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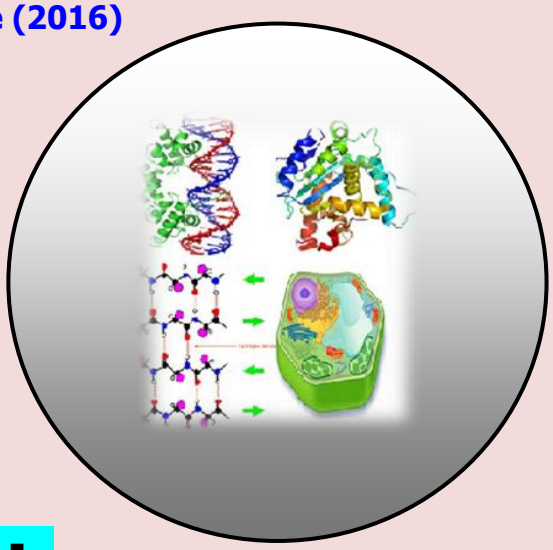

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RESEARCH PAPER

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Managing Post Harvest Diseases of Apple and Mango by UV-C and Hot Water Treatment

Madhu Prakash Srivastava, *Shashi Gupta,
Neeta Sharma and Yogesh Kumar Sharma

Center of Excellence, Department of Botany, University of Lucknow, Lucknow, U.P., India
***K.G.B.V. Mohanlalganj, Lucknow, U.P., India**

ABSTRACT

Postharvest losses have been reduced mainly through postharvest fungicides and to a lesser degree, through postharvest management practices to reduce inoculum or effective management of the cold chain system .We examined the possible use of a hot water treatment (HWT) with UV treatment to disinfect apple and mango fruits. In vitro studies showed than Penicillium expansum was sensitive to high temperature. It was observed that the treatment of 40ºC temperature was least effective in reducing spore germination of P. expansum at any duration of exposure to hot water treatment. Complete inhibition of P. expansum was achieved by exposing the spores to hot water treatment at 60ºC for duration of 2 min and at 50ºC for 20 min. 60ºC for duration of 2-20 min was 100% effective for both pathogens. The growth of B. theobromae was completely inhibited at 50ºC for 20 min and 60ºC for 2 min. UV-C treatment significantly decreased the spore-germination of P.expansum in accordance with duration of exposure and distance. UV-C treatment at 10 cm distance from the source of radiation for 2, 5, 10, 15 and 20 min showed 100% inhibition. In vivo treatments of hot water significantly decreased decay as compared to control. In vivo experiments showed that 60ºC for 1 min treatment was most effective in reducing decay. The exposure for 2 min or more at the same temperature reduced the appearance and commercial value of fruit. In apple fruits UV-C irradiation at the distance of 10 cm for 5 min was most effective to control decay caused by P. expansum. Increase in duration of exposure beyond 5 min at 10 cm distance injured both fruits and increased lesions instead of reducing them. In mango fruits effective control of B. theobromae was observed upon exposure for 5 min. HWT alone ye in combined which was applied separately were each able to reduce natural decay incidence to commercially acceptable levels (5%) and combining them showed no additional effect.
Key words. Hot-water Treatment, Physical Agent (UV), P. expansum, B. theobromae, Apples (Pyrus malus L.), Mango (Mangifera indica L.), Blue Mold Disease and Biological Control.

INTRODUCTION

Many of the fungicides such as benzimidazole and dicarboximide fungicides that are still available for use are losing their effectiveness because of the development of resistance in many postharvest pathogens

