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# Screening of some plant extract against *Lasiodiplodia theobromae* isolated from Musambi (*Citrus sinensis*)

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## ABSTRACT

The fruits of *Citrus sinensis* L. contain a wide range of active ingredients like vitamin C, flavonoids, acids and volatile oils. They have bergapten which sensitizes the skin to sunlight. Such valuable fruits of *Citrus sinensis* L. were collected from different fruit markets of Gorakhpur district of Uttar Pradesh. 21 fungi were isolated from fruits of *Citrus sinensis*. Isolated fungi were also tested for their pathogenic nature and weight loss in fruits. Out of 21 isolated fungi, 12 were confirmed Koch's postulate. *Aspergillus flavus*, *A. niger* and *Lasiodiplodia theobromae* caused highest loss of weight. Extract of different plant parts of different angiospermic families were tested for their antifungal activity against *Lasiodiplodia theobromae* by inverted plate method. *Apium graveolens*, *Erythrina indica*, *Foeniculum vulgare*, *Pimpenella anisum*, *Pouzolgia indica* and *Spillanthes acmella* were found to be 100% effective against test fungus. The Minimum Inhibitory Concentration (MIC) of extract was 5000 ppm.

**Keywords:** Fruits, Koch's postulate, Inverted plate method, antifungal activity, plant extracts, MIC.

## INTRODUCTION

Fruits are important part of human diet. They're commercially important and nutritionally essential food product [1]. Fruits have high water content, soft texture, sweet, sour and semi astringent flavours. Because of their exotic flavor and taste, considerable attention is paid in numerous parts of the globe.

The fruits are important for human being because they have organoleptic and chemical property. They play a vital role in human nutrition, by supplying the necessary growth factors essential for maintain normal health *Citrus sinensis* also known as sweet orange is the most popular of the citrus fruits. It is widely cultivated in most of the regions of the globe [2]. Sweet orange form a rich source of vitamin C, flavonoids, phenolic compounds and pectins. Hesperidine, narirutin, naringin and eriocitrin are main flavonoids found in citrus species [3]. Just one sweet orange provides 116% of the daily requirement for vitamin C. An upscale source of water-soluble vitamin, prevents atom generation within the body and damage to the tissues within the aqueous environment both inside and outdoors cells [4].

The improper handling, packaging, storage and transportation may change the physiological state of the fruits and vegetables which result in decay and growth of microorganisms [5]. Because of low pH, rich nutrients and high moisture content, fruits are very susceptible to attack by pathogenic fungi, which in addition to causing rot and by producing mycotoxins make the fruits unfit for their consumption [6]. The principle of spread of fungal infection in fruits supports that a single infected sweet orange fruits can be the source of infection to other sweet Orange fruits during storage and on transit [7]. Soil -infesting fungi and bacteria typically infect plants at the time of or just before harvesting. Mostly infection may occur during post-harvest handling and storage.

The aim of this study was to isolate and identify fungal pathogens associated with sweet orange spoilage and to discover some products as preservatives of this fruit against fungal deterioration.

## MATERIAL AND METHODS

Fruit samples of *Citrus sinensis* were collected time to time from fruit markets and brought to lab in sterile polythene bags. Symptoms of the disease caused by isolated fungi were observed.

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**Isolation of fungi**

Before isolation the spoiled fruits of *Citrus sinensis* were surface sterilized and the isolated fungi were placed to culture medium (PDA).

Inoculated culture plates were incubated at 24 ± 2 °C. Petri plates were examined daily for the fungi. All the isolated fungi were purified and cultures were identified with the help of literatures [8-11].

**Test for Pathogenicity**

To confirm the pathogenic nature of the isolated fungi pathogenicity tests were conducted. Injury made over the surface of *Citrus sinensis* fruits by the method of Granger & Horne, 1924. Five replicates of fruits were maintained [12].

The pathogenic nature of the isolated fungi were recognized when they confirm Koch's postulate.

**Loss in Weight**

To observe weight loss in infected fruits by pathogenic fungi, surface sterilized fresh fruits were inoculated with particular pathogenic species. In Control sets fruits were not inoculated with the pathogenic fungi.

After incubation for a week at 24±2ºC weight loss was calculated by the following formula:

$$\text{Weight loss} = \frac{W - w}{W \times 100}$$

Where,

W = weight of the fruit before incubation
w = weight of the fruit after incubation

**Plant parts extraction and their fungitoxicity against test fungus**

Plants part of different families of angiosperms were collected from district of Gorakhpur and identified with the help of many literatures [13-17].

