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Studies on standardization of hot water treatment technique to manage post-harvest anthracnose of mango

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Abstract
The limitations in using fungicide resources and probability of the development of resistance to the fungicides prompted us to search for viable alternatives that are non-chemical, eco-friendly, simple to implement, cheap and non-detrimental to human health. Consequently, we used hot air and hot water treatments at selected temperature and time combinations for the control of anthracnose disease in mango fruits and the study revealed that all the selected hot water treatments delayed anthracnose disease in the mango fruits to standardize a hot water treatment protocol to manage the post-harvest anthracnose. The experiment was clearly point out that the incidence of anthracnose on untreated control fruits was having disease incidence of 13.3% with disease severity of 60.0%. While, HWT @ 50 °C for 30 minutes caused minimum disease incidence of 3.3% showing minimum disease severity of 20.0% followed by 26.7% on fruits treated @ 52 °C for 10 minutes with the incidence of 4.4%.

Keywords: *Colletotrichum gloeosporioides*, *Mangifera indica*, anthracnose, post-harvest and heat water treatment technique

Introduction
Mango (*Mangifera indica* L.) is an important fruit crop of India and other tropical and subtropical countries of the world. It is believed to be originated within a large area including north western Myanmar, Bangladesh and north-eastern India. It is being called the "King of the fruits". In India, major mango growing states are Uttar Pradesh, Andhra Pradesh, Bihar, Gujarat, Karnataka, Maharashtra, Tamil Nadu, West Bengal and almost all states with total production of 20.79 million tons in 2293 thousand ha area. In Rajasthan, it is cultivated in 4.97 thousand hectares area with production of 87.37 thousand tons. Rajasthan holds the first place among all the Indian states in productivity of mango with 17.57 million tone/ha (Anonymous, 2017-18) [2].

Mango is prone to many fungal diseases like anthracnose, rhizopus rot, stem end rot, penicillum rot, black mould rot, mucor rot, phyllosticta rot, pestalotia rot, macrophoma rot and powdery mildew, leading to heavy lose in yield. Among these diseases, anthracnose is the major disease of mango as it occurs at all the growing parts including leaves, twigs, flowers, fruits except root and trunk throughout the year. Anthracnose caused by the fungus *Colletotrichum gloeosporiodes* also known by the name of its prefect stage as *Glomerella cingulata* is the most important disease of mango worldwide. It is the major pre and post-harvest disease of the fruit in all mango producing areas of the world.

The symptoms on leaves emerge as irregular shaped black necrotic spots on both the sides. Lesions repeatedly coalesce and form large necrotic areas, commonly along the leaf margins. Blossom blight or panicle anthracnose can affect both the individual flowers and inflorescence stalk. Elongated dark grey to black lesions appear on the stalk. Blighted flowers dry and fruits size smaller than pea-size can be infected and aborted. Fruit stalks also get infected lending to the fruit drop at various stages. Post-harvest anthracnose appears as rounded brown to black lesions with an unclear border on the fruit surface. Lesions of different size can coalesce and cover broad areas of the fruit. Lesions are usually limited to the peel, but in severe cases the fungus is recorded to attack the pulp also. In advanced stages of the disease, the fungus produces acervuli and abundant orange to salmon pink masses of conidia on the lesions (Arauz, 2000) [3].

C. gloeosporioides is known to infect several fruit crops including mango (Sanders and Korsten, 2003) [13]. Under favourable conditions; the fungus can invade the twigs and cause dieback (Ploetz *et al*., 1996) [12].

Blossom blight or panicle anthracnose can affect both the inflorescence stalk and the individual flowers (Arauz, 2000) [3]. Incidence of this disease can reach almost 100% in fruits produced under wet or very humid conditions (Arauz, 2000) [3], and yield losses ranged from 30 to 60% were recorded due to anthracnose of mango in several countries (Akem, 2006; Chowdhury and Rahim, 2009) [1]. In addition, post-harvest decay due to mango anthracnose can cause rejection of fruits in the market.

There are different strategies that can be employed for the management of postharvest diseases in mango fruits which include controlled environment, waxing, use of fungicides, handling and storage, irradiation and heat treatments. However, for fungicide and wax treatment, poisoning of the fruits due to chemical residue has been one of the limitations. Besides, concerns about environmental contamination and human health risks associated with fungicide residues periodically led to regulatory reviews and restrictions or cancellations. In addition, the wide spread and continuous use of these synthetic fungicides has led to the resistance of fungi which compromised the effectiveness of these fungicides [Oladele *et al.*, 2018] [11]. In fact, the cost of developing new pesticides to overcome resistance developed by pathogens and the withdrawal of some chemical pesticides, such as benomyl and captan for control of postharvest diseases in the USA and ethylene dibromide for sterilization of Queensland fruit fly in Australia, is a clear signal that new technology for control of plant diseases as an alternative to chemical fungicides is required. As a result, the use of non-chemical eco-friendly means of control such as heat treatments have emerged as viable alternatives.

