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# Antifungal Activity of Anise Oil against *Corticium rolfsii* Causing Papaya Fruit Rot

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**ABSTRACT: The sample of fruits of *Carica papaya* L. were collected from fruit vendors, fruit shops and wholesale fruit market of Gorakhpur and bought to the laboratory in pre sterilized polythene bags. A total of 19 fungi associated with the fruits were isolated by standard method. Dynamics of rotting, test for pathogenicity and weight loss were observed. Dynamics of rotting was highest for *Corticium rolfsii* Sacc. which also caused highest weight loss of the fruit. Essential oil of *Pimpinella anisum* Linn. was tested for its fungitoxicity against *Corticium rolfsii* Sacc. by Poisoned Food Technique. The oil was found fungitoxic against test fungus. The Minimum Inhibitory Concentration (MIC) of the oil of *Pimpinella anisum* Linn. was 2000 ppm.**

**Key words:** Fruits, pathogenicity, rotting, essential oil, MIC

## I. INTRODUCTION

Plants suffer severely from post-harvest diseases. Post-harvest diseases render heavy losses to perishables during harvesting, grading and packing, during transportation to market and to consumers, and while the produce is in possession of the consumer until the movement of actual consumption are huge. Fruits are an important part of our food from pre historic time. They are chief source of vitamins, minerals, protein, carbohydrate, fat, fiber, and other minor and major elements. Higher water contents, nutrient composition and pH of most of the perishables make them capable of supporting the growth of a number of microorganisms. Fruits due to their low pH are spoiled primarily by fungi which in addition to causing rot, may also contaminate the fruits by producing mycotoxins (Phillips 1984; Moss 2002). That make the remainder of the product unfit for consumption or lower its nutritional and sale values. Worldwide post-harvest loss of perishables due to fungi is between 10% and 50%.

*Corticium rolfsii* is a well known pathogen of plants especially in the sub-tropical and tropical countries of the world causing diseases ranging from root-rot to fruit-rot. Bailey (1966) listed about 20 plants of economic importance affected by this fungus in Nigeria . These include root-rots and root diseases in tobacco (*Nicotiana tabacum* L.) and lima beans (*Phaseolus lunatus* L.); collar rot in groundnut (*Arachis hypogaea* L.), chilli pepper (*Capsicum annuum* L. and *C. fructescens* L.) and tobacco; stem rot in sunflower (*Helianthus annuus* L.), bananas and plantains (*Musa* spp.), cowpea (*Vigna sinensis* (L) Savi ex Hassk.) and maize (*Zea mays* L.); southern blight in potato (*Solanum tuberosum* L.); wilt in tomato (*Lycopersicon esculentum* Mill.); corm rot in cocoyam (*Colocasia antiquorum* Schott); and fruit rot in oil palm (*Elaeis guineensis* [acq.) and garden egg (*Solanum melongena* L.). Furthermore, the pathogen causes fruit rot of tomatoes (Irvine, 1969; Onesirosan & Fatunla, 1976), collar rot of *S. melongena* (Irvine, 1969). Working with a cowpea isolate of the fungus, Maduewesi (1975) found that the pathogen has a wide host range affecting 116 species from 38 families out of 125 species from 48 families of tropical plants tested for susceptibility. Although *Corticium. rolfsii* has been implicated in both the fruit rot and blight of some other varieties of watermelon in the United States of America (Ramsey *et al*., 1959; Schenck, 1960; United States Department of Agriculture, 1960).

The present paper describes the rot disease of fruits of *Carica papaya* L. caused by *Corticium rolfsii* during storage and their effect on dynamics of rotting, test for pathogenicity and weight loss. The paper also describes fungitoxicity of essential oil of *Pimpinella anisum* Linn.

