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Anise Oil : Effective as Post-Harvest Botanical Fungicide in the Management of Pink Mould of Apples caused by *Trichothecium roseum*

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ABSTRACT: ***Malus sylvestris*** **(L) Mill. also known as Apple belonging to family Rosaceae. The fruit is pome, where the actual fruit is enclosed by fleshy edible part of the thalamus. Around 20 lakh tones of Apple is produced in India each year. The highest apple producing states of India are Jammu & Kashmir, Himachal Pradesh, Uttrakhand and Arunanchal Pradesh. Apples are good source of fiber and vit. C and they are less in calories. They are devoid of fat or cholesterol and have only few amount of sodium. Apples are rich in polyphenols and these work as antioxidant. Apple lowers the risk of asthma and alzheimer's disease. They are helpful in weight loss, good for bones, lungs function and act as gastrointestinal shield.**

The current research was conducted to analyze the assessment of fungal pathogens related to apple fruit rot and their control. Samples of fruits were collected from different fruit markets Gorakhpur. Total 24 fruit rotting fungi were found to be related to apple fruits. Rotting dynamics, pathogenicity test and loss in weight were observed. ***Trichothecium roseum*** **Pers. Link. Ex. Showed highest rotting dynamics and it also caused highest weight loss in apple fruits. Volatile oil of** ***Pimpinella anisum*** **Linn. was tested for its fungitoxicity against** ***Trichothecium roseum*****. The oil was found fungitoxic against test fungus. The MIC (Minimum Inhibitory Concentration) of** ***Pimpinella anisum*** **oil was 500 ppm. So as to seek out the efficacy of anise oil as preservative of fruits from fungal spoilage during storage, anise oil treated fruits were stored for 12 days at 500 ppm. The treated fruits of apple were healthier than the untreated ones and free from fungal growth.**

Keywords : Fruits, rotting, pathogenicity, Dynamics of rotting, volatile oil, fungitoxicity, MIC.

INTRODUCTION

During storage succulent part of fruits are affected by many fruit rotting micro organisms, among them fungi form the main group. Because of high moisture and nutrient, they tends to decay, as they mature. This may be due to mechanical injury or physiological changes in the host tissues, which make them more suitable substrate for speedy development of the microorganisms. Due to mechanical and physiological injuries during harvesting and marketing, microorganism enters into fruits and cause major losses in fresh fruits.

Fruits are spoiled by fungi due to their low pH and cause rot disease. Some fungi also produced mycotoxins which are harmful for human health, when they consume spoiled or contaminated fruits (Phillips 1984; Moss 2002).

The current paper pronounces the rot disease of *Malus sylvestris* (L) Mill. caused by pink mould *Trichothecium roseum* Pers. Link. Ex. during storage and their effect on rotting dynamics, pathogenicity test and loss in weight. The paper also describes fungitoxicity of essential oil of *Pimpinella anisum* Linn. The antifungal activity of some essential oils are reported by many authors (Deans and Ritchie 1987; Meepagala *et al.,* 2002; Reuveni *et al*., 1984) and some authors also work on the efficacy of essential oils on post-harvest pathogens. Vapours of essential oils are bioactive, due to this they are used as fumigants for storage of food materials.

MATERIAL AND METHODS

Samples of *Malus sylvestris* were congregate periodically from fruit vendors and fruit markets of Gorakhpur. Collected samples of rotted apple were brought to the lab in sterile polybags. Disease Symptoms of related fungi were noticed and documented.

Isolation of fungi from fruits of *Malus sylvestris*

The surface of rotted fruits were sterilized with 90 per cent ethyl alcohol, then associated fungi from them were isolated and transferred to PDA (Potato Dextrose Agar) medium. Hyphae of some fungi were directly transferred to medium. Petri plat ^{o}C and they were recognized with the assistance of existing books.

Confirmation of Pathogenic nature of isolated fungi

Pathogenicity tests were led to confirm the pathogenic nature of the isolated mycoflora from apple fruits. Fresh, sound and surface sterilized fruits of *Malus sylvestris* were taken and an injury of 10 mm depth was made over. The surface of the fruits was injured (~10mm depth) with the help of sterilized cork borer of 5 mm diameter. Three day old inoculum was placed in the pit and the taken out piece of the fruit tissue was inserted back to its position. Then wound was sealed with the help of sterilized cotton.

Five replicates of inoculated fruits were prepared and at the temperature of 24±2^{o}C these were kept in sterilized glass jars. Pathogenic nature of the isolated fungi was confirmed only when Koch's postulate were completely gratified.

Dynamics of rotting were also recorded by using data with the help of formula given by Bottcher (1986).

$$Y = \beta_1 (x-z)^2$$

Where,

x = duration of storage in days

Y= rot

β_1 = Linear

z = a period without macroscopic symptoms

Calculation of loss in weight of apple fruits

Surface sterilized fresh and healthy fruits were inoculated separately with pathogenic fungi and similar control sets without pathogenic fungi were prepared.

After incubating the controlled and inoculated sets at 24±2^{o}C for 7 days loss in weight was calculated by following formula:

Weight loss = W – w / W × 100

Where,

W = weight of the infested fruit before incubation

w = weight of the infested fruit after incubation

Extraction of volatile fungitoxic fraction from the seeds of *Pimpinella anisum* Linn.