(Lennox and Spotts, 2003). The use of fungicides has been becoming increasingly more restricted because of health concerns due to contamination with chemical residues (Ragsdale and Sisler, 1994). The onset of postharvest decay in fruits and vegetables in forms of stem end rot and surface rot is caused by a number of microorganisms (Snowdon, 1990). Postharvest rots cause serious economic loss of 25 to 50 % worldwide. Postharvest losses have been reduced mainly through postharvest fungicides (Eckert and Ogawa, 1988) and, to a lesser degree, through postharvest management practices to reduce inoculum or effective management of the cold chain system (Thompson *et al.,* 2002). Development of resistance to commonly used fungicides within populations of postharvest pathogens has become a significant problem (Reimann and Deising, 2000) and has generated negative public perception with increasing demand to discontinue the use of such chemicals. This negative perception has promoted governmental policies restricting the use of fungicides (Gullino and Kuijpers, 1994). It is therefore necessary to find alternatives to control postharvest pathogens. Various methods have been investigated, and although they show promise, none alone has been found to be as effective as fungicides. It is therefore necessary to develop a strategy which combines several of these alternatives that may equal the effectiveness of fungicides. Apples (*Pyrus malus* L.) of the family Rosaceae popularly known as 'hill fruit' or 'cold region fruit' originated in Middle East nearly 4000 years ago and are grown in temperate climates. Apple is called 'seb' in Hindi and 'malus' in Latin. Some varieties of apple grown in the country are Red Delicious, Golden Delicious, Baldwin, Ambri Kashmiri, Maharaji, Hazaratbali, Jonathan, Rome Beauty, Granny Smith, King of Pippins, Winter Banana, Richard, Starking Delicious, Yellow Newton, Golden Pippin, Early Shanburry and Irish Peach. Indian apple production averaged nearly 1.4 million tons during 2002-2004, making it the sixth largest apple producer in the world. Its area is estimated to be second largest in the world while its average yield, about 5.5 tons per hectare, is the lowest of the major world producers. Nearly all of India's apples are grown in three mountainous states in north India- Himachal Pradesh, Jammu and Kashmir, and Uttaranchal Pradesh- where they typically grow at an altitude of 4,000 to 11,000 feet. Jammu and Kashmir has the highest average yield and accounts for about two- thirds of total production. The remaining small amounts of production occur in the hill regions of north-eastern India, in the states of Arunachal Pradesh and Nagaland (Deodhar, 2006). Mango (*Mangifera indica* L.) a member of family Anacardiaceae, is one of the most ancient among the tropical fruits indigenous to India. It was established in India in the pre Christian era. Its cultivation in india is estimated to be more than 4,000-6,000 years old. 'Mango' comes from Portuguese manga, which is probably from Malayalam manga. Mango is called 'aam' in Hindi, 'mango' in English and Spanish, with only slight variations in French called 'mangot', 'mangue', 'manguier', Portuguese 'manga', 'mangueira', and Dutch 'manja'. Mangoes are ranked as one of the top five fruit crops in the world (Oosthuyse, 1993). The Food and Agriculture Organization of United Nation estimates worldwide production of mangoes at more than 23 million tones in 2001 with 12 million tones produced annually (2002-2003), India accounts for almost half of world production, followed by China (3 million tons), Pakistan (2.25 million tons), Mexico (1.5 million tons) and Thiland (1.35 million tons). India accounts for almost half of the world production followed by China, Pakistan, Mexico and Thiland. Mango is the most important fruit covering about 35 per cent of area and second largest fruit in the country next to banana accounting for 22 per cent of total production followed by citrus, apple, grapes etc (Singh, 2008), which is highest in the world with India's share of about 54%. India has the richest collection of mango cultivars. Major mango growing States are Uttar Pradesh, Bihar, Andhra Pradesh, Orissa, West Bengal, Maharashtra, Gujarat, Karnataka, Kerala and Tamil Nadu. Some varieties of mango grown in the country are Alphanso, Dashehari, Banglora, Banganpalli, Langra, Fajri, Chausa, Kisan bhog, Totapuri, Neelum, Malda, Mallika, Amrapali, Ratna, Manjeera, kesar, Neeeshwari and Neelphonso. Hot air treatment (38 ◦C for 4 d) of apples after inoculation with *Penicillium expansum* either reduced or completely eradicated decay caused by this pathogen (Leverentz *et al.,* 2000). Decay of apple fruit by *Colletotrichum acutatum* was also reduced by this same heat treatment but was not eradicated as in the case of *P. expansum* (Janisiewicz *et al.,* 2003). Heat treatment, while a good eradicant, has no residual activity (Fallik *et al.,* 1996). Although many alternatives to chemical control have been investigated, none, when used alone, is as effective as fungicides. The reduction of decay by biological control is generally more variable than for fungicides since biocontrol is affected more by environmental factors. There is also a narrower spectrum of activity than is found with chemical control. While heat treatment is very effective in eradicating decay if infection occurs prior to heating, it has little protective effect if infection occurs after heating (Klein *et al.,* 1997). Also, while heat treatment is effective in eradicating infection by *P. expansum*, it is much less effective against *C. acutatum* (Janisiewicz *et al.,* 2003).

The treatments described above have been shown to be complementary to one another when applied in combination, and therefore the combinations are more effective than any individual treatment. When an antagonist specific for *P. expansum* was combined with one specific for *B. cinerea*, the resulting mixture inhibited the development of both types of lesions (Janisiewicz, 1988). Among the proposed alternatives, hot water brushing (HWB) method for rinsing and disinfecting fruit and vegetables has been recently developed (Fallik *et al*., 1999). This technique simultaneously cleans and disinfects the fruit and improves their general appearance while maintaining their quality. HWB has already been adopted commercially to clean and disinfect peppers (Fallik *et al*., 1999), mangoes (Prusky *et al*., 1999), kumquat (Ben-Yehoshua *et al*., 1998), organically grown citrus (Porat *et al*., 2000) and melon (Fallik *et al*., 2000). Our research goal is to combine several alternatives to develop a control strategy that will be highly effective and reliable in reducing postharvest decay of apples. The objective of this investigation was to improve upon our previous decay control strategies by studying the effect of a mixture of antagonists, heat, and, alone and combined, on reducing postharvest decay caused by *P. expansum* and *B. theobromae.* The objective of the present study was to determine the effect of HWT alone and in combination with the application of U. V. for the control of blue mold rot of apple fruits caused by *P. expansum* and mango fruits caused by *B. theobromae.* An effort has been made in the present study to combine these disparate measures to achieve safe and more effective postharvest disease control measures against fruit rotting fungi.

MATERIAL AND METHODS

Apples (*Pyrus* mal*u*s L.) and Mango (*Mangifera indica* L.) used in the experiments at commercial were obtained from wholesale market and kept at 0ºC until use. *Penicillium expansum* Link and *B. theobromae* Link ware obtained from infected peach fruit and cultured on Potato Dextrose Agar ((PDA: extract of boiled potatoes, 200 ml; dextrose, 20 g; agar, 20 g and distilled water, 800 ml). Spore suspensions were prepared by removing the spores from the sporulating edges of a 2–3-week-old culture with a bacteriological loop, and suspending them in sterile distilled water. The spore concentration was determined with a hemocytometer and adjusted to 10^5 spores ml^{-1}. To obtain single spore isolate, method described by Manandhar *et al.* (1995) was followed. Petri dish containing the fungus was flooded with sterile distilled water and serial dilution was made. One ml of the suspension (50 conidia/ml) was spread on Petri dish containing water agar (25 ml). The inoculated Petri dishes were incubated at 25±2^{0}C for 12-24 h. The colony formed by single spore was picked and subculture on fresh medium for further studies. The purified pathogen was identified up to genus level based on cultural character and type of conidia produced. The culture was purified using hyphaltip method and maintained on PDA slants in the refrigerator at 6-8ºC for further studies (Brown, 1934).

