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Management of post-harvest spoilage of Aonla (*Emblica officinalis* Gaertn.) due to Blue Mold

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An attempt was made to explore the possibilities of managing post-harvest spoilage of aonla (*Emblica officinalis* Gaertn.) caused by *Penicillium islandicum* through use of botanicals, oils and chemicals. Botanicals like *Mentha piperata*, *Zanthoxylum oxiphyllum* and *Eucalyptus citriodora* at 15% concentration; three commercial oils viz. neem (*Azadirachta indica*), castor (*Ricinus communis*) and mustard (*Brassica campestris* var. Sarson Prain); and two fungicide chemicals carbendazim (Bavistin @ 0.2%) and mancozeb (Dithane M-45 @ 0.2%) were used in the present experiment. In the *in vitro* studies, leaf extracts of Mint, *Zanthoxylum* and *Eucalyptus* at concentrations viz., 5, 10 and 15 per cent showed significant effect on the mycelial growth and per cent inhibition of *P. islandicum*. It was found that the treatments were more effective in managing the disease when applied under pre-inoculated treatment. Amongst all the treatments, mint leaf extract (15%), neem oil (15 ml) and carbendazim (0.2%) were found most promising in managing the disease and were found statistically at par. Out of the three botanicals, leaf extract of mint recorded the least disease intensity followed by *Zanthoxylum* and *Eucalyptus* leaf extract. In case of oils, neem was the most effective which recorded mean PDI 31.11 and 37.78 in both pre- and post-inoculated treatment followed by mustard oil and closely by castor oil. Among the two chemicals tested, carbendazim (0.2%) was observed to be significantly superior to mancozeb (0.2%) for both pre and post inoculation treatments.

Key words : Aonla, post harvest, blue moulds, plant extract, control

INTRODUCTION

Aonla or Indian gooseberry belonging to *Euphorbiaceae* family is an important fruit crop and is valued for its high medicinal and nutritional content. Aonla fruit which is indigenous to tropical Asia is probably the richest known natural source of vitamin C. In Nagaland, aonla is considered as one of the important wild fruits which play a significant role in nutritional security and economic prosperity in the society especially for jhum cultivators, small land holders and landless families living near forests. It is a very hardy tree and can be grown under diverse soil and agro-climatic conditions without much care. The total acreage of aonla cultivation in India is estimated at 49, 620 ha with annual production of 1, 50,500 tonnes (Dhandar, 2003). Uttar Pradesh has the highest area of aonla cultivation in India with 19, 835 ha and a total production of 123390 metric tonnes (Ram, 1993). At present aonla ranks next to mango in terms of area and production and holds the first and eighth position among minor fruits and tropical fruits respectively. Aonla is affected by many pests and diseases. Post harvest study yielded a total of 13 fungi from deteriorating stored fruits of aonla (Mishra, 1988). Of these, blue mold caused by *Penicillium* spp., fruit rot caused by *Aspergillus* spp., and *Fusarium* spp. are the major causes of spoilage of fruits during storage resulting in considerable economic losses.

With the increasing awareness on toxic hazards of chemicals to crops, consumers and environment due to their phytotoxic residual and pollution effects, the importance of indigenous products in plant disease management has been emphasized.

Plant extracts have opened a new avenue for the control of plant disease. Besides, being safe and non-phytotoxic, the plant extracts are known to be effective against various plant pathogens. Similarly, oil can also be safely used for preventing storage fruit deterioration because of their water repellent action and stable nature. Moreover, oil treatment has no residual effect and can be thoroughly cleaned before consumption. With this background the present work has been taken up with the aim of finding out effective non-chemical management strategy for post-harvest spoilage of blue mold of aonla.

MATERIALS AND METHODS

Isolation of the pathogen

Diseased fruits of aonla showing characteristics symptoms of blue mold disease were collected from the experimental farm of the School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema campus and also from the local markets in Medziphema and Dimapur of Nagaland. The diseased specimens were then brought to the laboratory for isolation and identification.

