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Use of plant extracts and yeast antagonists in the management of storage scab and rots of apple fruits

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ABSTRACT: **Nine different plant extracts and three yeast antagonists were compared for their efficacy as post-harvest dip against the development of scab (*Venturia inaequalis*) lesions and rots on apple fruits during storage for 60 days at ambient conditions. Studies revealed that water extract of *Emblica officinalis* leaves (15%) was highly effective against storage scab and provided complete control up to 60 days of storage. Water extracts of *Artemisia vulgaris*, *Melia dubia* leaves and *Emblica officinalis* seeds @15 per cent, though were not much effective against storage scab, but were found effective to check fruit rotting with decay reduction index (DRI) of 85.7, 80.9 and 70.8 per cent, respectively after 60 days of storage. A yeast antagonist, *Rhodosporidum toruloides* ($1.0x10^5$ cell/ml) was also effective in checking the storage fruit rots and gave DRI of 74.1 per cent.**

KEY WORDS: Apple, fruit rots, management, plant extract, storage scab, yeast antagonists

In apple (*Malus domestica* Borkh.) storage scab and fruit rotting fungi result in post-harvest and storage losses to the tune of 20-30 per cent in Himachal Pradesh (Kaul, 1985). Storage scab is incited by *Venturia inaequalis* (Cke.)Wint and its incipient infection occurs in late season, often remain unnoticed at harvest and packing time. Scab lesions become visible only in transit and storage as jet black, well marginated, shiny and circular spot. Appearance of scab lesions on fruits during storage also result in their shrinkage or else predispose them to secondary pathogens causing their complete rotting (Gupta and Verma, 1986). Though use of fungicides forms one of the major component in the management of these storage diseases (Sharma and Kaul, 1997; Sharma and Kaul, 1999) but the scope of their regular use is limited due to their high cost and terminal residue in fruits, besides development of resistance in pathogens (Gupta and Sharma, 1996). It thus warrants the necessity of developing ecologically safe, effective and economic methods of disease management (Horst *et al.*, 1992). In this aspect, though plant extracts (Anandraj and Leela, 1996; Srivastva and Lal, 1997) and biological agents (Quarles, 1993; Wilson and Wisneiwski, 1994; Sharma and Kaul, 1999) have successfully been employed against number of post-harvest diseases yet extremely scanty or practically no such work has been conducted to check storage diseases in apple. Therefore, in the present studies, an attempt has been made to test the bioefficacy of plant extracts and yeast antagonists against the development of storage scab and fungal rots in apple fruits during storage.

MATERIALS AND METHODS

The present studies were undertaken at Regional Horticultural Research Station of

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Dr. Y. S. Parmar University of Horticulture and Forestry at Bajaura (Kullu), Himachal Pradesh for two consecutive years namely 1996 and 1997. The station is situated at latitude 31.8°N, longitude 77°E and altitude 1090m above sea level having monthly maximum and minimum temperature ranging between 8.5-31.8°C and 1.4-21.5°C, respectively with relative humidity ranging between 55-94 per cent and receives an average rainfall to the tune of 937mm.

Preparation of plant extracts and yeast species suspension

i. Plant extract

Fresh leaves and seeds of neem (*Azadirachta indica* A. Juss), aonla (*Emblica officinalis* Gaertn.), bottlebrush (*Clestimon lanceolatus* Linn.) and only leaves of *Artemisia vulgaris* Linn. and *Melia dubia* Linn. were washed with distilled water, dried in hot air oven for 48 hours at 50°C and finely powdered. Twenty- gram powder of each was suspended in 100ml of distilled water, vortexed in a mixer for 10 minutes and filtered through a cheesecloth. Filtrate so collected was centrifuged at 3000rpm for 30 minutes to get a clear supernatent, which was further diluted with water to prepare concentrations of 5, 10 and 15 per cent (Anandraj and Leela, 1996). Neem triterpenes solution obtained from Department of Mycology and Plant Pathology (UHF) Nauni; Solan, was dissolved in alcohol and above dilutions were made.

ii. Yeast species suspension

The culture of three yeast species namely *Torulospora delbrueckii*, *Rhodotorula glutinis* and *Rhodosporidum toruloides* obtained from the Department of Mycology and Plant Pathology were multiplied on potato dextrose agar (PDA) medium. The suspension of each antagonist ($1.0x10^5$ cell/ml) was prepared in 0.5 per cent molasses (Pusey, 1989). Plain water containing 0.5 per cent molasses was used as untreated check.

iii. Evaluation of plant extract and yeast antagonists against storage scab and fruit rots in apple

