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Original Research Article

Hot water and UV – C as methods of physical control in postharvest losses of *Emblica officinalis* Gaertn

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Emblica officinalis; Aspergillus niger; hot water; UV-C; disease incidence.

A B S T R A C T

Indian gooseberry (*Emblica officinalis*) is known for its ethno-botanical uses since ancient times and its medicinal uses are recognized since time immemorial. The fruits are rich source of Vitamin-C, but available for very short period, thus proper storage becomes a primary concern. *Aspergillus niger* is the main cause of the rotting during storage, which is primarily controlled by use of synthetic fungicides. Alternative to synthetic fungicides against many pathogens have been developed. In this communication we are presenting the use of hot water and UV-C for control of black mold rot caused by *Aspergillus niger* in Indian gooseberry fruits. The hot water treatment at 60°C for 2minutes could check spore germination by 100%. In *in vivo* studies dipping at 60°C for 45 seconds could control 76% disease incidence. Similarly UV-C treatment was done in relation to distance and time. Exposure of fruits for 15 minutes from a distance of 15cm could effectively control spore germination. 48.67% control of natural decay was observed in case of *in vivo* experiments. The results suggest that use of hot water and UV-C can be used as alternative to the chemicals, which besides being easily available, is also environment and consumer friendly.

Introduction

Aonla (*Emblica officinalis*) or Indian gooseberry is indigenous to Indian sub-continent. India ranks first in the world in area and production of this crop. Apart from India naturally growing trees are found in different parts of the world like Sri Lanka, Cuba, Puerto Rico, USA (Hawai & Florida), Iran, Iraq, Pakistan, China, Malaysia, Bhutan, Thailand, Vietnam, Philippines, Trinidad, Panama and Japan.

The fruits act as antitumor (Jose *et al*., 2001); cytoprotective against chromium (Sairam *et al*., 2002); protects against oxidative stress in ischemic reperfusion injury (Rajak *et al*., 2004); induction of apoptosis (Rajeshkumar *et al*., 2003) etc. The root, bark and leaves are also used for the treatment of indigestion, diarrhea, dysentery, eczema and warts (Zhang *et al*., 2002). It is also said to relieve thirst, burning sensation impurity of the blood

and to promote abundant hair growth (Luanpitpong *et al.,* 2011) and has been used to control common cold scurvy cancer and heart disease (Khopde, 2001). Aonla is richest source of vitamin C, containing 30 times the amount found in orange. High productivity/unit area (15-20tons/ha), nutritive and therapeutic value, amla is becoming more and more commercially important with every passing year. Postharvest black mold, caused by *Aspergillus niger*, are the most economically important postharvest diseases of gooseberry fruits. Diseases among fruits are primarily controlled by application of synthetic fungicides. Alternative methods are needed because of concerns about environmental contamination and human health risks associated with fungicide residues and because the widespread use of these chemicals at commercial level has led to the proliferation of resistant strains of the pathogens. Thermal treatment methods using hot water vapour or hot air have been investigated extensively as an alternative to chemical fungicides (Olesen *et al.,* 2004; Gramje *et al.,* 2009; Kim *et* ., 2009). Water is preferred medium for most applications since it is a more efficient heat transfer medium than air. This treatment has number of advantages which include relative ease of use, short treatment time, reliable monitoring of fruits and water temperature and the killing of skin borne decay causing agents. Non ionizing UV irradiations, although low energy and lacking penetrating power, affects a wide range of biological processes and has been reported to stimulate various plant responses. A relatively new crop protection technology that involved exposing fruits and vegetables low to dose ultraviolet light (UV-C, 254 nm) was first shown by Stevens and colleagues to induce resistance to postharvest storage rots (Stevens *et al*., 1996; Wilson *et al*., 1994).Ultraviolet irradiation is now widely used as an alternative strategy to control microorganism in food products (Shama and Alderson, 2005; Jiang *et al*., 2010).

Materials and Methods

Pathogen inoculums

Aspergillus niger was isolated from infected gooseberry fruits. This culture was maintained on potato-dextrose agar medium (PDA: potato extract of 200g; dextrose, 20 g; agar, 20 g and distilled water, 1000 ml) at 4 °C, and fresh cultures were grown on PDA plates before use. A spore suspension was prepared by removing the spores from the sporulating edges of a 15 day old culture with a loop, and suspending them in sterile distilled water. Spore concentrations were determined with a hemocytometer, and adjusted as required with sterile distilled water.