In vitro efficacy of physical treatments against fungal pathogens

Hot Water Treatment

To determine the effect of hot water treatment (HWT) on *P. expansum* conidia germination, the sterile screw capped glass tubes containing 1.8 ml sterile distilled water were placed in water bath at 40°C, 50°C or 60°C allowed to equilibrate for 30 min. Then 0.2 ml of concentrated conidial suspension was added to the tubes, to achieve a final concentration of 1×10^6 conidia ml^{-1}. After 2, 5, 10, 15 and 20 min of incubation tubes were removed from water bath and immediately placed on ice bath. Aliquots of 20µl of each suspension were plated on PDA plates (Karabulut *et al.,* 2002). After 72h of incubation at 25±2ºC the colonies were counted and percent inhibition over control was calculated. To observe the effect of same treatments on *B. theobromae* culture disks were used. The *B. theobromae* culture disks exposed to hot water treatments were placed in the center of the PDA plates and radial growth of treated disks was compared to control.

Percentage of spore germination inhibition = [dc-dt/ dc×100], where dc = Number of fungal colonies in control and dt = Number of fungal colonies in treatment

Percentage of radial growth inhibition = [dc-dt/ dc×100], where dc = radial growth of fungal colony in control and dt = radial growth of fungal colony in treatment

UV-C Treatment

A germicidal low pressure mercury vapour discharge lamp (Sankyo Denkii, Japan model G30T8) having nominal power output of 30 W, and a maximum amperage of 0-36 emitting quasi-monochromatic UV radiation energy at 254nm was used. The lamp had tube diameter of 2.5 cm and length of 88 cm. The lamp was mounted 2.5 cm apart on a cast iron frame with stainless steel reflector. The reflector was of parabolic design which has uniform distribution of UV light.

PDA plates seeded with test fungus (20µl/plate) and were exposed to UV-C irradiation for 2, 5, 10, 15 and 20 min duration at the distance of 10, 15, 30 and 45 cm from the surface of lamp. Average dose rate was 1.24mW/cm^2. A UVX digital radiometer was used to measure UV-C dose rate. All the PDA plates except non-irradiated control were irradiated with a UV-C lamp. Each UV-C irradiation experiment was carried out at room temperature. Petri plates were incubated in darkness for 72 h at 25±2ºC to avoid photoreactivation (Sharma *et al*., 2005). UV-C irradiation treatment was given to *B. theobromae* by exposing the culture disks facing the mycelia part towards source of irradiation. After treatment culture disk was kept inverted touching the medium with its UV-C exposed mycelia part and radial growth of treated disks was compared to control. Percentage of spore germination inhibition = [dc-dt/ dc×100], where dc = Number of fungal colonies in control and dt = Number of fungal colonies in treatment

Percentage of radial growth inhibition = [dc-dt/ dc×100], where dc = radial growth of fungal colony in control and dt = radial growth of fungal colony in treatment.

***In vivo* efficacy of physical treatments against fungal pathogens**

Hot Water Treatment

Trials were carried out on apple and mango fruits having no blemishes, injury or disease. Fruits were washed under running water and allowed to dry. The apple fruits were inoculated by 20 µl of spore suspension (10^6 spore ml^{-1}) of *P. expansum* in wounds at three sites. The mango fruits were inoculated at two sites by the culture disk of *B. theobromae.* After 24 h fruits were exposed to hot water treatment. Apple and mango fruits were treated with hot water at 50°C, 55°C and 60°C for 1, 2 and 5min. Fruits rinsed with tap water served as control. Air dried fruits were incubated at 25±2ºC (Karabulut *et al.,* 2002). Apple fruits were evaluated daily for decay symptoms over a period of 14 days while mango fruits over a period of 7 days. Lesion diameters were measured and compared to control. In all the experiments designed to control lesion development against respective pathogens in wounded fruits, each treatment consisted of three replicates, each containing 15 fruits. Each experiment was repeated three times.

UV-C Treatment

UV-C treatment was applied by keeping the fruits below the UV-C light. UV-C treatment was sub-divided into four smaller sub doses and fruits were individually rotated four times to expose four separate sides of the same fruit (Stevens *et al.,* 1997). Apple and mango fruits were exposed to UV-C at 10 cm distance from the source of radiation to doses [(in kJm^{-2}) and total time duration of exposure (in min)] of 1.48 KJ/m^2 for 2min, 3.72 KJ/m^2 for 5 min, 7.44 KJ/m^2 for 10 min, 11.16 KJ/m^2 for 15 min, 14.88KJ/m^2 for 20 min. After treatment, fruits were stored in darkness to avoid photoreactivation. After 24 h apple fruits were inoculated with 20 µl of spore suspension (10^6 spore ml^{-1}) of *P. expansum* in wounds and then kept in incubator at 25±2ºC along with (untreated but inoculated with pathogen) control fruits. The mango fruits were inoculated by the culture disk of *B. theobromae.* The fruits were incubated at 25±2ºC. Apple fruits were evaluated daily for decay symptoms over a period of 14 days while mango fruits over a period of 7 days. Lesion diameters were measured and compared to control.

Statistical analysis

Jandel Sigma Stat Version 2.0 statistical software (Jandel Corporation) was used for computation of statistical parameters. Statistically significance was judged at $P< 0.05$ level. To compare treatments Duncan's multiple range test was applied to separate means.