Freshly collected leaves and seeds of plants were sterilized with 0.1 per cent mercuric chloride. The 100 g of leaves and seeds were macerated with 100 ml of sterile water (1:1 w/v) in mortar and pestle. The pulp was filtered through a double layered muslin cloth and 2.0 mg streptopenicillin was added to it to prevent bacterial activity. The extract was then tested for its e antifungal activity by inverted plate method [18]. Control set was prepared, by using distilled and sterile water in place of filtrate. After incubation of six days at 24 ± 2 °C, diameter of colony of both the sets was measured. The above experiment contained five replicates and repeated thrice. Per cent mycelial inhibition was calculated by following formula [19].

$$\% \text{ inhibition of mycelia growth} = \frac{dc - dt}{dt \times 100}$$

Where,

dc = Average diameter of fungal colony in control sets and
dt = Average diameter of fungal colony in treatment sets.

**Minimum Inhibitory Concentration (MIC)**

By using inverted Petri plate method of Bocher (1938) minimum inhibitory concentration was observed. For MIC 1000-10,000 ppm of *Apium graveolens* L., *Erythrina indica* L., *Foeniculum vulgare* L. *Pimpinella anisum* L., *Pouzolgia indica* Ls. and *Spillanthus acmella* Murr. extract placed in the lower lid of Petri plates. The Petri plates were incubated for six days. On seventh day, colony diameter of treatment as well as of the control sets was measured [19].

## RESULTS AND DISCUSSION

After survey of local fruit markets of Gorakhpur, a number of rots causing fungi were collected from *Citrus sinenesis* fruits. Identification were made on the basis of morphology and symptom of the disease produced by isolated fungi (Table 1).

**Table 1:** Showing fungi isolated from fruits of *Citrus sinensis* their pathogenicity and effect on weight of fruits.

| Name of Fungi isolated | Parameters | |
|---|---|---|
| | Pathogenicity | Weightloss |
| *Alternaria alternata* Keissler | + | 2.29 |
| *Alternaria citrii* | - | 0.0 |
| *Aspergillus flavus* Link | + | 3.75 |
| *A. fumigatus* Fresenius | + | 1.87 |
| *A. niger* Van Tieghem | + | 4.06 |
| *A.ochraceous* Wilhelm | + | 2.50 |
| *A. sydowi* Bainier and Sartory Thom and Church | - | 0.0 |
| *A. terreus* Thom | + | 2.81 |
| *A.ustus* BainierThom and Church | - | 0.0 |
| *Cladosporium herbarum* (Persoon)Link | - | 0.0 |
| *Curvularia lunata* (Walker) Boedijin | + | 1.87 |
| *Fusarium moniliforme* Sheldom | + | 2.50 |
| *Fusarium* sp U.I. | - | 0.0 |
| *Lasiodiplodia theobromae* Pat. | + | 3.75 |
| *Penicillium chrysogenum* Thom | - | 0.0 |
| *P. citrinum* Thom | + | 2.50 |
| *P. oxalicum* Currie and Thom | + | 2.18 |
| *Rhizopus stolonifer* (Ehrenb ex. Fr.) Lind | + | 2.81 |
| Sterile mycelium (Black) | - | 0.0 |
| Sterile mycelium (Greenish black) | - | 0.0 |
| Sterile mycelium (White) | - | 0.0 |

+ = Present - = absent

During survey 21 fungi were isolated from the fruits of *Citrus sinenesis* (Table 1). Out of which 18 species i.e. *Alternaria citrii*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceous*, *A. sydowi*, *A. terreus*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium sp. U.I.*, *Penicillium chrysogenum*, *P. citrinum*, *P. oxalicum*, Rhizopus stolonifer, Sterile mycelium black, Sterile mycelium greenish black and sterile mycelium white are first time reported from this fruit. Out of 21 fungi 12 were pathogenic and 09 were non-pathogenic because they did not confirm Koch's postulate. Weight loss was noted to be 1.87, 1.87, 2.18, 2.50, 2.50, 2.50, 2.81, 2.81, 3.12, 3.75, 3.75 and 4.06 per cent when inoculated by *Aspergillus fumigatus*, *Curvularia lunata*, *Penicillium oxalicum*, *Aspergillus ochraceous*, *Fusarium moniliforme*, *Penicillum. citrinum*, *Aspergillus terreus*, *Rhizopus stolonifer*, *Alternaria alternata*, *Aspergillus flavus*, *Lasiodiplodia theobromae* and *Aspergillus niger*.

The result of screening of various plant extracts are given in the Table 2. It is evident from the Table that aqueous extracts of different parts of 30 plant species belonging to 20 angiospermic families were screened against test fungus. Most of the species showed moderate (above 50%) activity. *Apium graveolens* L., *Erythrina indica* L., *Foeniculum vulgare* L. *Pimpinella anisum* L., *Pouzolgia indica* L. and *Spillanthus acmella* Murr. were 100% effective against test fungus.

