) is the most important postharvest pathogen of fresh and stored citrus fruits. The pathogen being necrotroph, enters into the fruit through rind wounds caused during the pre- and postharvest. In the current investigation, pre-harvest experiments were carried out by using organic elicitor and inorganic salts while Topsin M was used as positive control. Treatments were made seven days before harvest. Ten fruits were harvested from each plant and these fruits were inoculated with P. italicum, placed in boxes and stored at 20°C for seven days. Data were recorded at daily basis up to the rotting of control. The results revealed that Potassium Bicarbonate and Salicylic Acid (9 mM) effectively reduced the lesion diameter and growth inhibition% of P. italicum as compared to control. However, Salicylic acid (5 mM) and Sodium carbonate were less effective as compared to other inorganic salts. Hence it is inferred from the present studies that application of inorganic salts may be utilized for the eco-friendly management of postharvest diseases in citrus. This will be helpful to reduce pesticides hazards and environmental degradation.

Key words: *inorganic salts, pre-harvest, postharvest, disease management, eco-friendly*

INTRODUCTION

Fruits constitute an important item of our food and they play a significant role in the human diet through the supply of vitamins and minerals (Prabhakar et al., 2004). In the world market, there is an increasing trend in demand of fruit and vegetables. That is why, these commodities have a fast growing market share (Kabas, 2010). In Pakistan, citrus is the major fruit crop that occupies an area of 199.9 thousand hectares, and gives 2.132 MMT annual production and 12 tons per hectare average yield. Punjab is the leading citrus producing province sharing about 96% of national production. Around 80% of the citrus especially Kinnow mandarin is grown in Sargodha and its peripheral areas including Toba Tek Singh, Faisalabad and Sahiwal districts (Johnson, 2002). Mandarins are a diversified group of citrus fruits characterized by excellent flavor, bright colored pulp and peel, easy-to-peel rind and segments that separate easily (Parashar, 2010). Although we are at 13th position among citrus producers, average yield of citrus in Pakistan is still 40% less than as in USA (Razi et al., 2012).

Besides yield losses that occur before crop harvest, postharvest losses also touch a bigger figure. According to an estimate, on worldwide basis, the postharvest losses are almost 30 to 40% that are even much more in developing countries (Bhattarai et al., 2013). Postharvest losses are those that occur during postharvest handling, shipping, storage and supply of commodities especially perishable fruits and vegetables. More to the point resulting in huge financial losses and low per capita availability, these amplify transport and marketing costs also. Major portion of the citrus is deteriorated by the fungal postharvest diseases which ultimately put economical burden on both grower and exporter (Spadaro et al., 2004)

Penicillium italicum (PI) and *Penicillium digitatum* (PD) are the causal organisms of most important postharvest diseases in fresh citrus fruit (Valencia-Chamorro et al., 2008). Blue mould (*P. italicum)* is one of the major postharvest diseases on citrus fruits that are cold stored (Iqbal et al., 2011). This pathogen is necrotrophs and gains entry into fruit through rind lesions produced during, before and after

crop harvest especially while transporting it (Palou, 2009). *Penicillium italicum* Whemer gives best growth at temperatures lower than 10°C.

Although, fungicides are considered most effective for controlling pre- and postharvest diseases causing deterioration of food, yet their indiscriminate use poses health and environmental risks mainly due to their carcinogenicity and teratogenicity, and overall toxic effects (Janisiewicz et al., 2002; Queiroz et al., 2006). This is especially true in case of postharvest diseases. Moreover, there is an increasing trend on imposing restrictions on the use of pesticides by the international consumer affairs institutions and regulatory agencies (Spadaro et al., 2004). It is therefore of utmost importance to identify the alternative technologies for management of postharvest diseases in fruits and other perishable crops. The use of other novel compounds in disease control is a viable option.

Valencia-Chamorro et al. (2008) used potassium sorbate (PS) for the control of *Penicillium italicum* and *Penicillium digitatum* (PD) and found it effective as potential commercial antifungal inorganic salt. Many inorganic salts were tested for postharvest decay of fruit and found Sodium bicarbonate (SBC) as an as effective alternative to chemicals (Fallanaj et al., 2013). Salicylic acid (SA) induces plant resistance and has been proved to have antifungal properties against few pathogens causing diseases in citrus, mango and pear (Zainuri et al., 2001; Shaat and Galal 2004; Cao et al., 2006; Iqbal et al., 2011).