Apparently, healthy fruits of cv. Royal Delicious were harvested from hot spot (endemic area) of apple scab namely Bajaura (Kullu), H. P. Twenty five fruits were dipped in already prepared dilutions of plant extracts and yeast species for 10 and 5 minutes, respectively. Triton sticker was also added in each solution of plant extract at the rate of 0.02 per cent. Each treatment was replicated thrice. A dip in plain water served as untreated check. Fruits were allowed to dry in shade, wrapped individually in newspaper, packed in wooden boxes and stored at room temperature (18-24°C). Data were recorded on the incidence of scab lesion development and fruit decay each after 30, 45 and 60 days of storage. Per cent disease control (PDC) over check in case of storage scab was calculated by using the formula used earlier by Sharma *et al.* (1993). Decay reduction index (DRI) percentage were calculated in case of fruit rots during storage with the formula suggested by Sharma and Kaul (1989). The pathogens developed in association with storage scab lesion and fruit rot were identified and per cent frequency of their occurrence was recorded.

Data obtained were pooled and subjected to statistical analysis by adopting the procedure suggested by Gomez and Gomez (1984) to find out the least significant differences amongst the treatments.

RESULTS AND DISCUSSION

Storage scab management

i. Plant extracts

Perusal of data (Table 1) revealed that amongst nine plant extracts, post-harvest fruit dip in water extract of *E. officinalis* leaves (15%) was found highly effective in checking the development of storage scab and provided complete disease control up to 60 days of storage in comparison to 23.5 per cent disease in check treatment after same duration of storage. It was closely followed by its second concentration (10%) and the per cent disease control decreased with further lowering down the concentration levels.

Asolkar *et al.* (1992) reported the antimicrobial activity of aonla leaves extract and found that it was due to the presence of high phenolic contents like lupeol, B-sitosterol and phyllembin. Fruit dip in *M. dubia* leaves extract was found more effective in the initial days of storage (30 days) in comparison to all other treatments but later on its efficacy was recorded to be at par with *A. vulgaris* leaves extract. Other treatments were less effective and leaf/seed extracts of bottlebrush were least effective.

ii. Yeast antagonists

Perusal of data (Table 2) revealed that none of the yeast species were effective to check the development of storage scab as these could give only 51.6 to 54.0 per cent disease control after 60 days of storage.

Table 1. Effect of post-harvest fruit dip in plant extracts on the development of storage scab

Plant Extract	Per cent disease control in different concentrations after days in storage								
	30			45			60		
	5	10	15	5	10	15	5	10	15
Neem leaf	44.6 (41.5)	51.8 (45.1)	54.4 (47.5)	30.2 (33.3)	41.3 (39.9)	45.2 (42.2)	20.0 (26.5)	28.6 (31.7)	40.2 (39.2)
Neem seed	54.4 (47.5)	63.2 (52.6)	65.2 (53.8)	51.1 (45.6)	58.3 (49.8)	60.4 (51.0)	50.1 (45.1)	60.1 (50.7)	61.4 (51.6)
Neem triterpene	40.4 (39.4)	42.5 (40.7)	47.5 (43.0)	43.4 (41.2)	51.0 (45.6)	52.7 (46.5)	47.3 (43.4)	55.6 (48.2)	57.3 (49.2)
Aonla leaf	97.2 (80.4)	100.0 (90.0)	100.0 (90.0)	90.6 (72.1)	96.2 (78.7)	100.0 (90.0)	82.7 (65.4)	88.5 (67.5)	100.0 (90.0)
Aonla seed	48.7 (44.3)	58.5 (49.9)	61.1 (51.4)	37.8 (37.9)	45.5 (42.4)	47.2 (43.4)	28.2 (32.1)	35.7 (36.5)	38.5 (38.3)
Bottlebrush leaf	47.1 (43.3)	49.2 (44.5)	49.2 (44.5)	39.5 (38.9)	43.4 (41.1)	47.2 (43.4)	30.2 (33.3)	37.3 (37.6)	41.1 (40.0)
Bottlebrush seed	44.6 (41.9)	51.8 (46.0)	54.4 (47.5)	43.3 (41.1)	47.2 (42.0)	48.7 (44.3)	34.4 (35.9)	40.2 (39.3)	43.1 (41.0)
Artemisia vulgaris leaf	47.1 (43.3)	56.4 (48.7)	61.1 (51.4)	47.6 (43.7)	58.7 (50.0)	62.5 (52.2)	60.3 (50.9)	67.1 (55.0)	68.2 (55.7)
Melia dubia leaf	72.5 (58.4)	77.2 (61.5)	79.2 (62.8)	54.9 (47.8)	58.8 (50.1)	61.1 (51.4)	60.1 (50.8)	63.0 (52.5)	64.0 (53.1)
Control (Water)*		12.0			19.3			23.5	

*Per cent scabbed fruits; Figures in parentheses are the angular transformed values.