Although the use of synthetic pesticides in plant protection had made a great contribution to plant protection, many are no longer used because of economic, environmental or health concerns, or due to development of resistant strains. Fungicides that are primarily used for controlling post-harvest diseases have recently come under special scrutiny as posing a potential oncogenic risk. Therefore, the scientific community at international level is looking for safer alternative products from plants for effective control of pests during storage. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents. Biologically active essential oils represent a rich potential source of an alternative and perhaps environmentally more acceptable disease management compounds. With a broad range of natural fungicidal plant volatiles, numerous opportunities exist to explore their usefulness in controlling post-harvest diseases. The general antifungal activity of essential oils is well documented (Avasthi *et al.,* 2010; Devkota and Sahu, 2017; Bhattarai and and Kumar, 2016; Reuveni *et al.*1984; Deans and Ritchie1987; Alankararao *et al.*1991; Baruah *et al.*1996; Gogoi *et al.*1997; Pitarokili *et al.*1999; Meepagala *et al.* 2002) and there have been some studies on the effects of essential oils on post-harvest pathogens (Bishop and Thornton 1997). The advantage of essential oils is their bioactivity in the vapour phase, a characteristic which makes them attractive as possible fumigants for stored product protection.

## II. MATERIAL AND METHODS

Sample of fruits of *Carica papaya* L. were collected periodically from fruit vendors, fruit shops and mandies as well as from the markets of nearby areas in pre sterilized polythene bags and brought to the laboratory. Symptoms of disease and associated mycoflora were observed.

*A. Isolation of the associated mycoflora*

Isolation of associated mycoflora with spoiled fruits was done after their surface sterilization with 90 per cent alcohol. The isolated fungi were transferred to Czapek-Dox Agar (CDA) medium. Some isolation were made by transferring the hyphae directly from the aerial mycelium present on the surface of the infested fruits.

Petri plates were incubated at the temperature of 24 ± 2°C. During the incubation period, Petri plates were examined daily from third day of the incubation for the fungi. All the fungi, thus isolated were purified by single spore technique. The pure cultures were maintained on CDA slants at 10°C. The cultures were identified with the help of available literature ( Raper and Thom, 1949; Raper and Fennel, 1965; Gilman, 1967; Booth, 1971; Ellis, 1971, 1976; Subramanian, 1971; Domsch and Gams,1972; George Barron, 1972).

*B. Test for Pathogenicity*

Pathogenicity tests were conducted to confirm the pathogenic nature of the isolated fungi on their respective hosts. Fresh and sound fruits of *Carica papaya* L. were surface sterilized with 90 per cent alcohol to remove the superficial mycoflora as well as to maintain the natural nature of the skin of fruits. An injury of 10 mm depth was made over the surface of the fruits with the help of sterilized cork borer of 5 mm diameter. A bit of tissue was taken out and three day old inoculum was placed in the pit. The piece of the fruit tissue taken out was inserted back to its position and the wound was then sealed with the sterilized cotton.

The inoculated fruits were placed in sterilized glass jars at the temperature of 24±2°C. The pathogenicity of the organism was considered established only when Koch's postulate were fully satisfied. For both the fruits five replicates were maintained.

Data were also recorded for the dynamics of rotting using following formula of Bottcher (1986).

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

Where,

x = duration of storage in days

Y= rot

$\beta_1$ = Linear rise

z = a period without macroscopic symptoms

**Weight loss:** To observe change in the weight of the fruits due to the infection caused by the pathogenic fungi, fresh and healthy fruits were surface sterilized and inoculated separately with respective pathogenic species as described above. Similar control sets were maintained in which the pathogenic fungi were not inoculated.

Weight loss was noted after incubating the controlled and inoculated sets for a week at 24±2°C. Loss in weight was determined by following formula :

Weight loss = W – w / W × 100

Where,

W = weight of the infested fruit before incubation

w = weight of the infested fruit incubation

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

The essential oil was isolated by hydro distillation through Clevengers apparatus. 500 g seed of *Pimpinella anisum* were thoroughly washed with sterilized water.

The seeds were then placed in the round-bottom flask of the Clavengers apparatus. The ratio between the plant material and water in the flask was maintained as 1:3. Water was heated to produce steam that carried the most volatile fractions of the aromatic material with it. The steam was then chilled (in a condenser) and the resulting distillate was collected. The essential oil was found to float on the top of the hydrosol (the distilled water component) and was separated off. The extracted oils were dehydrated by the addition of anhydrous sodium sulphate, followed by thorough shaking and standing for 6–8 h and filtration.