RESULTS

Pathogens

The pathogens isolated from the infected apple and mango grew readily on Potato Dextrose Agar (PDA). The fungal growth covered the Petri plates partially in case of *P. expansum* while completely in case of *B. theobromae* after one week of inoculation and incubation at 25±2ºC. The colony in culture appeared bluish green for *P. expansum* and grayish to white grayish black for *B. theobromae*. Besides the *P. expansum* and *B. theobromae* other fungal genera were also isolated from apple and mango fruits. Mango fruits were also suffering from the decay caused by *Aspergillus niger, Penicillium* sp., *Alternaria* sp., *Colletotrichum* sp. and *Rhizopus* sp. The cultures of pathogens thus isolated were identified on the basis of their morphological characters observed. The pathogens were maintained in PDA slants for further experiments (Table 1).

Pathogenicity tests and inoculum concentration

Pathogenicity test showed severe decay of apple and mango counter inoculated with their respective pathogens under laboratory conditions. On most affected fruits, fungal growth on fruit surface was clearly visible.

In apples counter inoculated with *P. expansum* disease started appearing after 24 h as small water soaked brown lesions which gradually increases in size and measured 16 mm in diameter after seven days. After fifteen days size of lesion diameter was 37 mm. In case of severe infection, the entire surface of rotten apple was covered with bluish green spore mass of the pathogen and later they become pulpy due to severe rotting. The fungus infects the fruits through injuries giving a soft brown lesion that enlarges very rapidly and it may also enter through stems, calyx and open lenticels. The isolate of *Botryodiplodia* spp. used in the present study were found to cause complete decay of mango within 4-7 days. The pathogenicity test of the fungus was carried out on fresh fruits of mango. Studies on the symptomatology of stem end rot disease revealed that *B. theobromae* usually infects fruit from the stem end of the fruit, leading to development of water soaked lesion that extends to the pulp. At later stage the central portion turns black brown. The initial symptoms of disease started after 24h of inoculation as dark brown lesion around wound which rapidly increases in size and measured 70 mm in diameter after seven days. These symptoms were similar to those observed in naturally infected fruits. The fruits inoculated with distilled water (D.W.) served as control and they remained free from infection. The colonies of *P.expansum* Link are bluish green powdery and with concentric zones. Conidiophores hyaline, penicillately branched at apex, the branches terminating in groups of 5 to 9 phialides. Conidial fructification in three stages consisting of one to three primary branches bearing verticils of metulae supporting crowded whorls of phialides. Conidia are blue-green, elliptical to globose in mass and smooth causes a soft brown rot of fruit, especially in storage, colonies appearing on watery, yellowish-brown areas which often originate at wounded site. In pure culture, the *B. theobromae* produces abundant dark grey-black, fluffy mycelium with septate hyphae on potato dextrose agar medium. Thick walled dark brown, osteolate pycnidia were produced accidently when heat treatment (45-50ºC) was given and treated pathogen was allowed to grow on potato dextrose agar medium. The pycnidia were formed only near the inoculated disk not in the whole plate. Conidia were having median septum, dark brown, ellipsoid in shape. Spores were lacking when fungus was grown on culture media without any treatment. Pycnidia were visible very rarely on rotten fruits.

Different concentrations of *P. expansum* showed significant impact on disease incidence on apple fruits. It was found that 10^6 spores/ml of *P. expansum* resulted in 87.5% infection while 69.5% infection was recorded in case of 10^4 spores/ml after 15 days of incubation . In case of *B. theobromae* 100% infection was found after 7 days of incubation.

***In vitro* efficacy of physical treatments against fungal pathogens**

Hot Water Treatment

The effect of various temperatures and exposure times on the viability of *P. expansum* and *B. theobromae* was tested. *In vitro* treatments of hot water significantly decreased spore germination of *P. expansum* (Table 2). It was observed that the treatment of 40ºC temperature was least effective in reducing spore germination of *P. expansum* at any duration of exposure to hot water treatment. Complete inhibition of *P. expansum* was achieved by exposing the spores to hot water treatment at 60ºC for duration of 2 min and at 50ºC for 20 min. As the temperature is reduced the required exposure time to inhibit spore germination increased. 60ºC for duration of 2-20 min was 100% effective for both pathogens. The growth of *B. theobromae* was completely inhibited at 50ºC for 20 min and 60ºC for 2 min. Hot water treatments in various time regimes effectively decreased radial growth except 40ºC that completely failed to inhibit the growth of *B. theobromae* at any exposure time period.

UV-C Treatment

The effects of UV-C treatment on spore germination of *P. expansum* and growth of *B. theobromae* are presented in table 3. UV-C treatment significantly decreased the spore-germination of *P. expansum* in accordance with duration of exposure and distance. UV-C treatment at 45 cm distance from the source of radiation showed least inhibition of spore germination at all exposure durations. UV-C treatment at 10 cm for 2, 5, 10, 15 and 20 min showed 100% inhibition of spore germination of *P. expansum*. In case of *P. expansum,* at 15 cm distance exposure for 2, 5, 10, 15 and 20 min showed 82.50%, 85.23%, 86.42%, 90.71% and 93.57% inhibition respectively while at 30 cm distance exposure for 2, 5, 10, 15 and 20 min showed 80.00%, 82.50%, 85.22%, 88.57% and 92.14% inhibition respectively. In case of *B. theobromae,* at 30 cm distance exposure for 2, 5 and 10 min showed 96.1%, 98.7% and 98.7% inhibition respectively while exposure for 15 min and 20 min at 15, 30 and 45 cm distance resulted in 100% inhibition. UV-C treatment at 10 cm distance from the source of radiation for 2, 5, 10, 15 and 20 min showed 100% inhibition.