CD (P=0.05)

Due to plant extract = 2.62

Days = 2.17

Concentration = 1.29

Plant extract x Conc.x Days = 3.09

iii. Secondary fungi associated with storage scab lesions

Six fungi namely *Penicillium expansum* Link, *Trichothecium roseum* (Pers.) Link ex Fr., *Monilinia laxa* (Aderhold & Ruhland) Honey, *Alternaria mali* Roberts, *A. alternata* (Fr.) Keissler and *Glomerella cingulata* (Stonem.) Spauld & Shrenk, were identified to be associated with the storage scab lesions. The occurrence of *T. roseum* was recorded in highest (60.4%) frequency. Kaul (1985) reported the association of this fungus to a greater extent with the storage scab lesions. Gupta and Verma (1986) also reported that 82 per cent scabbed fruits developed rotting due to entry of secondary pathogens namely *P. expansum*, *Monilinia* spp., and *G. cingulata* through scab lesions in Himachal Pradesh. The findings on the association of *A. mali* and *A. alternata* with storage scab lesions under present studies is a new record.

was found highly effective in comparison to eight other plant extracts to check the development of fungal rots during storage and gave 100.0, 93.3 and 85.7 per cent decay reduction index (DRI) after 30, 45 and 60 days of storage, respectively. It was followed by *M. dubia* leaves and aonla (*E. officinalis*) seeds, where 79.2, 80.9 and 68.8, 70.8 per cent DRI was recorded at 10 and 15 per cent concentrations, respectively after 60 days of storage. It shows that these two concentrations are almost at par with each other. Gade and Kaul (1996) reported the effectiveness of water extract of aonla seed against apple fruit rot caused by *P. expansum*. Inhibitory effect of aonla seed was due to the presence of tannins, polyphenols, certain acids (gallic acid and ellegic acid) and vitamin C, which have been reported to inhibit growth of many fungi (Asolkar *et al.*, 1992). Next best treatments were aonla leaf, neem seed and neem

Table 2. Effect of post harvest fruit dip in yeast antagonists on the development of storage scab

Yeast species	Disease control (%) after days in storage		
	30	45	60
Torulospora delbrueckii	35.75 (36.69)	39.48 (38.88)	53.27 (46.83)
Rhodotorula glutinis	37.45 (37.70)	37.94 (38.00)	51.63 (45.90)
Rhodosporidum toruloides	34.30 (38.85)	42.05 (39.23)	54.09 (47.29)
Control (Water)*	13.70	19.50	24.40

*Per cent scabbed fruits; Figures in the parentheses are the angular transformed values.

CD (P=0.05)

Due to plant extract = 2.62
Due to Yeast Species = 1.21
Days = 1.09
Yeast species x Days = 2.46

Storage rots management

i. Plant extracts

Data presented in Table 3 revealed that fruit dip in water extract of *A. vulgaris* leaves (15%) triterpenes. Further the DRI in each treatment decreased with lowering down the concentration of the extract. Fruit dip in water extract of bottlebrush leaf and seed was not effective.

Table 3 Effect of post-harvest fruit dip in plant extracts on the development of fruit rot in apple during storage