The purpose of this work was to investigate the ability of salicylic acid and inorganic salts of potassium and sodium as an alternate to commercially used chemicals and fungicides for sustainable management of citrus blue mould disease. This report would probably the first attempt towards using these salts for pre-harvest disease control of kinnow mandrain.

MATERIALS AND METHODS

Orchard selection

In mid-February 2013, 25 years old Kinnow mandarin (*Citrus reticulata* Blanco) orchid growing in Chak No. 104, Silanwali road, District Sargodha, Punjab, Pakistan was selected for this experiment. The trees were planted at row to row distance of 6.5 m and tree to tree distance of 2 m in north-south row direction. All the experimental trees received the similar cultural practices except for pre-harvest spraying of different inorganic salts. For pre-harvest spray trees were selected randomly in triplicates for each salt. Topsin M was used as positive control.

Preparation of salt solutions

Different concentrations of salts (Table 1) were applied one week before harvest. All salts except Salicylic acid were dissolved in distilled water. Salicylic acid was first dissolved in ethanol, then volume was made with water.

Table 1. Inorganic salts used for pre-harvest treatment and their concentration

Sr. No.	Salt	Concentrations
1	Salicylic acid	5mM (0.69g/L)
		7mM (0.97g/L)
		9mM (1.24g/L)
2	Potassium Carbonate	5g/L
3	Potassium Sorbate	5g/L
4	Sodium bicarbonate	5g/L
5	Sodium carbonate	5g/L
6	Potassium Bicarbonate	5g/L

Isolation, purification and culturing of *Penicillium italicum*

Penicillium italicum was isolated from diseased Kinnow mandarin fruits, collected from commercial orchard at Chak No. 18, Bhalwal, District Sargodha. Cultures were purified and multiplied on potato dextrose agar (PDA) media under sterilized condition and placed at 25°C in an incubator.

Inoculation

Spores of *P. italicum* were collected from 2-week old PDA cultures of *P. italicum* and 20 ml of sterile distilled water was poured into Petri plate culture. The spores were gently detached from the surface with a sterile glass rod, and suspension was used for cross inoculation. Surface-sterilization of pre-harvested sprayed fruits was carried out with 5% commercial bleach solution for 02 minutes, then thoroughly washed with tap water and allowed to dry at room temperature for 24 hours. Fruits were wounded by creating punctures of approximately 3 mm depth with a sterilized nail and inoculated with 10 µL of a conidial suspension of *P. italicum*. Inoculated fruits were arranged in Paper boxes and kept at 90-95% relative humidity and 20ºC until full coverage of control with test fungus.

Data recording

Data were recorded on lesion diameter upto nine days after inoculation of the *P. italicum*.

RESULTS

The results of pre-harvest application of different inorganic salts on citrus orchard varied significantly (*P=0.05*) in terms of lesion diameter produced by blue mould, *Penicillium italicum* (Table 2). Salicylic acid (9 mM) and Potassium bicarbonate effectively reduced the lesion diameter upto nine days after inoculation of fungus as compared to control and all other treatments. Salicylic acid (5 mM) and Sodium carbonate exhibited maximum lesion diameter as compared to other inorganic salts. Potassium carbonate revealed intermediate results. The two treatments, Salicylic acid (9 mM) and Potassium bicarbonate were also effective against *P. italicum* when their results were compared on the basis of mean lesion diameter (Table 2).

Growth inhibition % of *P. italicum* was also variable among all the treatments. Maximum growth inhibition % was exhibited by Salicylic acid (9 mM) and Potassium bicarbonate being 34.80 and 28.54% respectively. However minimum growth inhibition % was observed in treatment where Salicylic acid (5 mM) was applied.

