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Effect of Different Post-harvest Treatments of Fungicides, Botanical Oils, Food Preservatives and Packaging on Black Mould Fruit Rot of Pomegranate

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ABSTRACT

Background: Pomegranate is an important favourite fruit of tropical, subtropical and arid regions. In India, it is grown in almost all the states but a commercial orchard exists in Maharashtra and Gujarat. Some pathogens only attack the fruit from the inside, while the external surface of the fruit remains asymptomatic. Diseased fruit display poor shelf life and flavour quality attributes. Changes include losses in sugars, acids, characteristic aroma and development of off flavours. Therefore, identifying and quick characterization of these disease symptoms is paramount to their effective control and management. Since there is a lack of research work on pomegranate black mould fruit rot diseases caused by *Aspergillus niger*. Realizing the gaps concerns the environmental factors affecting the prevalence of disease development and management of disease, the present investigation was undertaken with the objective: Management of post-harvest black mould fruit rot diseases of pomegranate by different methods.

Methods: The experiments were carried out at Division of Horticulture, UAS, GKVK, Bengaluru by using the variety Bhagwa with 11 treatments which are replicated four times in Completely Randomized Design (CRD). Different chemical, physical methods, food preservatives and packaging were used to control the black mould fruit rot.

Result: The epidemiological studies revealed that injury to the pomegranate fruits was found to be a prerequisite for infection. All the fruits inoculated by cork-wounding, pin-pricking, scrapping and rubbing method exhibited symptoms of rot. Cork-wounding proved to be the most efficient method of inoculation. Post-harvest dipping in hot water, fungicides, oils and food preservatives provided effective control of the rot. Oil suspensions were found effective in preventing the rot. Food preservatives were also found effective in preventing rot, potassium metabisulphite (KMS) 0.5 percent followed by sodium benzoate 0.5 percent proved most effective against the rot in both pre-and post-inoculation treatments. Packaging for postharvest storage showed very good performance in maintaining the quality of pomegranate fruits up to 12 weeks.

Key words: Black mould fruit rot, Fungicides, Oils, Packaging, Pomegranate.

INTRODUCTION

Pomegranate is an important favourite fruit of tropical, subtropical and arid regions. It belongs to the family Punicaceae and is believed to be a native of the Middle East (Iran and adjoining countries) and spread to most tropical and subtropical countries of the world. It is extensively cultivated in Iran, India, Egypt, Pakistan, Spain, Morocco, and Afghanistan and in some places of Myanmar, China, Japan, California, South Italy, and Bulgaria (Mitra *et al.,* 1999). In India, it is grown in almost all the states but a commercial orchard exists in Maharashtra and Gujarat. The other important states are Karnataka, Rajasthan, Uttar Pradesh, Andhra Pradesh and Tamil Nadu.

Pomegranate is a hardy plant and can thrive even under desert conditions. The tree cannot produce sweet fruits unless the temperature is high for a sufficiently long period. The quality of fruit is adversely affected in humid climates. Tannin occurs in all parts of the tree, particularly upto 26 percent in the dried rind (Kanwar and Thakur, 1973). Fruits are an important part of the human diet because of supply essential nutrients such as vitamins, carbohydrates and minerals. They are also considered important to human

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health and well-being because they contain other necessary compounds such as antioxidants. Increased consumer awareness that diet and health are linked has therefore resulted in greater consumption of fruits. At the same time, however, consumers are equally concerned about the safety of the fruits they eat and want foods free from pesticide residues, toxins and harmful microorganisms. Losses due to pests and diseases in the field, during storage, as well as in transit amount up to 25 per cent of the total production in industrialized countries.

Infected pomegranate fruit trees display symptoms of wilting, stunted growth, reduced vigour, dieback of branches, and general tree decline (Kanwar and Thakur, 1973). Leaf symptoms (such as discoloration, spots and lesions) can at times be followed by wilting of the entire plant leading to death. Pomegranate fruit disorders can be visualized in the form of surface rots, shriveling, discolouration and the display of undesirable cosmetic attributes such as spots or lesions. Moulding can appear on the affected areas depending and on the type of pathogen the colour of the moulds can develop as either fluffy grey or powdery blue/ green mould. All fruit disorders eventually lead to rind breaks down, aril browning and decay.