The efficacy of pre storage heat treatments has been well documented. Nevertheless, earlier authors (Inkha *et al*., 2009) [8] who carried out extensive studies on the effects of post-harvest heat treatments on fruits had concentrated so much on citruses and storage at cool temperatures. So far there has been no report on the effects of hot air and hot water treatments on disease severity caused by *C. gloeosporioides* in infected mango fruits during storage at 28 ± 2 °C and 75 ± 5% relative humidity (RH). On the other hand, the geographical region can affect the response of mango cultivars to disease severity by *C. gloeosporioides* and heat treatments.

Material and methods

Source of fruits

Mature, green and healthy mango fruits were harvested from mango orchard at Agricultural Research Station, Banswara (Rajasthan).

Preparation and sterilization of culture medium

The culture medium used for isolation of fungi from the spoiled mango fruits and for preparation of pure cultures in the study was prepared by weighing 50 g of malt extract agar (MEA) into a conical flask to which was added 1 litre of water. The mixture was shaken together and sterilized in an autoclave at 121 °C for 15 minutes. After sterilization, it was poured into oven sterilized Petri dishes and allowed to solidify.

Isolations from infected fruits

Isolation of the choice/test fungus (characterized by appearance of black spots) on the surface of spoiled mango fruits was made by cutting out the interface between the healthy and the disease tissue and placing pieces of the affected fruit rind without surface sterilization on plates of solidified malt extract agar. The plates were then incubated at 28 ± 2 °C for 7 days. Sub culturing of the isolate was prepared by transferring agar cut with distinct mycelium to sterilized Petri dishes containing solidified MEA and then incubated at 28 ± 2 °C until pure cultures were obtained. The resulting pure culture was then used for morphological characterizations of the isolate.

Morphological identification

After incubation, identification of the isolate was based mainly on the structural features as seen in the culture plates as well as microscopic characteristics. A drop of cotton-in-blue lactophenol solution was put on a slide. The isolate was placed on a slide. This was covered with a cover slip. Excess liquid was drained with filter paper and the isolate was examined under microscope. Examination was done with x40 objective for the presence and type of hyphae, mycelium whether clear or dark and spore morphology.

Material and Methods

The experiment was conducted during the year 2018 on mango cultivar Kesar. The fruits were hand-picked from mango orchard at Agricultural Research Station, Banswara. There were three replications for each treatment. The design of the experiment was completely randomised block design. The treatments of the experiment were: T_1: HWT @ 52 °C for 10 minutes; T_2: HWT @ 50 °C for 30 minutes; T_3: HWT @ 48 °C for 60 minutes; T_4: HWT @ 46 °C for 65 minutes and T_5: Control (Fruit wash with tap water). Fruits of uniform size and maturity were selected for the experiment. The fruits were cleaned thoroughly. Sample size of each treatment was 20 fruits per replication (out of which 5 fruits was used for quality parameters). Hot water treatment (HWT) was carried out in big glass pot poured with hot water and maintained desired temperature by consistently monitoring by thermometer. After each HWT, the fruits were given hydro-cooling in normal water and kept for storage in CFB boxes at room temperature after cooling.

Observation on disease severity and disease incidence were recorded after 7 days of storage under ambient conditions of room temperature. Disease severity of anthracnose was rated using a scale given by Akhtar and Alam (2002) where: 1= No fruit lesion; 2= 3 fruit lesion; 3= 4-6 fruit lesion; 4= 7-15 fruit lesions and 5= >30 fruit surface covered with lesions. The scale for measuring disease incidences of anthracnose was used as given by Akhtar and Alam, (2002) where: 1= none; 3=traces; 5= slight, 7 = moderate and 9= severe.