Table 2. Effect of different inorganic salts and organic acids applied as pre-harvest spray on lesion diameter of *Penicillium italicum* on kinnow mandarin after different time intervals and stored at 25 ±1°C

Treatments	Lesion diameter (mm)									Mean LD (mm)	
	Time (days)									LD (mm)	GI %
	1	2	3	4	5	6	7	8	9		
Salicylic acid (5 mM)	16.5	21.8	28.8	34.7	41	46.7	52.2	57.9	62.8	40.27	8.94
Salicylic acid (7 mM)	12.7	18.4	25	29.9	35.9	41	47.7	53.2	58.7	35.83	18.97
Salicylic acid (9 mM)	8.7	10.6	16.1	22.1	27.5	33.6	39.4	45.7	55.8	28.83	34.80
Potassium Carbonate	9.6	15.4	21.2	27	33.1	39.2	44.4	50.1	55.7	32.86	25.70
Potassium Sorbate	11	16.7	22.6	27.8	33.6	38.8	44	49.2	55.2	33.21	24.90
Sodium bicarbonate	12	18.1	24.4	29.9	35.5	41.5	46.4	51.6	57.4	35.20	20.40
Sodium carbonate	13	18.9	25.1	30.5	36.3	42	47.7	53.3	59.7	36.28	17.96
Potassium Bicarbonate	8.6	14.2	20.5	26.4	31.7	37.3	42.4	48.4	54.9	31.60	28.54
Topsin M	14.6	21	26.5	32.2	38.1	43.7	48.9	54.9	61	37.88	14.34
Control	17	23.5	30.4	37.65	44.1	51	58	64.7	72.2	44.22	

LD Lesion diameter; GI Growth inhibition

DISCUSSION

Different salts have been extensively examined for their postharvest treatment by spraying or dipping. However, very few studies on their pre-harvest application have so far been done to control postharvest decay (Nigro et al., 2006; Teixidó et al., 2010; Iqbal et al., 2012). In order to solve the problems related to their application and to achieve their performance up to commercially acceptable level, several combinations of alternative means have been suggested by many authors to manage postharvest diseases of vegetables and fresh fruit (Palou et al., 2008; Sanzani et al., 2009; Janisiewicz and Conway, 2010).

A number of researches were done on fungitoxic activity of silicate, sorbate, carbonate, and bicarbonate and when applied in different fruits and vegetables (Smilanick et al., 2005; Guo et al., 2007; Gregori et al., 2008; Youssef et al., 2010b). The reduction in epiphytic fungal population i.e. disease incidence and severity, wound rotting, lesion diameter and mycelia growth may be due to direct and indirect activity of salts. Directly, these salts caused osmotic stress during field application which helped in reduction of blue mould growth (Elad and Kirshner, 1993; Ippolito et al., 2005). While some salts act by suppressing the germination of spores, germ tube elongation, and production of pectinolytic enzymes in several pathogens (Hervieux et al., 2002; Smilanick et al., 2005).

Indirectly, these salts act by increasing tissue resistance (Malamy et al., 1990; Yao and Tian, 2005). Youssef et al. (2010c) demonstrated an indirect effect of some salts arbitrated by tissue-induced resistance when salts were applied in wounds 5 mm apart from the pathogen. The results revealed that these salts caused a notable reduction in lesion diameter as compared to the water control. Venditti et al. (2005) noticed that sodium carbonate brought about structural changes in cell wall, production of scoparone and an increase in pH of citrus rind tissues. Some other experiments have proved the increase in the scoparone, phytoalexins, umbelliferone and scopoletin, in sodium carbonate and sodium bicarbonate treated orange tissues (Youssef et al., 2011). Similarly, Iqbal et al. (2012) reported the suppressive effect of salicylic acid (8 mM) and methyl jasmonate (3 mM) against *P. digitatum*. A pre-harvest application of these organic elicitors proved to be more effective in decreasing lesion diameter, wound rotting and spore mass density of *P. digitatum* compared to postharvest treatment.

In conclusion, the results revealed that postharvest disease caused by blue mould fungus can be effectively controlled by pre-harvest application of inorganic salts. However, proper pre-harvest management to reduce the inoculums potential, proper handling throughout the supply chain, proper packaging materials and suitable environment management during storage are equally important in reducing the postharvest disease.

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