Some pathogens only attack the fruit from the inside, while the external surface of the fruit remains asymptomatic. Diseased fruit display poor shelf life and flavour quality attributes. Changes include losses in sugars, acids, characteristic aroma and development of off flavours due to fermentative metabolism and transfer of undesirable odours, such as sulfurous compounds mainly from fungi (Mandal and Dasgupta, 1981). Close examination of affected fruits can at times reveal distinct symptoms of crown rot, and internal damage of the calyx area (Bilgrami *et al.,* 1979). Therefore, identifying and quick characterization of these disease symptoms is paramount to their effective control and management.

Post-harvest black mold fruit rot disease caused by *Aspergillus niger* in pomegranate is a major problem. Bhagwa, a variety of pomegranates is widely cultivated in the arid zone. Since there is a lack of research work on pomegranate black mold fruit rot diseases. In this experiment, we are interested to know the effect of different management practices to minimize the loss caused by post-harvest diseases and to improve the shelf life.

MATERIALS AND METHODS

The experiments were carried out at Laboratory, Division of Horticulture, UAS, GKVK, Bengaluru during September 2015 - May 2016.

Initially, the skin color slightly faded out at the site of infection was slightly depressed. Later, the lesion becomes water-soaked and gradually enlarges and coalesces to form bigger spots. The aril becomes soft and watery fluid. Spots covered by black spore masses. Finally, the fruit decays both externally and internally. Pure culture of pathogenic fungus showed well-developed hyphae profusely branched, septate and hyaline. The culture was initially dirty white, soon changing to carbonaceous black; Mycelium produced abundant conidiophores, which were non-septate, thick-walled light brown near the vesicle, conidial head dark brown to carbonaceous black, globose upto 400-410 µm in diameter, large number of sterigmata present in chains. Conidia were globose, thick-walled, light-colored, measuring 2.5- 4 µm.

Mature pomegranate fruits were used in the studies. The fruits were surface sterilized by dipping them in mercuric chloride solution for 1 to 2 minutes followed by 3 times of washings with sterilized water and inoculated with the test fungus. The inoculums consisted of 2 mm diameter disc that was cut away from the periphery of 7 days old culture of the fungus grown in petri-dishes. Each treatment was replicated four times having 2/3 of pomegranate fruit per replication.

The first study was conducted using four inoculation methods: cork-borer wounding method, rubbing method, scapping method and inoculation without injury. A hole of 2 mm diameter and 2 mm depth was made with the help of a sterilized cork borerin cork-borer wounding method. The inoculums were placed in the hole and the host tissue was replaced on the hole. In rubbing method; two fruits were rubbed with each other for a while and inoculums were placed on the rubbed area with the fungal growth facing the fruit surface. In scrapping method; The fruit skin within the periphery of 2 mm diameter was scrapped thrice with a razor blade and the inoculums were placed on the scraped area with fungal growth facing the fruit surface. The inoculums were placed on an uninjured fruit surface with fungal growth facing the fruit surface as control. In all the treatments, the inoculums were covered by a thin layer of sterilized cotton dipped in sterilized water. These inoculated pomegranate fruits were kept in new and moistened polythene bags. The bags were sealed and kept in the incubator at 25°C± 1°C. The fruits were examined daily till the appearance of the symptoms to record the incubation period

Second study, four chemical fungicides, five botanical oils, four food preservatives, hot water treatment and fruit packaging were used as treatments with relevant controls. Treatments were replicated four times in completely randomized design (CRD). Bhagwa pomegranate variety were used in these experiments. In chemical fungicide study, pomegranate fruit of uniform maturity was surface sterilized with mercuric chloride solution and inoculated with the pathogen by cork borer wounding method. Four chemical fungicides: 0.2% of Indofil M-45 (Zinc ion, Manganous ethylene and Bisdithio-carbama), 0.1 % of Bavistin 50% WP (Methyl-2-benzimidazole carbamate [MBC]), 0.2% of Captan 50% WP (N-Trichloromethyl thio-4 cyclo hexane 1,2 dicarboximide), 0.2% Kavach 75% WP (Tetrachloraisphthalo nitrite) and 0.2% Blitox-50 (Copper oxy chloride) were tested with unsprayed control.