The infected aonla fruits collected were taken into the inoculation chamber. Small portion of diseased tissue were then sliced out from the infected part and surface sterilized with 0.01% mercuric chloride for two minutes. These were then rinsed thoroughly with three changes of sterile distilled water. One slice was placed at the centre on each Petriplate already poured with PDA (Potato Dextrose Agar) medium. The inoculated Petriplates were incubated at 27±1°C. Pure cultures of the fungi were made through hyphal tip culture technique in Petri plates and then transferred to PDA slants and stored in refrigerator. The virulent isolate of *Penicillium islandicum* isolated from the infected aonla fruits were then used for the present study.

Efficacy of plant extracts in vitro

The effect of leaf extracts of five plants viz., Mint (*Mentha piperita* L.), Zanthoxylum (*Zanthoxylum oxiphyllum* DC.), Eucalyptus (*Eucalyptus citriodora* Hoch.), Periwinkle (*Catharanthus roseus* (L.) G. Don) and Wild sesamum (*Anisomeles indica*) were assessed on the radial growth of the pathogen, *Penicillium islandicum*.

Well grown leaves collected afresh were ground with sterile water (1ml/g) in a pestle and mortar. The extract was filtered through muslin cloth and finally through What man (No. 1) filter paper. This was passed through seitz filter to free the extract from the bacterial contaminants. This formed the standard plant extract solution (100 per cent). The extract was further diluted to different concentrations *viz.*, 5, 10 and 15 per cent with sterile distilled water. The extracts were added to the growth medium (PDA) just prior to plating by means of a sterile pipette under aseptic condition. Separate pipettes were used for each concentration. Each petriplate (90 cm diameter) was inoculated by placing a 5 mm disc prepared from the vigorously growing culture of *P. islandicum* at the centre. The inoculated PDA medium without incorporation of the extract served as control. The petriplates were then incubated at 27 $\pm$ 1 °C and observation on mycelial growth of the pathogen was recorded at 3, 7 and 10 days after inoculation. The experiment was placed in a Completely Randomized Design (CRD) and each treatment was replicated three times. The different treatment combinations were as follows:

T_1 - Mint leaf extract + *P. islandicum*
T_2 - Zanthoxylum leaf extract + *P. islandicum*
T_3 - Eucalyptus leaf extract + *P. islandicum*
T_4 - Periwinkle leaf extract + *P. islandicum*
T_5 - Wild sesamum leaf extract + *P. islandicum*
T_6 - *P. islandicum* (control)

Artificial inoculation to healthy fruits:

Healthy fruits were collected and surface sterilized with 0.01% mercuric chloride solution followed by thorough washing with two changes of sterile water. The fruits were then pricked 1-2 mm deep by a sterilized needle. A uniform of five pricks were maintained per fruit. A disc of 5 mm diameter of the pathogen isolated was then taken with sterilized cork borer and placed on the surface of the fruit. The culture was smeared with the help of sterilized soft camel hair brush and covered with cotton swab for 12 hours. The fruits were kept at room temperature (20-25°C) for initiation of infection.

Preparation of botanicals

The leaf extracts of plants viz. *Mentha piperata*, *Zanthoxylum oxiphyllum* and *Eucalyptus citriodora* at 15% concentration; three commercial oils viz. neem (*Azadirachta indica*), castor (*Ricinus com-*

munis) and mustard (*Brassica campestris* var. Sarson Prain); and two fungicide chemicals carbendazim (Bavistin @ 0.2%) and mancozeb (Dithane M-45 @0.2%) were used to test their effectiveness against *P. isolandicum* on aonla fruits.

The effect of botanicals, oils and chemicals were studied on highly susceptible aonla fruits (local variety) against *P. islandicum* in both pre-inoculated and post-inoculated conditions under room temperature. The experiment was laid in CRD and was replicated three times. There were 18 treatment combinations, where control was maintained under both pre- and post-inoculated conditions. A set of 20 fruits were maintained for each treatment which represented a single replication. In the treatments with botanicals and oils, a uniform dose of 15 ml each were applied in each treatment. In the pre-inoculated treatments botanicals, oils and chemicals (0.2%) were done 12 hrs prior to the inoculation of *P. islandicum*. In the post-inoculated treatments, botanicals, oils and chemicals (0.2%) were applied 13 hrs after the inoculation of *P. islandicum*. In control experiment, spraying of only sterilized water was done in both pre-inoculated and post-inoculated conditions. A hand atomizer was used for application of all the treatments. Observations on disease intensity were recorded at 3, 6 and 9 days after inoculation.