Fruits

Gooseberry fruits were purchased from a local markets of Lucknow and the fruits were used immediately. Fruits were disinfected with 0.1% sodium hypochlorite for 2 min, washed with tap water, air dried prior to wounding in a pre-sterilized chamber.

Efficacy of hot water treatment in controlling the mold decay of Indian gooseberry

Hot water treatment (*In vitro)*

The test fungi obtained from the infected fruits of gooseberry was cultured on the PDA plates and spore suspension was prepared by removing the spore from the

edge of 15 day old sporulating colonies of the fungi, and suspending it in sterile distilled water. The suspension was filtered through four layer of cheese cloth to remove the mycelia. Spore concentration was adjusted using heamocytometer to 10^5spores/ml. The tubes containing spore suspension were treated on water bath shaker (60 strokes /minute). Treatment of different temperature (30-60°C) and time duration (2-15 min) were taken. After each treatment tubes were kept in ice bath immediately for cooling. After treatment 50µl suspension was spread on pre poured solidified PDA plates with the help of a sterilized spreader for separation of spores. Untreated spore suspension was taken as control. The plates were kept in incubator at 25±2°C. Plates were observed daily and percent spore germination was calculated. Each treatment had three replicates.

Hot water treatment (*in vivo*)

Freshly harvested *Emblica* fruits were obtained from local market of Lucknow, India. Fruits were washed under running water then surface sterilized with 70% ethanol and allowed to dry. Hot water treatment was applied in a water bath shaker (60 strokes /minute). After treatment, the fruits were air dried and packed in polythene bags and kept at 20±2°C along with the control fruits (untreated). Each treatment had three replicates. Experiment was observed daily. Percentage of decayed or infected fruits was calculated by counting the infected and uninfected fruits (Fallik *et al* 2000)

Efficacy of UV-C treatment in controlling the mold decay of Indian gooseberry

UV-C treatment (*in vitro*)

50µl spore suspension (10^5 spore/ml) was spread on PDA plates. When the suspension completely settled on the medium, these plates were exposed to UV-C light. After treatment, treated and untreated (control) plates were kept in incubator at 25±2°C. Experiment was observed daily and spore germination was calculated. Each treatment had three replicates.

UV-C treatment (*in vivo*)

UV-C treatment was applied by keeping the fruits below the UV –C light. UV-C treatment was subdivided into four smaller sub doses and fruits were individually rotated four times to expose four separate sides of the same fruit. After treatment, fruits were packed in brown paper bag and then kept in incubator at 20±2°C along with the control (untreated fruits).Each treatment had three replicates. Experiment was observed daily. Percentage of decayed or infected fruits was calculated by counting the infected and uninfected fruits (Stevens *et al* 2005).

Statistical analysis

All observations were made in three replicates, using three different sets of samples and the average values were subjected to student's t-test and the values were plotted as mean ± SE. Statistical analysis was performed with Microsoft excel 2007, according to the method of DDPaterson (1939) and were considered significant when $P<0.05$.

Results and Discussion

Efficacy of hot water treatment in controlling *Aspergillus niger* infection of Indian gooseberry

In vitro *In vitro* treatments of hot water

significantly decreased spore germination rate. The table 1 shows that the treatment of 30°C temperature did reduce spore germination, but not significantly. However hot water treatment at the higher temperature, 50 and 60°C was highly effective in reducing spore germination. 60°C treatment reduced 100% spore germination at minimum tested time range. The study showed at lowest time range i.e. 2 min; 60°C temperature inhibited 100% spore germination. Hence 60°C was taken for *in vivo* studies.

In vivo: *In vivo* treatments of hot water significantly decreased decay comparatively to control. Gooseberry fruits were treated with hot water, as shown in table 2. In *in vivo* experiments , 60°C temperature treatment was highly effective in reducing decay (according to time range) 15 second and 30 second treatment reduced infection but not significantly , 45 second treatment showed 76% control while 60 second treatment showed 100% control.