In the pre inoculation treatment, the fruits were first dipped in the test chemical for 5 minutes and then inoculated with the fungus. Where in the post-inoculation treatment, the fruits were first inoculated and then treated with the chemical. The interval between inoculation and dip treatment and vice-versa was 12 hours. The experiment was carried out using completely randomized design. There were four replications in each treatment. Proper controls were maintained for the method of inoculation for pomegranate fruits and their incubation at 25±1°C.

In botanical oil study, pomegranate fruits of uniform size were surface sterilized with mercuric chloride solution and inoculated with the pathogen by cork borer wounding method. Oils tested against the disease were Mustard oil, Neem oil, Castor oil, Soybean oil and Linseed oil at the concentration 4%. Teepol was used as a solubilizing agent in each oil. The oils were tested by dipping the fruits in the oil suspension. The oils were used in both pre-and post-inoculation treatment. In the pre-inoculation treatment, the fruits were first dipped in the test oil for 5 minutes and then inoculated, while in the post-inoculation treatment, the fruits were first inoculated and then dipped in the oils. The interval between inoculation and treatment with oils or vice-versa was 12 hours. The experiment was conducted by following a complete randomized design. There were four replications in each treatment. Proper controls with and without teepol were also maintained. The method of inoculation of pomegranate fruits, their incubation at 25±1°C.

For hot water treatment; pomegranate fruits of uniform size were surface sterilized and inoculated with the pathogen by a suitable inoculation method. After 12 hours of inoculation, the pomegranate fruits were dipped in hot water maintained at 40, 45 and 50± 1 C for 10 minutes. The treated fruits were then incubated at 25±2°C with 90 percent relative humidity. In all the above epidemiological and control studies, described against inoculums after inoculation, the fruits were covered by a thin layer of sterilized cotton dipped in sterilized water and the inoculated fruits were examined on 3rd and 6th days after inoculation to record the disease incidence and severity by using standard formulae and disease assessment keys (Mayee and Datar, 1986). Before spray the food preservatives, pomegranate fruits of uniform size were surface sterilized with mercuric chloride solution and inoculated with the pathogen by cork-borer wounding method. Food preservatives tested for the disease were 0.5% Potassium metabisulphite (KMS), 0.5% sodium benzonate, 0.5% acetic acid and 10% common salt with untreated control. The food preservatives were tested by dipping the fruit in the solution of food preservatives. The food preservatives were used in both pre-and post-inoculation treatment.

Fruits were first dipped in the test food preservatives for 10 minutes in an airtight jar and then inoculated, while in post-inoculation treatment, the fruits were first inoculated and then treated with food preservatives in an airtight jar. The interval between inoculation and with food preservatives or vice-versa was 24 hours. There were four replications in each treatment. Control was also maintained. *X*tend® bags were used as modified atmospheric breathebla pacakaing for the preservation of pomegranates fruits up to 3 to 4 months after harvest. For commercial storage, modified atmospheric packaging technology is simple and cost-effective. It is also important to seal the bags only after transferring the fruit at room temperature to avoid water condensation and undesirable gas compositions inside the bags. Besides, the application of approved postharvest fungicides is recommended to enhance the performance of the packaging in preventing decay. For long-term storage in bulk packaging, it is recommended that fruit harvested during the middle of the harvest season be packed and not early immature or late over-ripe fruits

RESULTS AND DISCUSSION

Pomegranate is an important fruit crop being grown commercially in many countries. Pomegranates are susceptible to various decay-causing pathogens during post-harvest by many diseases such as black mould, grey mould, and blue mould fruit rot. The main problem in this fruit is at the maturity stage. Here we discussed losses caused by the black mould fruit rot of pomegranate during the transportation and storage as well as the protection by different management methods.