RESULTS AND DISCUSSION

In *in vitro* evaluation of the leaf extracts of Mint, *Zanthoxylum, Eucalyptus,* Periwinkle and Wild sesamum (Table 1) showed fungicidal activity by way of inhibiting mycelial growth of the pathogen at all concentrations *viz.*, leaf extracts (LE) of 5, 10 and 15 per cent. At 10 DAI, the mean mycelial growth of *P. islandicum* in the control plates measured 8.60 cm but the treated plates *viz.*, Mint, *Zanthoxylum, Eucalyptus*, Periwinkle and wild sesamum were only 2.43 cm, 3.11 cm, 3.65 cm, 4.44 cm and 4.96 cm respectively. However, their performance was better at 15 per cent concentration. Mint at 15 per cent concentration (82.61 %, 81.34 % and 82.56 %) exhibited the highest inhibition of *P. islandicum* followed by *Zanthoxylum* with 71.74 %, 73.28 % and 75.58 % and *Eucalyptus* with 60.87 %, 64.63 % and 69.42 % at 3, 7 and 10 DAI respectively. The least per cent inhibition was recorded in Wild sesamum with only 43.48 %, 47.91 % and 56.63 % respectively. Hence, among the botanicals leaf extracts of Mint, *Zanthoxylum* and *Eucalyptus* showed highest inhibition against *P. islandicum* and thus were selected for further investigation under *in vivo* condition against *P. islandicum*.

Fungicidal activity of plant extracts against various fungi have been reported by several workers (Skinner, 1955 and Dubey *et al.*, 2002). Singh and Sumbali (2003), reported that the LE of *Mentha piperita*, *M. spicata* and *M. longifolia* were quite effective in arresting the development of *P. expansum* rot of apples. They also reported that the LE of *Eucalyptus globulus* and *Zanthoxylum armatum* effectively controlled fruit rot of apples. Complete inhibition of the mycelial growth of *Fusarium oxysporum* with Mint LE has also been reported by Singh *et al.* (1994). The inhibitory effect of different leaf extracts on mycelial growth of *P. islandicum* in the present investigation may be attributed to the presence of antifungal compounds. The presence of antifungal compounds such as alkaloids, phenols, resins, steroids and essential oils have also been reported by Kishore *et al.* (1989) and Roy (1995).

The data presented in the Table 2 indicate that under both pre- and post-inoculated conditions, initiation of the disease was recorded 3 days after inoculation (DAI) in control with 50.00 and 63.33 PDI respectively. However, fruits treated with neem oil, mint and carbendazim took 6 days to initiate the disease under both pre-inoculated (36.67, 36.67 and 40.00 PDI) and post-inoculated (53.33, 45.56 and 50.00 PDI) conditions.

The PDI in control at 9 DAI achieved 93.99 under pre-inoculated conditions and 97.33 under post-inoculated conditions. The PDIs under both the inoculated conditions in the different treatments were 56.67 and 60.00 (neem oil), 66.11 and 71.67 (castor oil), 70.00 and 70.00 (mustard oil), 64.45 and 63.33 (*Zanthoxylum* LE), 52.22 and 55.00 (mint LE), 68.33 and 71.67 (*Eucalyptus* LE), 55.55 and 60.00 (Carbendazim) and 80.00 and 78.67 (Dithane M-45) respectively.