Table: 1. Percent occurrence of isolated pathogens of apple and mango fruits.

Infected fruits	Isolated pathogens	Occurrence (%)*
Apple	*Botrytis cinerea*	1
	Penicillium expansum	80
	Alternaria sp.	40
	Rhizopus sp.	69
	Aspergillus sp.	1
Mango	*Aspergillus niger*	80
	Botryodiplodia theobromae	90
	Penicillium sp.	2
	Colletotrichum sp.	75
	Rhizopus sp.	20
	Alternaria sp.	10

* The values are mean of four years viz., 2007-08, 2008-09, 2009-20010 and 2010-2011.

Table 2. *In vitro* efficacy of hot water treatment on percent inhibition of *P. expansum* and *B. theobromae*

Temp (ºC)	Percent inhibition									
	Time (min)									
	P. expansum					*B. theobromae*				
	2	5	10	15	20	2	5	10	15	20
40	0.02	0.60	10.77	15.05	17.05	0.0	0.0	0.0	0.0	0.0
50	50.00	52.00	54.20	80.20	100.00	37.0	44.0	52.0	84.0	100.0
60	100.00	100.00	100.00	100.00	100.00	100.0	100.0	100.0	100.0	100.0
Control	0.00					0.00				

Table 3. *In vitro* efficacy of UV-C light on percent inhibition of *P. expansum* and *B. theobromae*

Distance (cm)	Percent inhibition									
	Exposure time (min)									
	P. expansum					*B. theobromae*				
	2	5	10	15	20	2	5	10	15	20
10	100.00	100.00	100.00	100.00	100.00	100.0	100.00	100.00	100.00	100.00
15	82.50	85.23	86.42	90.71	93.57	94.8	96.0	99.1	100.00	100.00
30	80.00	82.50	85.22	88.57	92.14	96.1	98.7	98.7	100.00	100.00
45	80.71	80.71	83.57	86.42	91.42	89.6	98.7	98.7	100.00	100.00
Control	0.00					0.00				

Table. 4. *In vivo* efficacy of hot water treatment (HWT) on lesion diameter in apple against *P. expansum* and in mango against *B. theobromae*

HWT(°C)	Lesion Diameter (mm)					
	P. expansum			*B. theobromae*		
	1 min	2 min	5 min	1 min	2 min	5 min
50	20.2^{b}±1.102	19.9^{b}±1.504	19.7^{b}±1.155	18.5^{c}±0.000	20.2^{cd}±0.577	23.7^{bc}±1.617
55	18.5^{b}±0.133	17.5^{b}±0.577	17.2^{b}±1.000	14.9^{e}±0.000	23.6^{bd}±1.732	25.5^{b}±2.000
60	7.0^{c}±0.473	Injury	Injury	Injury	Injury	Injury
Control		33.9^{a}±1.328			69.6^{a}±1.212	

Values in the same column followed by different superscript's letters are significantly different (P<0.05) according to Duncan's Multiple Range Test. ±SE are given along the mean values.

***In vivo* efficacy of physical treatments against fungal pathogens**

Hot Water Treatment

In vivo treatments of hot water significantly decreased decay as compared to control. *In vivo* experiments showed that 60ºC for 1 min treatment was most effective in reducing decay. The exposure for 2 min or more at the same temperature reduced the appearance and commercial value of fruit (table 4).

Hot water treatment at 50ºC for 20 min and 60ºC for 2 min completely inhibited the growth of *B. theobromae* during *in vitro* treatments .When these temperatures with defined time regimes were applied to mango fruits they resulted in poor appearance of fruit with low commercial value. Exposure of fruits at 55ºC for 1 min was found to be the most effective against *B. theobromae* without causing any injury to fruits. Hot water treatment at 60ºC for any duration of exposure injured the fruits.

UV-C Treatment

In apple fruits UV-C irradiation at the distance of 10 cm for 5 min was most effective to control decay caused by *P. expansum.* Increase in duration of exposure beyond 5 min at 10 cm distance injured both fruits and increased lesions instead of reducing them. In mango fruits effective control of *B. theobromae* was observed upon exposure for 5 min (table 5).

