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Prevention of Rot Development: in Mechanically Injured Apple (Baldwin) Fruits by using Indigenous Plant Extracts

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Abstract :- **In a hungry and increasingly competitive world reducing post harvest food losses is major agricultural goal. Even a partial reduction in postharvest losses can significantly reduce the overall cost of production and lessen our dependence on marginal land and other scarce resources. The objective of this work was prevention of rot development in mechanically injured apples (baldwin) fruits by treating with extracts of some indigenous plants. The fungi isolated from rotten apples were *Penicillium* spp. (Green rotting agent fungi), *Colletotrichum* spp.(Bitter rotting agent fungi) and *Monilia* spp.(Brown rotting agent fungi). The water and methnol extracts of plants namely: azardirachta indica, piper nigrum, lawsonia inermis, capsicum annum and citrus limon, suppressed fungal growth and reduced the rot development in the apples.**

Key words: Plant extracts, rot fungi, rot inhibition, isolation, antifungal effect, antifungal properties, mechanical injury.

1. INTRODUCTION

The apple is the most ubiquitous of temperate fruits and has been cultivated in century B.C Europe and Asia from antiquity. About 69 million tones of apples are grown worldwide. India produces about 2.05% of total world apple production and around1.6% of the country's productions get exported. A large portion of all fresh produce is lost worldwide after harvest. For highly perishable commodities, 30 percent of the harvested crop may be lost to postharvest diseases before it reaches the consumer. Nowadays reduction in postharvest food losses is a major agricultural goal. Postharvest losses include damage caused by environmental conditions such as heat or drought, improper postharvest sanitation, poor cooling and environmental control and mechanical damage during harvesting and handling. Mechanical injury can lead to postharvest decay; losses are estimated at 20-40% in developing countries and 10-15% in developed countries. Efforts to control these factors are often very successful in reducing the incidence of disease. Reducing mechanical damage during grading and packing greatly decreases the occurrence of postharvest disease by preventing the entry of disease-causing organisms (pathogens) through wounds. Many chemicals have been effectively used to reduce the incidence of postharvest disease. In recent years because of economic, environmental, and health concerns many of these materials have lost their popularity and major efforts are made to reduce post harvest losses. This paper reports on reduction of post harvest losses occurring to apple fruits due to mechanical injuries by preventing the growth of fungi associated with their decay.

2. MATERIALS AND METHODS

2.1. Source of apples

Rotted apples were collected from local markets in kanpur and packaged into polythene bags and taken to the laboratory of biochemical department, Harcourt butler technical institute, Kanpur, India. Healthy apples were also collected from the markets.The local plants; *Azardirachta Indica* (leaves),*Piper Nigrum* (seeds), *Allium Sativum* (bulb), *Lawsonia Inermis* (leaves), *Citrus Limon* (leaves) used in this study were collected from CSA kanpur. These plants were verified and authenticated in the herbarium of N.B.R.I., Lucknow.

2.2. Isolation of fungi associated with rotted apples

Rotted apples were washed in tap water, and cut into sections with sterilized scalpel. The sections were surface sterilized in 1% sodium hypochlorite and rinsed with several changes of sterile distilled water. Sections of the sterilized apples were plated out on development. The developing fungi were identified and pure cultures were obtained and stored in slants for further use

2.3. Pathogenecity test

Fresh, healthy apples were washed with tap water, rinsed with distilled water followed by surface sterilization with 70% ethanol and allowed to dry in a laminar air flow hood. Cylindrical discs were removed from the apple with a sterile 5 mm cork borer. A disc of five days old culture of the isolated fungi was transferred into holes created in the apples by using another cork borer of 4mm. Vaseline was used to completely seal each side and pieces of cotton were placed on the Vaseline. The inoculated apples were placed in separate airtight containers and incubated for 10 days at room temperature (28 ± 2°C). The same procedure was used for the control except that discs of uninoculated PDA were placed in the holes created in the apples [1]. After

incubation period, the apples were examined for infection and disease development the positive length and girth of the rot area and those of the entire apples were measured and recorded.

2.4. Preparation of plant extracts

The following local plants: *Azardirachita indica* (leaves); *Allium Sativum* (bulb)*; Lawsonia Inermis* (leaves); *piper nigrum* (seeds) and *Citrus Lemon* (leaves) were sundried and grounded separately. The extracts of different plant material were prepared by using cold solvent method [2]. Thirty grams of each sample was added to 30 ml of distilled water and 95% methanol in separate flasks. This was vigorously stirred and left to stand for 24 hours. The sample was filtered with a whatman filter paper (1µ) and the filtrate is used as the crude extract. Concentrations of 25, 50, 75, 100 per cent and the crude extract were used for the experiments. Along with different concentrations 10% solution of crude extract was also prepared by dissolving 10 ml of crude extract into 90 ml of distilled water.