It is also obvious from the data provided in the table that the intensity of disease gradually increased proportionately with the progress in time. However, the disease intensity was observed to be maximum in the post-inoculated treatments as compared to the pre-inoculated treatments. The mean PDI recorded under post-inoculated condition was 51.07,

Leaf extracts	Concentrations (%) 5 Mycelial growth (cm)	5 Inhibition over control (%)	10 Mycelial growth (cm)	10 Inhibition over control (%)	15 Mycelial growth (cm)	15 Inhibition over control (%)	Mean (cm)
Mint	3.20	62.79	2.60	69.77	1.50	82.56	2.43
Zanthoxylum	3.97	53.84	3.27	61.98	2.10	75.58	3.11
Eucalyptus	4.50	47.67	3.82	55.58	2.63	69.42	3.65
Periwinkle	5.50	36.05	4.53	47.33	3.30	61.63	4.44
Wild sesamum	6.10	29.07	5.06	41.16	3.73	56.63	4.44
Control (Sterile water)	8.60	-	8.60	-	8.60	-	8.60
Mean	5.31	-	4.65	-	3.64	-	-

Mean of the replications

	CD (P = 0.05)
Leaf extract	0.15
Concentration	0.11
Leaf extract x concentration	0.26

Table 3: Average effect of the oils, botanicals and chemicals on blue mould disease of aonla under pre- and post-inoculated conditions

Inoculation	Treatment: Neem oil	Castor oil	Mustard oil	Zanthoxylum leaf extract	Mint leaf extract	Eucalyptus leaf extract	Carbendazim	Mancozeb	Control	Means of inoculated conditions over treatments
Pre	31.11 (30.04)	47.59 (43.39)	44.44 (40.29)	37.04 (34.73)	29.63 (29.18)	46.85 (42.97)	31.85 (30.49)	57.04 (49.39)	76.00 (62.45)	44.62
Post	37.78 (33.92)	57.22 (49.26)	51.11 (45.39)	44.81 (40.43)	33.52 (31.46)	53.89 (47.21)	36.67 (33.28)	62.15 (52.42)	82.44 (67.75)	51.07
Means of treatments over inoculated conditions	34.45 (31.98)	52.41 (46.33)	47.78 (42.84)	40.93 (37.58)	31.93 (30.32)	50.37 (45.09)	22.84 (31.89)	59.59 (50.91)	79.22 (65.10)	

Figures in parentheses represent angular transformed values

Table 4: Biomass of leaf extracts against Penicillium islandicum (Poisoned food technique) per cent inhibition at 10 days after inoculation (DAI)

Table 2: Efficacy of some oils, botanicals and chemicals on blue mold disease of aonla under both pre- and post-inoculated conditions

Treatment	Concentration %	Percent disease index after days						Mean	
		3		6		9			
		Pr I	Ps I	Pr I	Ps I	Pr I	Ps I	Pr I	Ps I
Neem oil	15	0.00 (4.05)	-	36.67 (37.22)	-	56.67 (48.84)	-	31.11 (30.04)	-
Neem oil	15	-	0.00 (4.05)	-	53.33 (46.92)	-	60.00 (50.79)	-	37.78 (33.92)
Castor oil	15	23.33 (28.78)	-	53.33 (46.92)	-	61.11 (54.47)	-	47.59 (43.39)	-
Castor oil	15	-	30.00 (33.00)	-	70.00 (56.79)	-	71.67 (57.98)	-	57.22 (49.26)
Mustard oil	15	13.33 (19.06)	-	50.00 (45.00)	-	70.00 (56.81)	-	44.44 (40.29)	-
Mustard oil	15	-	20.00 (26.57)	-	63.33 (52.78)	-	70.00 (56.84)	-	51.11 (45.39)
Zanthoxylum leaf extract	15	6.67 (11.56)	-	40.00 (39.23)	-	64.45 (53.41)	-	37.04 (34.73)	-
Zanthoxylum leaf extract	15	-	13.33 (19.06)	-	57.78 (49.48)	-	63.33 (52.74)	-	44.81 (40.43)
Mint leaf extract	15	0.00 (4.05)	-	36.67 (37.22)	-	52.22 (46.27)	-	29.63 (29.18)	-
Mint leaf extract	15	-	0.00 (4.05)	-	45.56 (42.44)	-	55.00 (47.88)	-	33.52 (31.46)
Eucalyptus leaf extract	15	23.33 (28.78)	-	48.89 (44.36)	-	68.33 (55.77)	-	46.85 (42.97)	-
Eucalyptus leaf extract	15	-	26.67 (30.99)	-	63.33 (52.78)	-	71.67 (57.86)	-	53.89 (47.21)
Carbendazim	0.2	0.00 (4.05)	-	40.00 (39.23)	-	55.55 (48.19)	-	31.85 (30.49)	-
Carbendazim	0.2	-	0.00 (4.05)	-	50.00 (45.00)	-	60.00 (50.79)	-	36.67 (33.28)
Dithane M-45	0.2	33.33 (35.22)	-	57.78 (49.53)	-	80.00 (63.43)	-	57.04 (49.39)	-
Dithane M-45	0.2	-	36.67 (37.22)	-	71.11 (57.52)	-	78.67 (62.51)	-	62.15 (52.42)
Control (Water spray)	15	50.00 (45.00)	-	84.67 (67.14)	-	93.33 (75.20)	-	76.00 (62.45)	-
Control (Water spray)	15	-	63.33 (52.78)	-	86.67 (68.66)	-	97.33 (81.82)	-	82.44 (67.75)
CD at 5%		9.78		5.43		5.04			