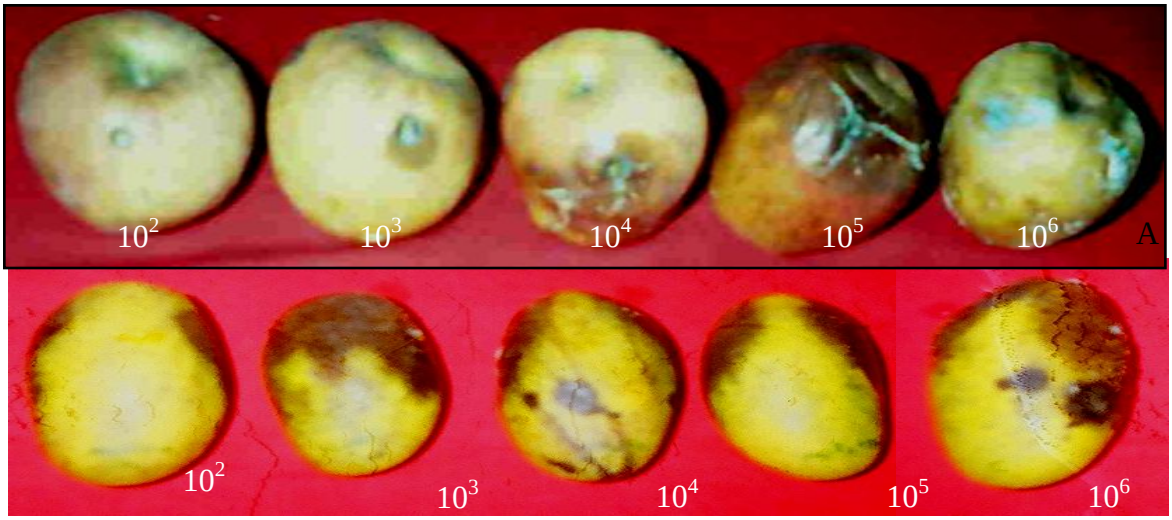

Figure 1. (A) Effect of different inoculum concentrations of *P. expansum* in wounded apple fruits (B) Effect of *B. theobromae* in wounded mango fruits.

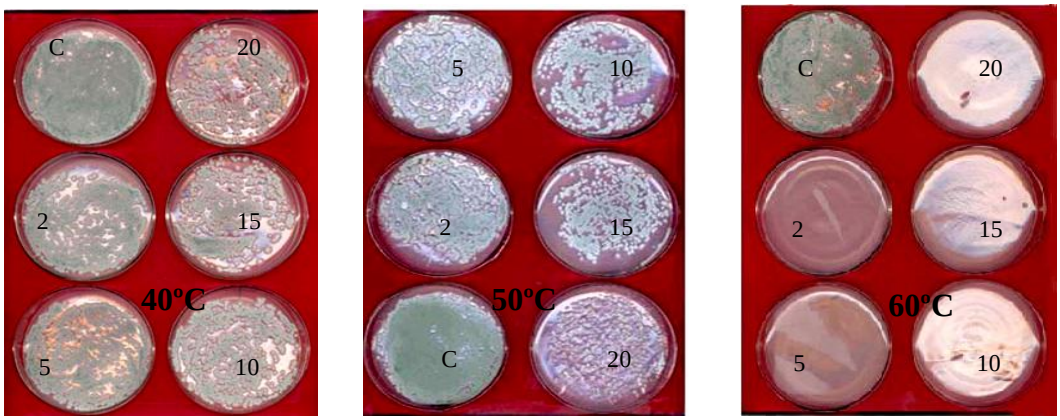

Fig 2 A. *In vitro* efficacy of hot water treatment (40°C, 50°C and 60°C for 2, 5, 10, 15, 20 min and C= control) on spore germination of *P. expansum*

Table. 5. *In vivo* efficacy of UV-C light on lesion diameter in apple against *P. expansum* and in mango against *B. theobromae*

UV-C exposure time (min)	Lesion diameter (mm)	
	P. expansum	***B. theobromae***
1	14.9^{c}±0.173	21.9^{b}±0.346
2	12.1^{d}±0.115	20.0^{c}± 0.173
5	10.0^{e}±0.057	17.9^{d}±0.410
10	22.2^{b}±1.270	20.0^{c}±0.231
Control	35.6^{a}±0.633	69.3^{a}±0.338

Values in the same column followed by different superscript's letters are significantly different (P<0.05) according to Duncan's Multiple Range Test. ±SE are given along the mean values.

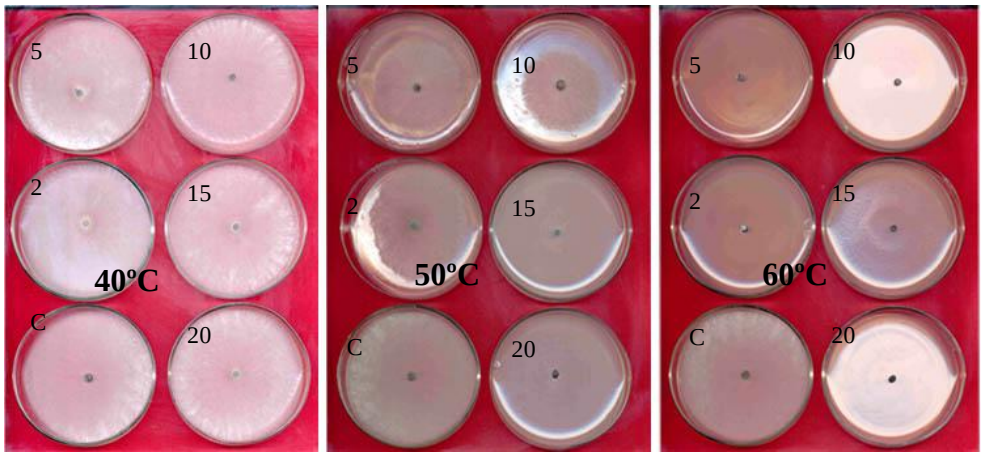

Fig 2 B. *In vitro* efficacy of hot water treatment (40°C, 50°C and 60°C for 2, 5, 10, 15, 20 min and C= control) on radial growth of *B. Theobromae.*