*D. Fungitoxicity of the oil against Corticium rolfsii*

Fungitoxic activity of the oil was tested by the poisoned food technique of Grover and Moore (1962) using Czapek-Dox Agar (CDA) medium against the test fungus *Corticium rolfsii* at 2000 ppm. The concentration of the essential oil was prepared by dissolving requisite amount of oil in 0.5 ml of acetone and mixing it with 9.5 ml of Czapek-Dox Agar (CDA) medium. In the control sets were prepared similarly using equal amounts of sterilized distilled water in place of the oil. The prepared plates were inoculated aseptically with assay discs of the test fungus and incubated for 6 days. The observations were recorded on the seventh day and the percentage mycelial inhibition was calculated by the following formula:

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

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

*E. Physico- Chemical properties of the essential oil*

The oils were standardized through GLC and physicochemical properties viz. acid value, phenolic content, optical rotation, refractive index, specific gravity and solubility in organic solvent were estimated following Langenau (1948).

*F. 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, experiments were carried out by the above mentioned poisoned food technique of Grover and Moore (1962) using graded concentration of essential oil below 2000 ppm. The nature of the toxicity (fungistatic/fungicidal) of the oil against the test fungus was determined following Garber and Houston (1959). The inhibited fungal discs of the oil treated sets were reinoculated into fresh medium and revival of their growth was observed.

*G. In vivo applicability of the oil of Pimpinella anisum*

Fresh, healthy and nearly same age fruits of *Carica papaya* L. 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 help of 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 fungi 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 at 24 ± 2°C temperature for 12 days. The development of rot was measured after four, eight and twelve days following the method of Thind *et al.* (1976). The formula used was -

$$\text{Percent rot} = \frac{W - w}{W} \times 100$$

Where,

W = The weight of fruits before inoculation

w = The weight of the fruit after removal of rotted portions

## III. RESULTS AND DISCUSSION

A survey of local markets as well as of the markets of nearby areas was periodically conducted for two years. During this period a number of rot causing fungi were collected from the fruits of *Carica papaya.* Symptoms of the diseases encountered and the morphological characters of the fungi isolated were taken into consideration for their identification. The results are given in Table 1.

Out of 19 fungi isolated from fruits of *Carica papaya* (Table 1). Among them 11 species, viz., *Aspergillus fumigatus*, *A. funiculosus, A. nidulans*, *A. ochraceous*, *A. tamarii*, *A. terreus*, *Cladosporium herbarum*, *Corticium rolfsii*, *F. semitectum*, *Penicillium chrysogenum* and *P. oxalicum*, are new record from this fruit.

The dynamics of rotting was highest (250) by *Corticium rolfsii* followed by *Fusarium moniliforme* (240) and it was least (37) by *Cladosporium herbarum.*

**Table 1: Showing Name of Fungi Isolated from Fruits of *Carica Papaya* their Dynamics of Rotting, Pathogenicity and Effect on Weight.**

| Name of Fungi isolated | Parameters | | |
|---|---|---|---|
| | **Dynamics of Rotting** | **Pathogenicity** | **Weightloss** |
| *Alternaria alternata* Keissler | 38.0 | + | 2.29 |
| *Aspergillus flavus* Link | 235.0 | + | 3.25 |
| *A. fumigatus* Fresenius | 147.0 | + | 1.91 |
| *A. funiculosus* | 147.0 | - | 0.0 |
| *A. nidulans* (Eidam) Winter | 48.0 | + | 1.34 |
| *A. niger* Van Tieghem | 196.0 | + | 4.02 |
| *A. ochraceous* Wilhelm | 192.0 | + | 2.68 |
| *A. tamarii* Kita | 147.0 | + | 2.29 |
| *A. terreus* Thom | 173.0 | + | 3.63 |
| *Cladosporium herbarum*(Persoon)Link | 37.0 | - | 0.0 |
| *Corticium rolfsii* Sacc. | 250.0 | + | 3.44 |
| *Curvularia lunata*(Walker) Boedijin | 133.0 | + | 1.91 |
| *Fusarium moniliforme* Sheldom | 240.0 | + | 1.34 |
| *F. oxysporum* Schlecht | 196.0 | + | 2.29 |
| *F. semitectum* Berk. And Rav. | 201.0 | + | 1.72 |
| *F. solani* App. Et. Wr. | 206.0 | - | 0.0 |
| *Penicillium chrysogenum* Thom | 144.0 | + | 2.87 |
| *P. citrinum* Thom | 64.0 | + | 3.82 |
| *P. oxalicum* Currie and Thom | 90.0 | - | 0.0 |