Figures in the parentheses represent angular transformed values
Pr I= pre-inoculated treatment
Ps I= post-inoculated treatment

whereas under pre-inoculated condition, PDI was recorded 44.62 (Table 3) which evidently indicated that the treatments were more effective when applied as pre-inoculation than as post-inoculation.

Among the botanicals, treatments with Mint LE recorded the least fruit rot incidence under both the inoculated conditions with 0.00, 0.00 at 3 DAI; 36.67, 45.56 at 6 DAI and 52.22, 55.00 at 9 DAI respectively. The maximum disease intensity, on the other hand was recorded in treatment with *Eucalyptus* LE under both the inoculated conditions with 23.33, 26.69, at 3 DAI; 48.87 and 63.33 at 6 DAI and 68.33, 71.67 at 9 DAI respectively.

However, the disease intensity in treatments with Mint LE and *Zanthoxylum* LE under pre-inoculated condition at 3 DAI (0.00 and 6.67) and 6 DAI (36.67 and 40.00) were statistically at par, while the disease intensity in treatments with *Eucalyptus* LE and *Zanthoxylum* LE at 9 DAI (68.33 and 64.45) was observed to be statistically at par.

Treatment with neem oil was recorded with the least disease intensity amongst the oil under both the inoculated conditions. At 3 DAI, neem oil recorded 0.00 and 0.00 while at 6 DAI and 9 DAI, it was recorded 36.67, 53.33 and 56.67, 60.00 respectively. Maximum disease intensity among the oils was recorded in treatment with Castor oil with 23.33 and 30.00 at 3 DAI and 53.33 and 70.00 at 6 DAI in both the inoculated conditions. At 9 DAI, castor oil recorded the maximum disease intensity under post inoculated condition with 71.67 while mustard oil recorded the maximum under pre-inoculated condition with 70.00. Analysis of variance however indicated that PDI in treatments with castor oil and mustard oil at 9 DAI were statistically at par under both the inoculated conditions.

The least disease intensity between the treatments with carbendazim and Dithane M-45 was recorded with carbendazim with 0.00 and 0.00 at 3 DAI, 40.00 and 50.00 at 6 DAI and 31.85 and 36.67 at 9 DAI under both the inoculated conditions as against 33.33 and 36.67 at 3 DAI, 57.78 and 71.11 at 6 DAI and 80.00 and 78.67 at 9 DAI with Dithane M-45 under both the inoculated conditions respectively.

Amongst all the treatments, Mint LE recorded the least disease intensity under both pre-inoculated (0.00 at 3 DAI, 36.67 at 6 DAI and 52.22 at 9 DAI) and post-inoculated (0.00 at 3 DAI, 36.67 at 6 DAI and 55.00 at 9 DAI) conditions followed by treatment with neem oil with 0.00 and 0.00 at 3 DAI, 36.67 and 36.67 at 6 DAI and 56.67 and 60.00 at 9 DAI under pre-inoculated and post-inoculated conditions respectively. In contrast, the maximum disease intensity among all the treatments was recorded in treatment with Dithane M-45 under both pre-inoculated (33.33, 57.78, 80.00) and post-inoculated (36.67, 71.11, 78.67) conditions at 3, 6 and 9 DAI respectively.