2.5. Effect of plant extract on fungal growth

Petri plates were used for the assay by using hole-plate diffusion method. Different percentages of the extract solution were poured into separate wells prepared on the Petri plates, containing sterilized potato dextrose agar and inoculated with different fungi. Inoculated Petri plates were incubated at room temperature (28 ± 2oC) for 7 days. After the incubation period, zone of inhibition were measured and noted. For the control, sterile water was added to the wells on potato dextrose agar plates. The effectiveness of each plant extract was recorded in terms of percentage inhibition in linear growth rate (LGR), which is calculated by using the following formula [3]:

$$LGR\ (\%) = (n - x)/n \times 100$$

Where; *n is* Growth in control and *x is* Growth in treatment.

2.6. Effect of plant extract on rot development

The method of "reference [4]" was used to determine the effect of extract on rot development. Freshly harvested healthy apples were washed with water, surface sterilized with 1% sodium hypochlorite solution and rinsed in five changes of sterile distilled water. The apples were soaked in10% plant extract and allowed to stand in the solution for overnight. In the control, apples were soaked in sterile distilled water for overnight. The apples were removed from the extract and water (for control) and incubated at room temperature for 24 h. Using a 1.1 cm cork borer, discs were removed from the extract treated and water treated control apples, and replaced with 1.1 cm discs of a 5 day old culture of each test fungi. Vaseline jelly was used to completely seal each hole. The inoculated apples were placed in sterile sealed containers and incubated at room temperature (28 ± 2oC) for 7 days. After the incubation period, the apples were incised horizontally with sterile knife. The length of rotted portion from each hole was measured over the total surface length with a meter rule. Fungi toxicity was determined in form of percentage growth inhibition was calculated according to the formula [5].

$$G.I.\ (\%) = \{(LC - LT) \div LC\} \times 100$$

Where, GI= Growth inhibition, LC = average length of unrotted portion of control and LT = average length of unrotted portion with treatment.

3. RESULTS

3.1. Occurrence of fungal pathogens isolated from samples of apples: Five fungi were isolated from the rotted apples *Alternaria alternata*, *Aspergillus niger*, *Penicillium italicum* ,*monilina fructigena,rhizopus sp.*The most frequently occurring fungi were *Colletotrichum* spp.(Table 1)

Table1. Occurrence of fungi isolated from diseased apples.

Isolates	Occurrence
Alternaria alternata	1.48
Colleoptrichum sp.	44.00
Penicillium italicum	38.15
Monilina fructigena	15.67
Aspergillus niger	2.00

3.2. Pathogenicity test: The pathogenecity test showed that all the three test fungi were pathogenic, hence causes rot in healthy apples after 7 days of inoculation. The most virulent among the three was *Colleoptrichum sp*, with rot incidence of 96.86 % followed by *Monilina fructigena*(46.12%) while the least virulent was *Penicillium italicum* with rot incidence of 30% (Table 2).

Table 2.pathogencity test/ mean percentage of rot by test isolates on apples

Isolates/Inoculated Fungus	Percentage Rot
Penicillium italicum	30.00
Monilina fructigena	46.12
Colleoptrichum sp.	96.86

3.3. Effect of extracts, distilled water and methanol on the growth of the three test fungi:

The effect of concentrations of extracts on the test organisms was significant ($p<0.05$).Colony diameter of the inhibition increased as the concentration of the extract increased as follows (25%, 50%, 75%, 100%) The interaction of extraction medium and concentration of extract was also significant ($P<0.05$) on the inhibition of both test fungi. Aqueous extracts of *A.indica* gave the highest inhibitory effect of *Monilia frucigena* by 79.12%, followed by with *A. sativum* (73.68%), while *L. inermis*, *P. nigrum and C. limon* showed the least inhibition of 73.68%, 72.22% and 66.67% respectively (Table.3).