+ = Present - = absent

Out of 20 species 15 were pathogenic because they confirmed Koch's postulate and 05 were non-pathogenic because they did not confirm Koch's postulate. Weight loss was noted to be 2.29, 3.25, 1.91, 1.34, 4.02, 2.68, 2.29, 3.63, 3.44, 1.91, 1.34, 2.29, 1.72, 2.87 and 3.82 per cent when inoculated by *Alternaria alternate*, *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. ochraceous*, *A. tamari*, *A. terreus*, *Corticium rolfsii*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporium*, *F. semitectum*, *Penicillium chrysogenum and P. citrinum.*

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 found to be soluble in all the tested organic solvents. The acid value, optical rotation, refractive index and specific gravity of oil was found to be14.40, $+60^{\circ}$ , 1.557 and 0.945 respectively. The phenolic content was present in the oil. The GLC of oil indicated it to be a mixture of 4 major and 6 minor components (Fig. 1).

The Minimum inhibitory concentration (MIC) of the oil of *Pimpinella anisum* at which it checked the mycelial growth of the test fungus was 2000 ppm. It may be noticed from Table 2 that the oil of *Pimpinella anisum* was fungicidal for the test fungus because the re-inoculated discs did not show growth of test fungus at the MIC.

The result given in Table 2 show that there was no rotting of the fruits of *Corticium rolfsii* 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.

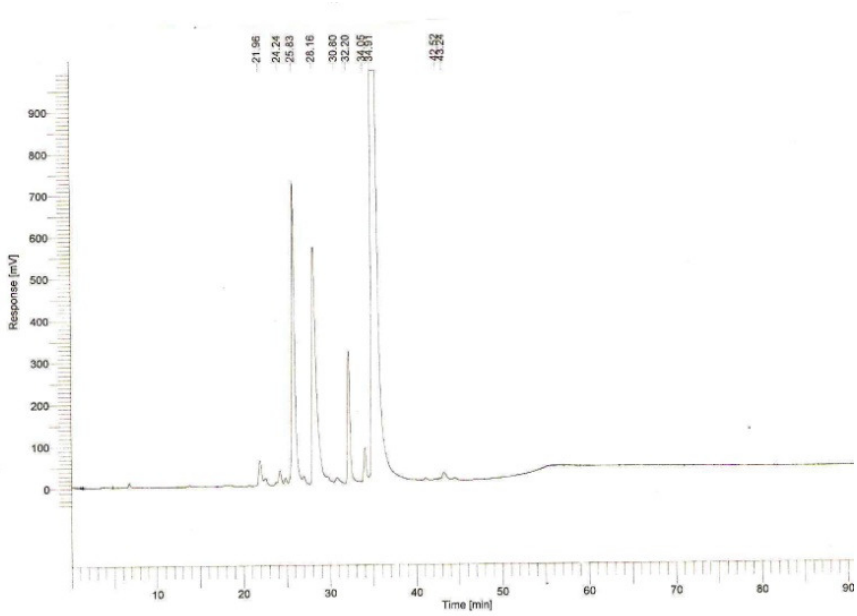


**Fig. 1.** Showing G.L.C. of essential oil of *Pimpinella anisum* L.

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

| Concentrations(ppm) | Percent inhibition of mycelial growth of *Corticium rolfsii* |
|---|---|
| 5000 | 100 |
| 4000 | 100 |
| 3000 | 100 |
| **2000** | **100** |
| 1000 | 80 |
| 500 | 66 |
| 100 | 49 |

**Table 3: Showing Nature of toxicity of *Pimpinella anisum* Linn. Oil against *Corticium rolfsii.***

| Concentrations (ppm) | Percent inhibition mycelia growth | |
|---|---|---|
| | Treatment set | Reinoculated set |
| 500 | 66 | 66 |
| 1000 | 80 | 80 |
| 2000 | 100 | 100 |
| 3000 | 100 | 100 |