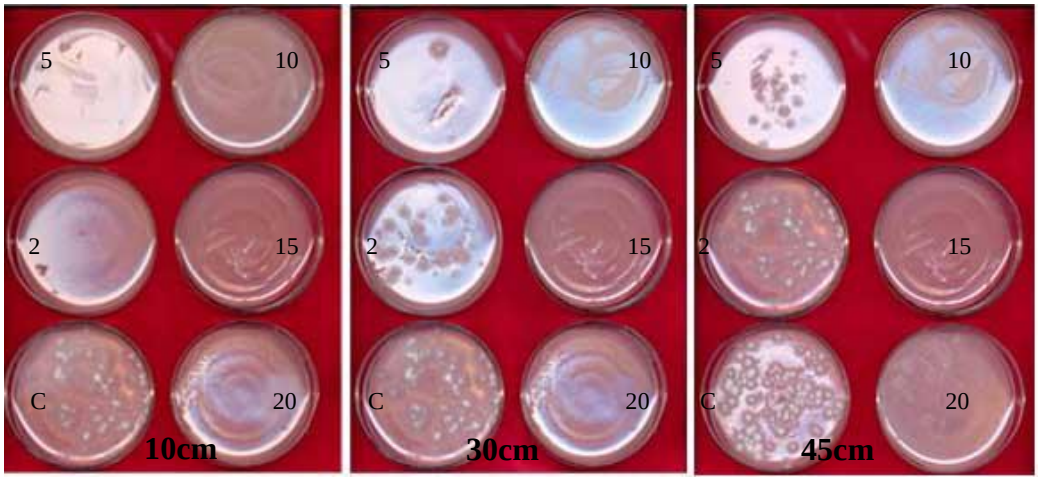

Fig 2C. *In vitro* efficacy of UV-C light (15cm, 30cm and 45cm for 2, 5, 10, 15, 20 min and C= control) on spore germination of *P. expansum.*

DISSCUSION

Fruits are highly perishable products particularly once they have been harvested from the parent plants. Considerable postharvest losses of fruits and vegetables are brought about by decay caused by fungal pathogens. Fruits, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots may also make them unfit for consumption. Although it is very difficult to determine the full extent of postharvest losses due to decay, which varies widely with commodity, production area and seasonal changes, it is well known that these losses are significant (Eckert, 1975; Pathak, 1997). Conservative estimates place losses of perishable commodities at 50% in under developing and tropical countries (Jeffries and Jeger, 1990). The conventional approach to the control of fungi has been the use of synthetic antifungal compounds. However, the development of fungicide resistance by pathogens and increasing environmental concern over fungicidal residues in fruits and vegetables has stimulated a search for alternative measures for disease control (Zhang *et al*., 2002). The present investigation was undertaken with the view that due to ban on fungicides they can be can sensibly replaced by ecofriendly, cheap, safe, easy to use and effective alternatives. During survey of local markets apple fruits were found heavily infected with *P. expansum*.