Injury to fruits is one of cause to fruit rots in pomegranate (Pathak, 1980; Whiteside, 1982: Khatri and Godara, 1999 and McDonald *et al.,* 1985). The present study also injury helped in the development of black mould rot on pomegranate fruits. Whereas, uninjured fruits were not infected, suggesting the rot can be minimized by careful handling of the fruits during harvesting, transport, and storage. Careful handling for the control of black mould rot of citrus caused by *Aspergillus niger* has also been suggested by Khatri and Godara (1999).

Symptoms of various rots in different fruits did not appear when inoculated at the immature stage as have been reported by other workers (Mehta *et al.*, 1975; Garg and Gupta,1979 and Blancard *et al*., 1984) However, in the present study *Aspergillus niger* was found to exhibit symptom in all the stages and maximum severity was found at the ripe stage of fruits. Mature fruits of lemon are reported to be more susceptible to sour rot caused by *Geotrichumcandidum* (Baudoin and Eckert, 1985).

Pomegranate has high commercial value and also hike in market price depends on season. After harvesting, fruits suffer from the huge loss that occurs during transportation and storage. Black mold fruit rot is an important post-harvest disease of pomegranate. The loss is upto 10-20 per cent. Hence, we discussed the survey of black mould fruit rot disease and its management by different methods to minimize the losses and to extend the shelf life of pomegranate fruits.

The surface-sterilized ripe fruits inoculated separately with isolated incitants (*Aspergillus niger*) showed symptoms of rot on the 6th day of inoculation, Un-inoculated fruits remained healthy upto the 6th day of inoculation. The symptoms observed in each inoculated fruit with separate incitants were similar to those seen in naturally infected fruits. Re-isolation from infected areas of inoculated fruits in each rot yielded the same culture which was identical to the one used for inoculation of the fruits. Symptoms of the disease and identity of the pathogens were as follows.

Mode of infection

The inoculation by cork-borer wounding method proved significantly most effective for infection and disease development of *Aspergillus niger* than the rest of the methods (Table 1). It resulted in the highest disease severity with the lowest incubation period. The next efficient method of inoculation was pin pricking. This method produced significantly higher disease than the scrapping and rubbing method. The disease severity was maximum on both the third and sixth days after inoculation. Symptoms of the disease did not appear when fruits were inoculated without injury. The incidence of the rot was cent percent on both the third and sixth days after inoculation. Rubbing was a less effective method of inoculation.

Effect of chemical fungicides

The fungicides tested were significantly superior in reducing the disease severity as compared to control in the pre-inoculation treatment at both 3rd and 6th day of inoculation (Table 2). Bavistin proved to be the most effective followed by Indofil M-45, Captan, Kavach and Blitox-50. Similar results were also observed in post-inoculation treatment at both the third and sixth days after inoculation (Table 2).

Different fungicides like Bavistin, Indofil M-45, Blitox, Captan and Kavach, *etc*., are effective in checking the storage rots of citrus fruits caused by a different fungal pathogen (Majumdar and Pathak 1995). In the present study, Bavistin proved most effective against *Aspergillus niger* in both pre-as well as in post-inoculation treatment followed by Indofil M-45 in checking the rot in both the treatments.

Effect of botanical oils

In the pre-inoculation treatment, the rot could not be completely checked by any oil (Table 3). However, the severity was significantly reduced with all the oils tried after the sixth day of inoculation. Castor oil was significantly most effective followed by neem oil, mustard oil, soybean oil and linseed oil tried in controlling the rotting, in the pre-inoculation treatment 6 days after inoculation. Similar results were also

Table 1: Incidence and severity of black mould fruit rot in pomegranate fruits inoculated by different methods and incubated for 3 and 6 days after inoculation at 25±1°C.