**Table 4: Effect of *Pimpinella anisum* oil applied as pre- and post-inoculation treatments on the percentage of rotting of the fruits of *Carica papaya* L.**

| Essential Oil | Test fungus incubation period (days) | Percent rotting | | |
|---|---|---|---|---|
| | | 4 | 8 | 12 |
| *Pimpinella anisum* | Pre-inoculation | 0 | 0 | 0 |
| | Post-inoculation | 0 | 0 | 0 |
| Control | | 10 | 25 | 50 |

In present study experiment was designed to find out the possibilities of utilizing volatile constituents of the higher plants to preserve the qualities of fruits deterioration during storage. The essential oils are thought to play a role in the plant defence mechanism against phytopathogenic microorganisms (Mihaliak *et al*. 1991). Most of the essential oils have been reported to inhibit post-harvest fungi in in-vitro conditions (Bishop and Reagon 1998; Singh and Tripathi1999; Bellerbeck *et al*. 2001; Hidalgo *et al*. 2002). In vitro antifungal activity of the essential oils from *Monarda citridora* and *Melaleuca alternifolia* was evaluated against various post-harvest pathogens. Both the oils exhibited a high level of antifungal activity (Bishop and Thornton 1997). Recent findings on the success of essential oils as biodegradable and ecofriendly fungitoxicants have shown the possibilities for their exploitation as natural fungicides (Dixit *et al.* 1995; Tripathi *et al.* 2004). In the present investigation the essential oil of *Pimpinella anisum* was selected for further study due to its fungitoxic nature at their lower MIC and was subsequently standardized through physicochemical properties, fungitoxic properties and practical applicability in controlling the fungus *Corticium rolfsii* causing papaya fruit rot. This fungus caused highest dynamics of rotting, highest loss of weight of papaya fruits during storage. Such investigations are essential with most of the fungitoxic plant products and are also required to recommend them to agrochemical firms for their formulation. The quality of essential oils depend on a number of physical parameters such as acid value, phenolic contents, optical rotation, refractive index, specific gravity and solubility in different organic solvents. A number of papers on the biological activity of essential oils have been published. Their data however show much variation between the same essences. The reason for this variability can be understood if we take in to account all the factors influencing the chemical composition of the oils such as climatic, seasonal and geographical conditions, harvest period and distillation techniques (Panizzi *et al.* 1993). The GLC of the essential oil of *Pimpinella anisum* showed it to be a mixture of 4 major and 6 minor constituents. Thus the activity of the oils seems likely to be due to the synergistic effect of major and minor components of the oils. The oils have a fungicidal action at lower MIC, which is a positive indication that they would not have any negative effect on host tissues. Generally fungi toxicants of plant origin have been found to be non-injurious to the treated food commodities and in some cases they have shown enhancement in the shelf life of the commodities. The essential oils of *Pimpinella anisum* has shown significant fungitoxic activity and enhanced the shelf life of papaya fruits during storage by protecting them from *Corticium rolfsii*. The fruits were fumigated by the essential oils at their respective MIC. The fumigated fruits with the oils of *Pimpinella anisum* of treated sets showed enhancement of shelf life up to 4 ,6 and 12 days, respectively (Dixit *et al*, 1983; Dubey *et al*, 1983; Asthana 1984; Chandra *et al*. 1982; Arora and Pandey,1977). . The oils did not showed any adverse symptom on the fruit peel. Therefore, the use of essential oils as antimicrobial agents can be an interesting field of investigation as the toxicity to mammals is mostly quite low, and their degree of volatility allows their use for fumigation in cold storage or for active packing. The essential oil of *Pimpinella anisum* with strong fungitoxicity, low MIC, thermostable nature, long shelf life, fungicidal nature against the test fungus as well as against other common fruit-rotting fungi have all the desired characters of an ideal fungicide and could be recommended as botanical fungitoxicant. However, the potential use of essential oils to control post harvest diseases requires a detailed examination of their biological activity and dispersion in fruit tissues and the development of a formulation which inhibits the growth of pathogens at non-phytotoxic concentrations.

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