The essential oil was extracted with the help of Clevenger's apparatus which works on hydrodistillation technique thoroughly washed 500 g seed of *Pimpinella anisum* with sterilized water were positioned in the flask of the above apparatus. The ratio was maintained as 1:3 respectively. Water was heated to The steam produced have volatile romatic fraction. After chilling of steam the distillate was collected. The essential oil was accumulated on the top of the distillate. The oil was separated and dehydrated by the use of anhydrous sodium sulphate. It was left for 6–8 hrs and then filtered.

Fungitoxicity of the oil against *Trichothecium roseum*

Fungitoxic activity of *Pimpinella anisum* oil was tested by poisoned food technique of Grover and Moore (1962) against the test fungus *Trichothecium roseum* at 1500 ppm. For preparing the concentration of essential oil, requisite amount of oil dissolved in 0.5 ml of acetone and mixed it with 9.5 ml of Potatodextrose Agar (PDA) medium. In the control sets equal amount of sterilized distilled water was used in place of the oil. Prepared Petri plates were inoculated with discs of the test fungus and incubated for 6 days. On the seventh day the percentage of mycelial inhibition was calculated by the following formula:

Percentage of mycelial inhibition = $\frac{dc - dt}{dc} \times 100$

Where dc is mean colony diameter of control sets and dt is mean colony diameter of treatment sets.

Physico- Chemical properties of the essential oil

Gas Liquid Chromatography of *Pimpinella anisum* oil were done and physicochemical properties like acid value, phenolic content, optical rotation, refractive index, specific gravity and solubility in organic solvent were assessed by method of Langenau (1948).

Minimum inhibitory concentration and nature of toxicity of essential oil of *Pimpinella anisum*

To find out the minimum inhibitory concentration at which the oil showed absolute fungitoxicity, tests were carried out by method of Grover and Moore (1962) by using arranged concentration of essential oil below 1500 ppm. The fungistatic and fungicidal nature of *Pimpinella* anisum oil against the test funguswas determined by the method of Garber and Houston (1959). Fungal discs of the oil treated sets were reinoculated into fresh medium and revival of their growth was detected.

In vivo applicability of the oil of *Pimpinella anisum*

Fresh, healthy and nearly same age fruits of *Malus sylvestris* L. Mill were purchased

from the local market and fungus inoculated on fruits by knife injury method of Tandon and Mishra (1969) were fumigated with essential oil (w/v).

For each treatment fruits were surface sterilized by wiping the fruit surface with cotton swab soaked in 90 per cent alcohol. Surface sterilized fruits were then injured with the sterilized knife. For pre- inoculation surface sterilized and injured fruits were fumigated with the oil of *Pimpinella anisum* Linn. For this a small piece of sterilized cotton was wetted with the oil at MIC and kept at the base of the pre- sterilized dessicator. Fruits were incubated inside the dessicator on sterilized wire gauge placed above the soaked cotton. The lid of dessicator was tightened. For post-inoculation treatment surface sterilized and injured fruits were first, separately inoculated with test fungus and then incubated for twelve hours at 24 ± 2°C under sterilized bell jars. After the incubation period, they were fumigated with the essential oil as described for pre-inoculation treatment. Proper control sets were also maintained, where surface sterilized and inoculated

fruits were not fumigated with oil. In all the treatments three replicates were taken.

Pre-inoculated, post-inoculated and control sets were incubated for 12 days at 24 ± 2°C temperature. The development of rot was measured after four, eight and twelve days by the method of Thind *et al*., (1976).

RESULTS AND DISCUSSION

During two years survey of local fruit markets so many rot causing fungi were isolated from fruits of *Malus sylvestris* L. Mill. The isolated fungi were identified on the basis of symptoms produce by them and their morphological features. The outcomes are given in Table 1.

Table 1: Showing Name of fungi isolated from fruits of *Malus sylvestris* L. Mill. their Dynamics of rotting, Pathogenicity and Effect on Weight.

Name of Fungi isolated	Parameters		
	Dynamics of Rotting	Pathogenicity	Weightloss
Alternaria alternate Keissler	33.0	+	2.30
Aspergillus flavus Link	157.0	+	3.46
A. Fumigatus Fresenius	134.0	-	0.0
A. nidulans (Eidam) Winter	37.0	+	1.15
A. niger Van Tieghem	132.0	+	4.23
A. ochraceous Wilhelm	147.0	+	2.69
A. parasiticus Speare	128.0	-	0.0
A. sydowi Bainier and Sartory Thom and Church	152.0	-	0.0
A. tamarii Kita	103.0	-	0.0
A. terreus Thom	148.0	+	21.30
A. versicolor (Vuillemin) Tiraboschi	12.0	+	1.53
Aspergillus sp. I.I.	105.0	-	0.0
*Botrytis cinerea*Persoon	43.0	+	1.92
Cladosporium oxysporum Berkeley and Curtis	186.0	-	0.0
F. oxysporum Schlecht	147.0	+	2.30
F. semitectum Berk. And Rav.	132.0	-	0.0
F. solani App. Et. Wr.	88.0	-	0.0
Mucor racemosus Fresenius	186.0	+	1.92
*Mucor*sp.U.I.	191.0	+	1.53
Penicillium chrysogenum Thom	101.0	-	0.0
P. citrinum Thom	35.0	-	0.0
P. oxalicum Currie and Thom	68.0	-	0.0
Rhizopus stolonifer (Ehrenb ex Fr.) Lind	147.0	+	2.34
Trichothecium roseum Pers. Link. Ex	284.0	+	4.23