Table 3.Effect of plant extract extracted with distilled water on 7 days old culture of *Monilia fructigena*

Extract and Control	Concentration (%)				
	0	25	50	75	100
Water	0.00				
A.indica		50	68	76	79.12
P. nigrum		66.67	68.75	70.59	72.22
L.inermis		50	66.67	68.75	73.68
A. sativum		61.54	66.67	72.22	75
C. limon		50	58.33	61.54	66.67

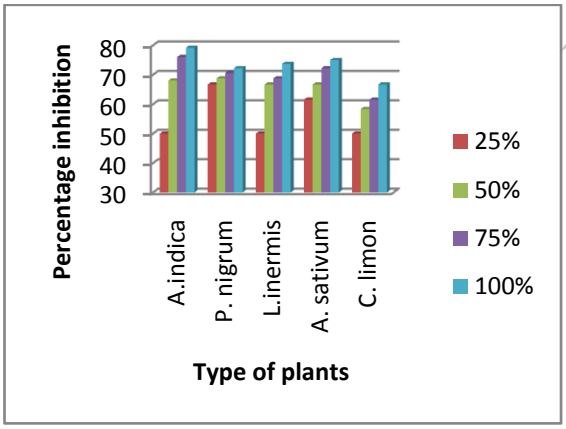

Fig1 Effect of plant extracts at different concentration in distilled water on *Monilia fructigena*

The methanol extract of *Allium sativum* gave the highest inhibition on *Monilia frucigena* (72%), which is significantly ($P<0.05$) greater than 59.42% recorded by *A.indica*, *P. nigrum*, *L.inermis*, *C. limon* which gave an inhibition percentages of 66.67%,68.18% and 56.25% on the growth of the fungus (Table 4).

Table 4.Effect of plant extract extracted with methanol on 7 days old culture of *Monilina fructigena*

Extract and Control	Concentration (%)				
	0	25	50	75	100
Water	0.00				
A.indica		61.11	63.16	65	66.67
P. nigrum		53.33	58.8	65	68.18
L.inermis		30	53.33	56.25	58.8
A. sativum		63.16	69.56	70.83	72
C. limon		30	50	53.33	56.25

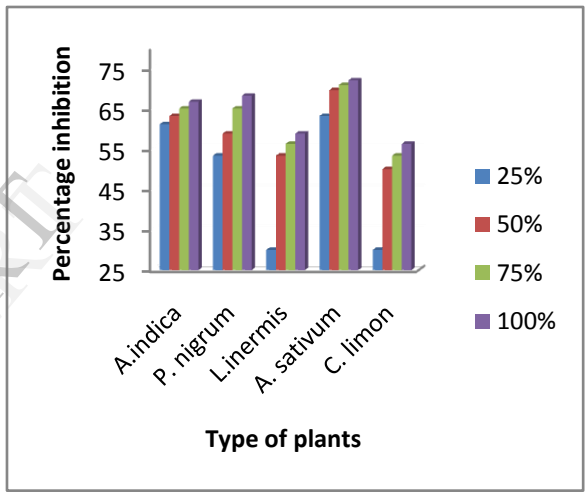

Fig2- Effect of plant extracts at different concentration in methanol on *Monilia fructigena*

The aqueous extracts of *Allium sativum* had the highest inhibitory effect on *Colleoptrichum sp.* by (61.90%), followed by *Azadirachta indica* (55.55%) and the least were *P. nigrum*(42.86), *L.inermis* (51.36%) and *C.limon* (50%) (Table 5).

Table 5.Effect of plant extract extracted with distilled water on 7 days old culture of *Colleoptrichum sp.*

Extract and Control	Concentration (%)				
	0	25	50	75	100
Water	0.00				
A.indica		46.67	50	52.94	55.55
P. nigrum		27.27	46.67	52.94	42.86
L.inermis		20	46.67	_	55.55
A.sativum		52.94	20	50	61.90
C.limon		20	33.33	46.67	50

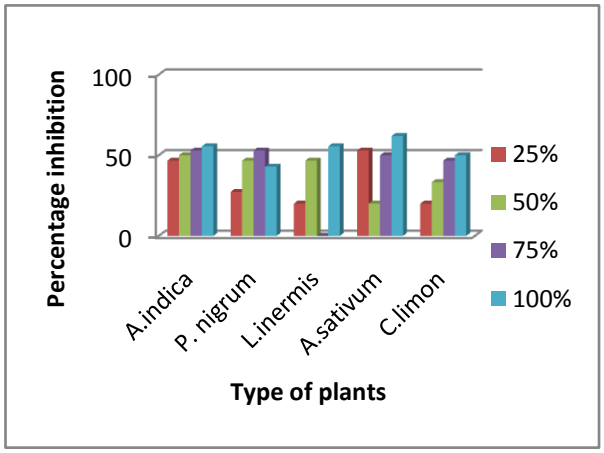

Fig3- Effect of plant extracts at different concentration in distilled water on *Colleoptrichum sp.*

The methanol extracts *A.indica* with percentage inhibition of (60.87%) had the highest inhibitory effect on *Colleoptrichum sp*. followed by *A.sativum* (57.14%), the least inhibitory effect was observed in *L.inermis* ,*C.limon* which gave percentage inhibition of 35.71 and 30.77 respectively, hence not significantly different from each other. *P. nigrum* showed moderate inhibitory effect of (50.00%), while the negative control showed uninhibited growth of *Colleoptrichum sp.* (Table 6).