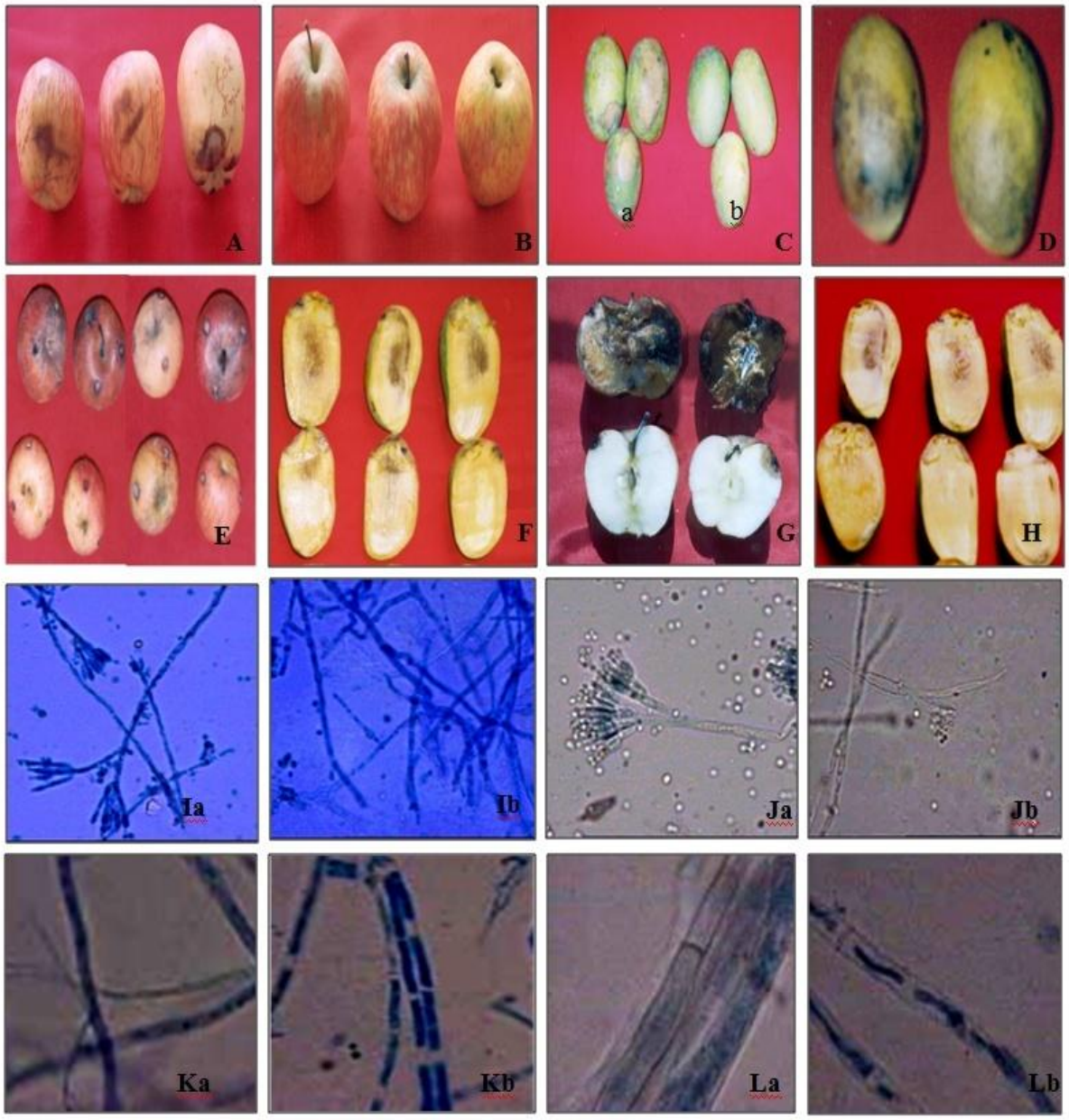

Fig 3 A. Effect of longer exposure period of hot water treatment (60°C for 2 min) on apple leads to injury.
Fig 3 B. Effect of hot water treatment (60°C for 1 min) to control Blue mold of apple.
Fig 3 C. A- Effect of high temperature of hot water treatment (60°C for 1 min) on mango leads to injury.
Fig 3 C. B- Effect of hot water treatment (55°C for 1 min) on mango.
Fig 3 D. Effect of high doses of UV-C light on mango.
Fig 3 E. Effect of hot water treatment (60°C for 1 min) on apple (upper row control, lower row treatment).
Fig 3 F. Effect of hot water treatment (55°C for 1 min) to control stem end rot of mango (upper row control, lower row treatment).
Fig 3 G. Effect of UV-C light on apple against blue mold (upper row control, lower row treatment).
Fig 3 H. Effect of UV-C light on stem end rot of mango (upper row control, lower row treatment).
Fig 3 I. Effect of hot water treatment on hyphae of *P. expansum* (40X) (Ia- control, Ib- treatment).
Fig 3 J. Effect of hot water treatment on hyphae of *P. expansum* (100X) (Ia- control, Ib- treatment).
Fig 3 K. Effect of UV-C light treatment on hyphae of *B. theobromae* (40X) (Ka- control, Kb- treatment).
Fig 3 L. Effect of UV-C light treatment on hyphae of *B. theobromae* (100X) (La- control, Lb- treatment).

The pathogen produces light colored, soft and watery lesions that in later stages of advancement are found covered with bluish green cushions of spores. These results are in agreement with the findings of Borecka (1977) who found several species of *Penicillium* as pathogen of apples and *P. expansum* was more prevalent and more virulent than other species. Conway (1983) isolated five species of *Penicillium* from apple dump tank water, but only *P. expansum* was pathogenic. The result of survey of apple fruits are in agreement with the previous finding that among various postharvest fungal pathogens of pome fruits, *P. expansum* is the major one that has been reported from almost every Indian fruit market and is known to contribute substantially to over all losses in stored pomaceous fruits (Singh and Sumbali, 2002). *B. theobromae* Pat is an important post harvest pathogen on mango and other tropical fruits (Alam and Nahar, 1990). The pathogen colonizes floral parts, develops endophytically in the fruit pedicel and remains quiescent until the fruit matures. Studies on the symptomatology of disease revealed that *B. theobromae* usually infects mango fruit from the stem end. Circular black patch appear around at the basal part of pedicel. These symptoms are in resemblance with the symptoms described by Srivastava (1968). The fungus was readily and invariably isolated from the affected portion of the fruits. The pathogenicity test was performed using fungal disk as inoculums (Jadeja and Bhatt, 2008) and placing them in wounds made on both sides of stem end to confirm the postulates given by Robert Koch. *In vitro* efficacy treatments of pathogens with hot water or UV-C clearly showed that these measures have ability to check spore germination of the test pathogen. Hot water treatment at the higher temperature (50ºC and 60ºC) was highly effective in reducing spore germination. Treatments at 60ºC reduced 100% spore germination of *P. expansum* at minimum tested time range (2 min). *In vitro* results showed that exposure of fruits at 50ºC for 20 min and 60ºC for 2 min completely inhibited the growth of *B. theobromae.* The similar results are reported by Sharma et al. (2005), which showed significant decrease in spore germination of *A. niger* and *P. expansum*. Hot water treatment at 60ºC for 1 min was found to be most effective in reducing decay of apple while 1 min exposure at 55ºC for was effective in reducing the decay of mango fruits. Several studies have demonstrated that hot water treatment has the potential to control postharvest diseases of pepper, mango, kumquat, citrus and melon (Fallik *et al*., 2000; Porat *et al*., 2000). In the present study *in vitro* experiments of UV-C against spore germination of *P. expansum* clearly showed that the distance of 10 cm and exposure for 2 min and above were found to be effective in complete inhibition of spore germination. The radial growth of *B. theobromae* was cent percent inhibited when kept at 10 cm distance and the plate was exposed for 2 min under UV-C light. Sharma *et al.* (2005) also reported similar results using UV-C light to inhibit spore germination. UV-C irradiation at the distance of 10 cm for 5 min was most effective to control decay caused by *P. expansum* in apples. Similar results were reported for mango. UV-C light has already been tested in many fruit mainly to control postharvest diseases and to delay ripening associated processes (D'hallewin *et al.,* 2000). Thus, alternative methods to control postharvest diseases are urgently needed. The need arose for the search of compounds, which were effective, natural in origin, safe for humans and were compatible with other control methods.

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Corresponding author: Dr. Madhu Prakash Srivastava, Center of Excellence, Department of Botany, University of Lucknow, Lucknow 226 007, U.P., India
Email: madhusrivastava2010@gmail.com