Method of inoculation	*Aspergillus niger*			
	Incidence (%)		Severity (%)	
	3 days	6 days	3 days	6 days
Cork borer wounding method	100	100	9.00	18.0
Rubbing method	100	100	1.50	4.50
Scrapping method	100	100	2.00	6.50
Pricking with Alpines	100	100	3.00	13.00
Inoculation without injury	0.00	0.00	0.00	0.00
SEm±	-	-	0.06	0.37
CD at 5%	-	-	0.18	1.12

Table 2: Effect of pre and post inoculation treatment with fungicides on the severity of black mould rot of pomegranate fruits incubated for 3 and 6 days after inoculation at 25±1°C.

Fungicides	Concentration (%)	*Aspergillus niger* (pre-inoculation)		*Aspergillus niger* (post-inoculation)	
		Severity (%)		Severity (%)	
		3 days	6 days	3 days	6 days
Indofil M-45	0.2	4.60	6.00	6.00	8.00
Bavistin 50% WP	0.1	3.75	4.25	5.00	7.50
Captan 50% WP	0.2	5.20	7.00	7.50	9.25
Kavach 75% WP	0.2	6.09	9.00	8.25	10.50
Blitox-50	0.2	8.50	10.50	9.00	11.50
Control		10.00	20.50	11.00	21.50
SEm±		0.15	0.26	0.20	0.29
CD at 5%		0.44	0.78	0.58	0.87

Table 3: Effect of pre and post inoculation treatment with oils on the severity of black mould fruit rot of pomegranate fruits incubated for 3 and 6 days after inoculation at 25± 1°C.

Oils	Concentration (%)	*Aspergillus niger*(pre-inoculation)		*Aspergillus niger*(post-inoculation)	
		Severity (%)		Severity (%)	
		3 days	6 days	3 days	6 days
Mustard oil	4.0	8.00	12.50	9.50	12.50
Neem oil	4.0	6.50	9.50	8.00	11.50
Castor oil	4.0	4.50	6.75	5.00	7.50
Soybean oil	4.0	8.50	13.50	12.50	18.50
Linseed oil	4.0	9.50	16.50	10.50	17.50
Control	-	10.50	20.00	11.00	22.00
SEm±	-	0.20	0.34	0.25	0.41
CD at 5%	-	0.58	0.90	0.75	1.20

observed in post-inoculation treatment at 3 and 6 days after inoculation (Table 3).

Because of environmental pollution and associated health hazards as well as the development of fungicide resistant strains of the pathogen, the emphasis is now gradually shifting from synthetic fungicide to natural products for the management of various plant diseases. The effectiveness of commercial oils against storage rots of fruits has been reported by different workers (Aulakh and Grover, 1968). In the present investigation, castor oil proved effective in controlling the rot in pre-as well as in post-inoculation treatments followed by neem oil in controlling the rot in both treatments.

Castor oil has been also reported to be effective against several fruit rots by Pathak *et al*. (1976); although neem oil against fruit rots have not been reported so far it proved to be effective against rot and this may be due to the presence of *nimbicidin* an antifungal substance (Khatri and Godara, 1999) was reported to inhibit the growth of *Aspergillus niger* causing fruit rots in citrus, papaya and mango.

Effect of hot water treatment

The rotting was not completely checked by hot water treatment on the third day of inoculation at 50 ±1°C. The severity was significantly reduced. Although no rot was recorded as compared to control, when fruits were treated with hot water at all temperatures *i.e*. 40±1°C, 45±1°C and 50 ±1°C on the 6thday of inoculation (Table 4). Hot water treatment has been reported to be effective against various fruit rots (Pathak *et al.,* 1976; Pathak and Shekhawat, 1977; Majumdar and Pathak, 1991). In the present investigation hot water treatment at 40±1°C to 50±1°C for 10 minutes reduced the severity of the rot significantly but at this temperature burning of fruit rind was observed. However, further investigation must be carried out to enhance the efficiency of hot water treatment. Hot water treatment at 52°C for 15 minutes has also been found to be effective against post-harvest fungal rot of orange (Godara, 1994).