Efficacy of UV-C treatment in controlling *Aspergillus niger* infection of Indian gooseberry

In vitro **Table 3** shows effect of UV-C treatment on spore germination. *In vitro* UV-C treatment significantly decreased the spore germination rate in accordance with time duration and distance. UV-C treatment at 45cm distance showed measurable inhibition of spore germination at all time ranges. UV-C treatment at 30cm for 5min, 10 min, and 15min showed good inhibitory effect on spore germination of the test fungus. In case of *A. niger* 30cm, 5 min, 10min 15min showed 10.66%, 66.66%, 85.66% inhibition on spore germination respectively, 15cm; 2 min 5 min 10min 15min showed 13.33%, 42.33%, 85.33% and 100% inhibition of spore germination respectively. In different combinations of distance and time 15cm-15min showed excellent effect on spore germination inhibition. 100% spore germination inhibition was recorded in the test fungi.

In vivo: *In vitro* studies showed 100% inhibition on spore germination at 15cm-15min so 15 cm distance and different time range was taken for *in vivo* studies. 15cm; 5min, 10min, 15min treatment showed 26.00%, 35.34%, and 48.67% control of natural decay respectively. Result is presented in table 4.

Fruit and vegetables are an important for mankind, and for the requirements to be met successfully all fresh harvested commodities need to be free of disease agents, insects, and synthetic chemicals, and cleaned of any dirt or dust before being consumed or being packed for export. The susceptibility of freshly harvested fruits to postharvest diseases increases during prolonged storage. However, since there are very few, or, in many cases, no registered postharvest fungicides for control of decay-causing agents, postharvest rot is the major factor limiting the extension of storage life of many freshly harvested fruit and vegetables.

Control of postharvest infection by heat treatment has been reported for number of fruits and vegetables. (Kim *et al* 2009, Wasker and Gaikwad 2005, Mirshekari 2012) the result presented here demonstrate that hot water dip (HWD) treatment has potential to control natural infection of *Emblica* fruits . 60° C temperature treatment was highly effective in reducing decay depending upon the time duration of treatment and 100% control was observed at 60second treatment.

Table.1 Effect of hot water treatment on spore germination of *A. niger*

Test fungus	Treatment time	Temperature °C			
		30	40	50	60
A niger	2minutes	100±00	90.02 ±0.09	60.34 ±0.12	00±00
	5minutes	100±00	87.00±0.01	35.5±0.07	00 ±00
	10minutes	100±00	58.54±0.11	00 ± 00	00±00
	15minutes	100±00	43.00 ±0.12	00± 00	00 ±00
	20minutes	100±00	11.34 ±0.06	00± 00	00 ±00
	Control	100±00	100 ± 00	100 ± 00	100 ± 00

Table.2 Effect of hot water treatment on natural infection of *Emblica officinalis*

S .No.	**Treatment time(in seconds)**	**Percent of fruit infected at 60°C**
1.	15	100.00
2.	30	99.10
3.	45	24.00
4	60	00.00

Table.3 Effect of UV-C treatment on spore germination of *A. niger*

Test fungus	**Treatment time**	**Height in cm**		
		15	**30**	**45**
A.niger	2minutes	86.67±0.15	95.67 ±0.07	100.00 ± 00
	5minutes	57.67± 0.04	89.34± 0.04	97.00± 0.37
	10minutes	14.67± 0.18	33.34 ±0.26	88.67± 0.14
	15minutes	00± 00	14.34±.08	81.34±0.93
	20minutes	00± 00	00±00	00± 00
	Control	100± 00	100±00	100± 00

Table.4 Effect of UV-C treatment on natural infection of *Emblica officinalis*

S .No.	**Treatment time**	**Percent of fruit infected at 15cm**
1.	Control	100.00
2.	5min	74.00
3.	10min	64.66
4	15min	41.33

UV-C could also be used to significantly reduce the natural infection of *Emblica* fruits. Exposure to UV-C radiation at different distance for different time duration gave a range of inhibition effectively controlling the rot at 15cm; 15min treatment (*in vitro*) (Alawami 2010). Possible mode of action of UV may be interference with hyphal wall metabolism, germicidal effect on microbes and induce resistance in post harvest commodities as a result rate of physiological changes may be altered , which may favour increased shelf life delayed senescence and resistance to pathogens.

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