**Table 2:** Showing screening of extracts of some plants for their activity against mycelial growth of test fungus.

| ***Plant*** | **Family** | ***Percent inhibition of mycelial growth*** |
|---|---|---|
| *Achyranthus aspera* L. | Amranthaceae | 94 |
| *Adenocalyma alliceae Mart ex Meisser* | *Bignoneaceae* | 98 |
| *Apium graveolens* L. | *Apiaceae* | 100 |
| *Azadirachta indica A. Juss* | *Meliaceae* | 55 |
| *Calotropis procera* (L.) *R. Br.* | *Asclepiadaceae* | 94 |
| *Croton bonplandianum Baill* | *Euphorbiaceae* | 78 |
| *Embelica ribes Burm. f* | *Myrsineae* | 77 |
| *Erythrina indica* L. | *Fabaceae* | 100 |
| *Foeniculum vulgare* Mill. | *Apiaceae* | 100 |
| *Hamelia patens Jacq.* | *Rubiaceae* | 0 |
| *Hibiscus rosa sinensis* L. | *Malvaceae* | 0 |
| *Hyptis suaveolens* (L.) *Poit.* | *Lamiaceae* | 4 |
| *Leucas aspera Spreng* | *Lamiaceae* | 97 |
| *Murraya exotica* L. | *Rutaceae* | 76 |
| *Murrya koenigii* (L.) *Sprang* | *Rutaceae* | 77 |
| *Myristica fragrans Houtt.* | *Myristicaceae* | 67 |
| *Nerium indicum* Mill. | *Apocynaceae* | 58 |
| *Papaver sominiferum* L. | *Papaveraceae* | 55 |
| *Parthenium hesterophorus* L. | *Asteraceae* | 79 |
| *Peristrophy bicalyculata Nees.* | *Acanthaceae* | 92 |
| *Phyllanthus niruri* L. | *Euphorbiaceae* | 66 |
| *Pimpinella anisum* L. | *Apiaceae* | 100 |
| *Pouzolgia indica* L. | *Urticaceae* | 100 |
| *Ranunculus scleratus* L. | *Ranunculaceae* | 3 |
| *Ricinus communis* L. | *Euphorbiaceae* | 0 |
| *Ruellia tuberose* L. | *Acanthaceae* | 60 |
| *Spillanthes acmella* (L.) *Murr.* | *Asteraceae* | 100 |
| *Triumpheta rhomboids Jacq.* | *Tiliaceae* | 64 |
| *Vinca rosea* L. | *Apocynaceae* | 64 |
| *Zingiber officinale* (Rose.) | *Zingiberaceae* | 54 |

**Table 3:** Showing minimum inhibitory concentration of the extracts of *Apium graveolens*, *Erythrina indica*, *Foeniculum vulgare*, *Pimpinella anisum*, *Pouzolgia indica* and *Spillanthus acmella* against test fungi, *Lasiodiplodia theobromae*.

| **Extract** | **Concentration (ppm)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **1. *Apium graveolens*** | **10000** | **9000** | **8000** | **7000** | **6000** | **5000** | **4000** | **3000** | **2000** | **1000** |
| Percent mycelial inhibition | 100 | 100 | 100 | 100 | 100 | 100 | 93 | 90 | 82 | 66 |
| 2. ***Erythrina indica*** | | | | | | | | | | |
| Percent mycelial inhibition | 100 | 100 | 100 | 100 | 100 | 100 | 88 | 81 | 73 | 70 |
| **3. *Foeniculum vulgare*** | | | | | | | | | | |
| Percent mycelial inhibition | 100 | 100 | 100 | 100 | 100 | 100 | 96 | 90 | 87 | 81 |
| **4. *Pimpinella anisum*** | | | | | | | | | | |
| Percent mycelial inhibition | 100 | 100 | 100 | 100 | 100 | 100 | 98 | 92 | 84 | 79 |
| **5. *Pouzolgia indica*** | | | | | | | | | | |
| Percent mycelial inhibition | 100 | 100 | 100 | 100 | 100 | 100 | 91 | 86 | 80 | 74 |
| **6. *Spillanthus acmella*** | | | | | | | | | | |
| Percent mycelial inhibition | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 90 | 75 | 69 |

On the basis of results, it is suggested that the extract of *Apium graveolens* L., *Erythrina indica* L., *Foeniculum vulgare* L. *Pimpinella anisum* L., *Pouzolgia indica* L. and *Spillanthus acmella* Murr. after in vivo studies may be used as effective preservative of fruits during storage.

## CONCLUSION

Mostly vascular plants have secondary metabolites which play an active role in plant disease resistance [20-22]. Secondary metabolites are compounds that are not necessary for a cell or organism to live, but they play an important role in the interaction of the cell or organism with its environment [23-25]. These compounds are often effective in plant protection against biotic or abiotic stresses. Some from these metabolites purified, characterized and used to control different types of plant diseases [25-27].

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