The least disease intensity recorded was in treatment with mint LE (mean PDI 29.63 and 33.52), which was closely followed by neem oil (mean PDI 31.11 and 37.78) and carbendazim (mean PDI 31.85 and 36.67). Analysis of variance of the data obtained however, indicated that treatment with mint LE, neem oil and carbendazim were all statistically at par. These were followed by *Zanthoxylum* LE and mustard oil which was also found to be statistically at par. The maximum disease intensity among all the treatments was recorded in treatment with Dithane M-45 (mean PDI 57.04 and 62.15) under both the inoculated condition. The order of performance was found to be similar under both the inoculated conditions.

The present study revealed that amongst all the treatments mint leaf extract, neem oil and carbendazim were the most promising in controlling blue mold disease of aonla followed by *Zanthoxylum*, mustard oil, *Eucalyptus* and castor oil. However, Dithane M-45 under both the inoculated condition could not yield any positive result to control the disease. Analysis of variance of the data obtained, indicated that treatment with mint LE, neem oil and carbendazim were all statistically at par.

Singh and Sumbali (2003) have demonstrated that pre- and post-infection dip treatment in leaf extract of *Mentha piperata*, *M. spicata* and *M. longifolia* was most effective against *Penicillium expansum* rot of apples. The fungicidal activity of *Mentha* sp. has also been demonstrated against a number of pathogenic fungi (Alice and Rao, 1986 and Jeyarajan, 1988) causing different disease in varieties of crops.

The efficacy of leaf extracts of *Eucalyptus* tree against *Penicillium* spp. has been reported by Singh and Sumbali (2003). They showed that pre-

infection dip treatment of apples in *Eucalyptus globules* leaf extract effectively controls fruit rot of apple. Singh and Sumbali (2003) reported that pre- and post-infection dip treatments of apples in leaf extracts of *Zanthoxylum* significantly controlled disease occurrence caused by *Penicillium expansum*. This implied that the mechanism of anti disease substances extracted from *Zanthoxylum* leaves enhanced disease tolerance of post-harvest fruits by exogenous application. The leaf extract of *Zanthoxylum* could be successfully used in the present study against blue mold of aonla and hence substantiate the reports of the earlier workers.

The variation in the efficacy of the botanicals in controlling different crop diseases (Godara and Pathak, 1995; Singh and Sumbali, 2003 and Yadav *et al.*, 2007) has already been documented. In the present study, water extracts of the botanicals were used and hence only the water soluble portions of the components present in the leaf of the botanicals encountered with the disease. This may be the reason as to why the variation in the efficacy of the botanicals was observed with those of others.

Yadav *et al.* (2007) reported that neem oil at 0.5% effectively reduced the severity of *Aspergillus* rot in aonla fruits in pre- as well as in post-inoculation treatments. Bhaskaran and Narasimhan (1994) also reported that neem oil and castor oil effectively inhibited the growth of *Fusarium* rot and *Alternaria* rot in tomato. Mustard oil and castor oil was also reported earlier as effective in the control of peaches infected by *Aspergillus niger*, and apples infected by *Gliocladium roseum* and pears infected by *Sclerotium rolfsii* (Sumbali and Mehrotra, 1980). The application of these oils viz. neem, castor and mustard in controlling aonla fruit rot is recommended because of the fact that it is readily available and cheap. Treated fruits would have no residue effects and oil treated fruits when washed with dilute soap emulsions are known to remain firm and healthy.

The effectiveness of carbendazim in controlling post-harvest spoilage of fruits caused by *Penicillium* spp. has been reported by several workers. Chib *et al.* (1983) suggested the control of blue mold rot incited by *Penicillium expansum* with carbendazim (0.1%). Rathod and Patel (2005) also recorded pre- and post-inoculation treatments of aonla fruits with carbendazim (500, 1000 ppm) and mancozeb (2000, 4000 ppm) effectively reduced the incidence of *Penicillium* rot of aonla. Maurya (2007) also suggested pre-harvest spray of aonla fruits with carbendazim at 0.1% to control *Penicillium* fruit rot. After harvest, pre-infectional dips with chemicals was more effective than post-infectional dips was also reported by Sumbali and Mehrotra (1983).

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