The data were subjected to analysis of variance (ANOVA) and the percent disease severity and percent disease incidence were calculated.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of inoculated fruits}} \times 100$$

Results and Discussion

Hot water treatment is a safer way of preserving fruits from postharvest diseases because it leaves no residue on the fruit after treatment and it is environmentally safe unlike the use of fungicides. The experimental findings clearly reveal that the untreated control fruits were having anthracnose disease

incidence of 13.3% with disease severity of 60.0%. While, HWT @ 50 °C for 30 minutes caused minimum disease incidence of 3.3% and also showing minimum disease severity of 20.0% followed by 26.7% disease severity on fruits treated @ 52 °C for 10 minutes with the anthracnose disease incidence of 4.4%. The subsequent treatments HWT @ 48 °C for 60 minutes and HWT @ 46 °C for 45 minutes significantly prevented incidence of anthracnose on fruits as compared with control during storage. Heat treatments have been used commercially to control fungal diseases and pest infestation since the first decades of the 20th century, when the effectiveness of hot water (44 °C – 48 °C) in controlling molds in citrus was reported (Escribano *et al*., 2014) [6]. Hot air has been used for both fungal and insect control and to study the response of commodities to high temperatures (Kshiesh *et al*., 2010) [9]. For instance, hot air treatment after inoculation had been discovered to increase the disease resistance of whole 'Red Fuji apple fruit' (Chen *et al.*, 2006) [4] and could completely control blue mold disease on the fruit while in 1992, for the first time, hot water treatment was employed to control rot on citrus fruits in Iran (Fatemi *et al.*, 2011) [7]. Also, immersing orange fruits of 'Clemenules' variety in 60 °C hot water for 1 minute decreased activity of green mold (Montesinos *et al*., 2009) [10].

Table 1: Effect of hot water treatment on the incidence of Anthracnose on mango fruits stored at ambient temperature

Treatments	Anthracnose disease incidence (%)			
	2016	2017	2018	Pooled
T_1- HWT @ 52 °C for 10 min	11.7 (19.9)c	12.3 (20.4)d	4.4 (11.9)c	9.5 (17.5)c
T_2- HWT @ 50 °C for 30 min	10.0 (18.0)bc	7.3 (15.7)e	3.3 (10.4)c	6.9 (14.8)c
T_3- HWT @ 48 °C for 60 min	25.0 (29.9)b	33.3 (35.2)c	7.5 (15.8)b	21.9 (27.0)bc
T_4- HWT @ 46 °C for 65 min	56.7 (48.9)a	54.3 (47.5)b	9.2 (17.6)b	40.1 (38.0)ab
Control	66.7 (54.8)a	76.3 (60.9)a	13.3 (21.3)a	52.1 (45.7)a
C.D. (P=0.05)	10.2	4.5	2.1	14.9

*Disease severity was recorded after 7 days of storage.

Table 2: Effect of hot water treatment on the disease severity of Anthracnose on mango fruits stored at ambient temperature

Treatments	Anthracnose disease severity (%)			
	2016	2017	2018	Pooled
T_1- HWT @ 52 °C for 10 min	31.1 (33.9)cd	26.7 (31.1)c	26.7 (31.1)c	28.2 (32.0)b
T_2- HWT @ 50 °C for 30 min	28.4 (32.2)d	21.3 (27.5)c	20.0 (26.5)d	23.2 (30.8)c
T_3- HWT @ 48 °C for 60 min	33.6 (35.4)bc	34.7 (36.1)b	29.3 (34.8)bc	32.5 (34.8)b
T_4- HWT @ 46 °C for 65 min	36.9 (37.4)b	37.3 (37.7)b	33.3 (35.2)b	35.8 (36.8)b
Control	54.0 (47.3)a	66.7 (54.8)a	60.0 (50.7)a	60.2 (50.9)a
C.D. (P=0.05)	4.2	4.8	4.1	4.7

*Disease incidence was recorded after 7 days of storage

Conclusion

The limitations in using fungicide resources and probability of the development of resistance to the fungicides prompted us to search for viable alternatives that are non-chemical, eco-friendly, simple to implement, cheap and non-detrimental to human health. Consequently, we used hot water treatments at selected temperature and time combinations for the control of anthracnose disease in mango fruits and the study revealed that all the selected hot water treatments delayed anthracnose disease in the mango fruits. However, HWT at @ 50 °C for 30 minutes resulted with minimum anthracnose disease incidence of 3.3% with showing minimum disease severity of 20.0% followed by 26.7% disease severity on fruits treated @ 52 °C for 10 minutes with the incidence of 4.4%. These effective heat protocols could be applied as part of an integrated pesticide-free alternative for the control of anthracnose decay by the fruit industry. In the same manner, post-harvest hot water dips have been considered effective against mango anthracnose. This was equally confirmed in this work as the effective Hot Water protocols delayed anthracnose disease during storage till after 7 days.

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