Plant Extract	Decay reduction index (%) in different concentrations after days in storage								
	30			45			60		
	5	10	15	5	10	15	5	10	15
Neem leaf	36.1 (36.9)	41.6 (40.1)	54.2 (47.4)	44.2 (41.6)	53.1 (46.8)	57.1 (49.1)	45.0 (42.1)	55.0 (47.8)	56.6 (48.8)
Neem seed	54.8 (47.7)	61.1 (51.4)	63.8 (53.0)	56.2 (48.5)	63.9 (53.1)	67.8 (55.4)	56.6 (48.8)	61.6 (51.7)	65.0 (53.7)
Neem triterpene	52.7 (46.5)	59.7 (50.6)	62.5 (52.5)	54.4 (47.5)	62.5 (52.2)	65.1 (53.8)	56.1 (48.5)	60.8 (51.2)	63.8 (53.0)
Aonla leaf	63.8 (53.0)	69.4 (56.4)	75.7 (60.5)	60.2 (50.9)	66.1 (54.4)	72.3 (58.2)	58.8 (50.1)	64.1 (53.2)	67.2 (55.1)
Aonla seed	75.0 (60.0)	80.5 (63.7)	82.8 (64.7)	64.7 (53.5)	69.6 (56.5)	76.0 (60.6)	64.1 (53.2)	68.8 (56.0)	70.8 (57.3)
Bottlebrush leaf	25.0 (30.0)	33.3 (35.2)	40.2 (39.3)	37.5 (37.7)	35.6 (36.6)	46.4 (42.9)	30.2 (33.3)	28.5 (32.3)	28.0 (31.9)
Bottlebrush seed	29.1 (32.6)	37.5 (37.7)	44.4 (41.7)	30.8 (33.7)	33.0 (35.1)	37.1 (37.5)	32.0 (34.4)	30.2 (33.3)	30.0 (33.2)
Artemisia vulgaris leaf	91.6 (73.1)	97.2 (80.4)	100.0 (90.0)	84.3 (66.6)	91.1 (72.6)	93.3 (75.0)	79.4 (63.0)	84.4 (66.7)	85.7 (67.8)
Melia dubia leaf	81.8 (64.7)	87.5 (69.3)	92.1 (73.7)	75.8 (60.5)	86.6 (68.5)	88.2 (69.9)	72.7 (58.5)	79.2 (62.9)	80.9 (64.1)
Control (Water)*		17.5			29.0			41.3	

*Per cent scabbed fruits; Figures in parentheses are the angular transformed values.

CD (P=0.05)

Due to plant extract = 2.14
Days = 1.96
Conc. = 1.03
Plant extract x Conc.x Days = 2.84

ii. Yeast antagonists

Fruit dip in *R. toruloides* was highly effective and provided 88.1, 80.0 and 74.1 per cent DRI after 30, 45 and 60 days of storage, respectively (Table 4). It may due to its antagonistic effect on apple fruit rot causing pathogens. It was followed by *R. glutinis*, whereas *T. delbrueckii* was not effective.

ii. Fungal pathogens associated with fruit rot in apple during storage

Eleven fungi namely *Penicillium expansum* Link, *Trichothecium roseum* (Pers.) Link ex Fr., *Botrytis cinerea* Pers., *Monilinia laxa* (Aderhold & Ruhland) Honey, *M. fructigena* Honey, *Alternaria mali* Roberts, *A. alternata* (Fr.) Keissler, *Glomerella cingulata* (Stonem.) Spauld

& Shrenk, *Botryosphaeria obtusa* (Sch.) Shoemaker, *Botryosphaeria dothidea* (Mong Fr.) Ces.& deNot and *Rhizopus arrhizus* Fischer were identified to cause fruit rot in apple during storage. The prevalence of *P. expansum* was highest (25.5%) followed by *T. roseum* (16.9%), *B. cinerea* (15.8%), *M. laxa* and *M. fructigena* (10.6%) and *G. cingulata* (8.0%). Other fungi were present in low frequencies (3.7-7.2%). Aggarwala and Sharma (1968) reported that *B. cinerea* causes major losses during storage. However, Sharma and Kaul (1999) reported that *P. expansum*, *T. roseum*, *M. laxa*, *B. cinerea* and *G. cingulata* are mostly associated with apple fruit rot after harvest and during storage.

Table 4. Effect of post harvest fruit dip in yeast antagonists on the development of fruit rots in apple during storage

Yeast species	Decay Reduction Index (%) after days in storage		
	30	45	60
Torulospora delbrueckii	22.04 (27.97)	26.03 (30.66)	26.68 (31.05)
Rhodotorula glutinis	75.26 (60.13)	69.43 (56.42)	65.80 (54.21)
Rhodosporidum toruloides	88.17 (69.82)	80.00 (63.43)	74.10 (59.23)
Control (Water)*	18.60	26.50	38.60

*Per cent cumulative fruit rot; Figures in the parentheses are the angular transformed values.

CD (P=0.05)

Due to Yeast Species = 1.64
Days = 1.39
Yeast species x Days = 2.74

It is, therefore, concluded that fruit dip in water extract of aonla (*E. officinalis*) leaves effectively checked the development of storage scab, whereas fruit dip in *A. vulgaris* and *M. dubia* leaf extracts was most effective against the occurrence of fruit rot in apple during storage. Further the yeast antagonists namely *R. toruloides* and *R. glutinis* showed antagonistic effect against the development of fruit rots in apple during storage. The findings on the association of *Alternaria mali* and *A. alternata* with storage scab lesions under present studies is a new record.

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