+ = Present - = absent

24 fungi were isolated from fruits of *Malus sylvestris* L. Mill. (Table 1). Of which *Trichothecium roseum* showed **highest (284)** dynamics of rotting which is followed by *Mucor* sp. (191) and it was least (12) by *Aspergillus versicolor*.

Out of 24 species 13 were found to be pathogenic and 11 were non-pathogenic because they did not confirm Koch's postulate. Estimated weight loss was 4.2, 4.2, 3.4, 2.6, 2.3, 2.3, 2.3, 2.3, 1.9, 1.9, 1.5, 1.5 and 1.1 per cent for *Aspergillus niger*, *Trichothecium roseum*, *Aspergillus flavus*, *A. ochraceous*, *Rhizopus stolonifer*, *Alternaria alternata*, *Aspergillus terreus*, *Fusarium oxysporium*, *Botrytis cinerea*, *Mucor racemosus*, *Aspergillus versicolor*, *Mucor* sp. and *Aspergillus nidulans*.

The yield of oil of *Pimpinella anisum* was 1.91%. The essential oil was nearly colourless and has a pungent liquor rice-like smell. The oil was soluble in different tested organic solvents. The acid value, optical rotation, refractive index and specific gravity of oil was noted to be 14.40, +60°, 1.557 and 0.945 respectively. The oil contains phenolic content. The GLC of oil shows mixture of 4 major and 6 minor components (Fig. 1).

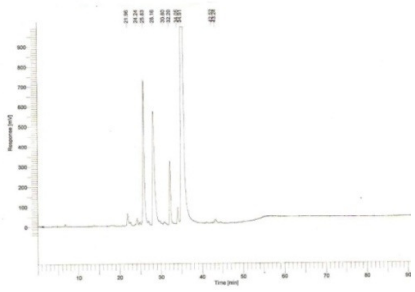

Fig. 1. Showing G.L.C. OF Essential Oil of *Pimpinellaanisum* L.

The MIC of the oil of *Pimpinella anisum* oil was 500 ppm at which it checked the mycelial growth of the test fungus *Trichothecium roseum*. It may be noticed from Table 2 that the oil of *Pimpinella anisum* was fungicidal for thetest fungus because the re-inoculated discs did not show growth of test fungus at the MIC.

The result given in Table 3 show that there was no rotting of the fruits of *Malus sylvestris* when treated with the oil of *Pimpinella anisum* while the fruits exhibited rotting in control sets where no oil was applied. The percentage of rotting of fruits increased with the increase in incubation period. The oil did not affect the appearance of the fruits. The treated fruits appeared much more healthy and fresh than the untreated ones.

Table 2: Showing MIC of *Pimpinella anisum* l. oil against mycelial growth of test fungus.

Concentrations (ppm)	Percent inhibition of mycelial growth of *Trichothecium roseum*
1500	100
1000	100
500	**100**
400	90
300	76
200	65
100	49

Table 3: Showing effect of *Pimpinella anisum* oil applied as pre and post inoculation treatments on the percentage of rotting of the Malus sylvestris (L.) Mill by *Trichothecium roseum*.

	Test fungus *Trichothecium roseum*			
Pimpinella anisum Oil	Incubation period (Days)	4	8	12
	Pre-inoculation	**0**	**0**	**0**
	Post- inoculation	**0**	**0**	**0**
Control set		**15**	**48**	**80**

Due to fungitoxic nature at its lower MIC the essential oil of *Pimpinella anisum* was selected for further studies. The oil was standardized through physicochemical, fungitoxic properties and practically applied in controlling the pink mould of apples. This fungus caused highest dynamics of rotting, highest loss of weight of apple fruits during storage. The quality of essential oils checked by a number of physical parameters such as acid value, phenolic contents, optical rotation, refractive index, specific gravity and solubility in different organic solvents. The GLC of oil of *Pimpinella anisum* oil showed mixture of 4 major and 6 minor components. The oil showed the synergistic effect. The essential oil of *Pimpinella anisum* exhibited substantial fungitoxic activity and it boosted the shelf life of apples during storage and protecting them from pink mould. The fruits were treated by the essential oils at their separate MIC. This treatment showed enhancement of shelf life of fruits up to 4, 6 and 12 days, respectively. The oils did not affect the peel of fruits and not showed any contrary symptom. Due to this the essential oil of *Pimpinella anisum* is used as antifungal agent. The *Pimpinella anisum* oil with its strong fungitoxicity, lower MIC, extensive shelf life and killing effect could be suggested to control apple rot causing fungi *Trichothecium roseum*.

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