Effect of food preservatives

In pre-inoculation treatment, the rotting was not completely checked by any food preservative (Table 5). The severity was significantly reduced with all the food preservatives on the sixth day of inoculation. However, potassium metabisulphite was significantly superior to other treatments in controlling the rot on sixth day after inoculation. Similarly, the post-inoculation treatment at 3 and 6 days of inoculation, the potassium metabisulphite and sodium benzoate treatments were on par but superior to the other three treatments (Table 5).

Table 4: Effect of hot water treatment on the severity of black mould fruit rot of pomegranate fruits incubated for 3 and 6 days after inoculation at 25± 1°C.

Hot water Temperature (%)	*Aspergillus niger*	
	Severity (%)	
	3 days	6 days
40± 1°C	3.57	12.40
45± 1°C	2.00	7.50
50± 1°C	0.00	4.00
Control	10.50	21.00
SEm±	0.06	0.17
CD at 5%	0.18	0.52

In food-preservatives treatment, Potassium metabisulphite (KMS) proved effective in controlling the rot in pre-as well as in post-inoculation treatments followed by sodium benzoate. Use of KMS alone and in combination was also observed to be effective against mango malformation and white specks of Aonla (Mehta *et al.,* 1986 and Pramod *et al*., 2007).

Effect of packaging materials on shelf-life of pomegranates fruits

In Table 6, demonstrates the effect of breathable modified atmospheric packaging materials on the shelf-life of the pomegranate fruits. Effect of breathable modified atmospheric on postharvest storage performance and quality of pomegranate fruits observations were made after 12 weeks of storage at room temperature. The fruits were not treated with any post-harvest chemical or fungicides before packing.

Table 5: Effect of pre and post inoculation treatment with food preservatives on the severity of black mould fruit rot of pomegranate fruits incubated for 3 and 6 days after inoculation at 25± 1°C.

Food preservatives	Concentration (%)	*Aspergillus niger* (pre-inoculation)		*Aspergillus niger* (post-inoculation)	
		Severity (%)		Severity (%)	
		3 days after Inoculation	6 days after Inoculation	3 days after Inoculation	6 days after Inoculation
Potassium metabisulphite	0.5	7.50	10.30	8.50	11.00
Sodium benzoate	0.5	9.50	11.50	9 .00	11.50
Acetic acid	0.5	8.00	12.00	10.00	15.50
Common salt	10.0	9.50	12.50	10.50	16.50
Control	-	11.50	21.50	12.50	22.00
SEm±	-	0.19	0.29	0.20	0.30
CD at 5%	-	0.57	0.87	0.57	0.85

Table 6: Effect of breathable modified atmospheric packaging on pomegranate fruits after 12 weeks of storage.

	Control (untreated)	Breathable modified atmospheric
Weight loss (%)	12.3	3.6
Decay (%)	25.0	2.2
Scald (%)	29.0	6.0
Taste score (0-10)	8	9

Breathable modified atmospheric packaging for post-harvest storage showed a very good response in maintaining the quality of pomegranate fruits by shrink and cling wrap. Observations were made after 12 weeks of storage at room temperature. Note that fruits were not treated with any postharvest chemical or fungicides before packing. The *X*tend packaging reduced the magnitude of changes during storage *i.e.*, ripening process drastically as evident from lower total soluble solids, higher total sugars, physiological loss of weight (PLW) was less than 3.6%. Very less decay (2.2%) and scald (6%).

CONCLUSION

Fruits inoculated without injury did not show symptoms of rot. All the fruits inoculated by cork-wounding, pin-pricking, scrapping and rubbing method exhibited symptoms of rot. Cork-wounding proved to be the most efficient method of inoculation. Post-harvest dipping in hot water, chemicals, fungicides, botanical oils and food preservatives provided effective control of the rot. Oil suspensions were found effective in preventing the rot. Food preservatives were also found effective in preventing rot, potassium metabisulphite 0.5 per cent followed by sodium benzoate 0.5 per cent proved most effective against the rot in both pre-and post-inoculation treatments. Breathable modified atmospheric packaging for postharvest storage showed very good performance in maintaining the quality of pomegranate fruits up to 12 weeks.

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