Table 6.Effect of plant extract extracted with methanol on 7 days old culture of *Colleoptrichum sp.*

Extract and Control	Concentration (%)				
	0	25	50	75	100
Water	0.00				
A.indica		18.18	30.77	40	60.87
P. nigrum		25	40	43.75	50
L.inermis		18.18	25	30.77	35.71
A.sativum		40	47.06	55	57.14
C.limon		10	18.18	25	30.77

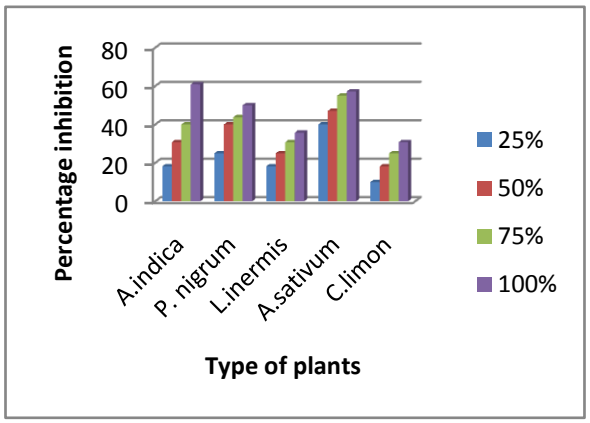

Fig4- Effect of plant extracts at different concentration in methanol on *Colleoptrichum sp.*

4. DISCUSSION

The organisms associated with post-harvest rot of apples found in this study were *Alternaria alternata*, *Aspergillus niger*, *Penicillium expansum*, *Monilina fructigena*, *Colleotrichum sp.* These were frequently isolated from rotten apples and have been reported to cause extensive rot of in storage and other post harvesting process [6],[7],[8],[9] . The involvements of the test fungi (Monilina fructigena, Penicillium italicum and Colleoptrichum sp.) in pathogenesis were also confirmed. The isolation of more than one pathogenic organisms from a particular apple confirms the possibility of multiple infections whose cumulative effect may cause rapid rottening of fruit, this agrees with the reports on yam [10]. In most cases fungi gain entrance into apples through natural opening and wounds created during harvesting, transportation, handling and marketing. This study revealed that fungi toxic compounds were present in *A.indica*, *P. nigrum*, *L.inermis*, *A. sativum* and *C. limon*, since they were able to inhibit the growth of the test fungi, this result is in consonance with the earlier reports of several researches but on different fungal organisms [11],[12], hence the four plant extracts used have the potential application in the protection of mechanically injured apples against rot fungi. However, the efficacy of the extracts differed with the plant material, concentration, and solvent of extraction and with each test fungus. Methanol extracts were more effective than aqueous extract, this suggests that water used in the extraction process was probably not able to dissolve all the principles compounds present in the plants, which are contained in the methanol extract. Being an organic solvent the methanol extract gave higher yield in all the plants [13] because of ability to dissolve organic compounds better, hence liberate the active compounds (phytochemical) required for antifungal activity. The difference in the fungitoxic between the extraction medium can also be as a result of the different susceptibility of each of the test isolates to different concentrations of the extracts [14].The present observations showed that *A .sativum and A. indica* are highly effective against mycelia growth of almost all the test fungi with inhibition ranging from 40.57% to 79.63% while extract of *P. nigrum* ranged from slightly to moderately effective inhibition (42.86 to 71.00%) [15].

5. CONCLUSION

This study have revealed the potentials of botanicals (*A. sativum, A. indica, P. nigrum, L.inermis* and *C.limon*) in the control of apple rot after mechanical injuries, with *A. sativum and A .indica* exhibiting the most fungitoxic activity. This study also depicted that methanol extracts demonstrated a higher antifungal activity over aqueous extract, indicating that methanol extract of *A. sativum and A.indica* could be an alternative or complimentary to synthetic chemicals in controlling apple rot. In absence of *A. sativum* and *A .Indica*, the extracts of *P. nigrum*, *L. inermis* and *C. limon* can also be used as a second option. However, the result of this study has gone a long way in providing better alternative to the over dependence on synthetic fungicides, and reduction of over reliance on one

source of agricultural chemicals to the farmers, that are reported to have long term harmful consequences on environment, Man and wildlife, as well as reduce production cost.

6. ACKNOWLEDMENT

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