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Original Article

Efficacy of plant extracts and fungicides against fungi associated with lemon in storage

Shamim Shamsi[1], Trisha Saha[2] and Najmun Naher[3]

[1, 2]Department of Botany, University of Dhaka and [3]Earth Science Group, National University, Gazipur-1704

ABSTRACT: A total of six species of fungi were isolated from *Citrus lemon*. The isolated fungi were *Aspergillus niger*, *Candida krusei, Fusarium* sp., *Rhizopus stolonifer*, *Penicillium digitatum* and *Trichoderma* sp. during the period of April 2013 to December 2013. Ethanol leaf extracts of five plants viz. *Azadirachta indica* L., *Citrus limon* L., *Mangifera indica* L., *Polyalthia longifolia* L. and *Tagetes erecta* L. were evaluated against the pathogenic fungi at 5, 10 and 20% concentrations. *In vitro* treatment showed that *A. indica* completely inhibited growth of the fungi at all the concentrations used. Similarly efficacy of four fungicides namely Bavistin DF, Green gel, Ridomil MZ Gold and Tall 25 EC were evaluated against the isolated fungi at 100 ppm, 200 ppm and 400 ppm concentrations. Tall 25 EC inhibited growth of the test fungi at all the concentrations used.

KEYWORDS: Efficacy, Plant extracts, Fungicides, fungi, lemon, Storage

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CORRESPONDENCE: Shamim Shamsi, E-mail: prof.shamsi@gmail.com

INTRODUCTION

Botanically, the citrus fruit belongs to the family of *Rutaceae*, in the genus, *Citrus*. The lemon (*Citrus limon* L) is a species of small evergreen tree native to Asia. The tree's ellipsoidal yellow fruit is used for culinary and non-culinary purposes throughout the world, primarily for its juice, which has both culinary and cleaning uses[1]. The pulp and rind (zest) are also used in cooking and baking[2]. The juice of the lemon is about 5% to 6% citric acid, which gives a sour taste. The distinctive sour taste of lemon juice makes it a key ingredient in drinks and foods such as lemonade and lemon meringue pie. It has been known that fruits constitute commercially and nutritionally important indispensable food commodity. Lemon is also used for the common cold and flu, H1N1 (swine) flu, ringing in the ears (tinnitus), Meniere's disease, and kidney stones. It is also used to aid digestion, reduce pain and swelling (inflammation), improve the function of blood vessels, and increase urination to reduce fluid retention[3].

Lemon plays a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health. The fruit, juice, and peel are used to make medicine. Lemon is used to treat scurvy, a condition caused by not having enough vitamin C[4].

Short shelf-life period caused by pathogen attack is one of the limiting factors that influence the fruits economic value. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal infection[5]. Considerable research has been done on ethnobotanical and medicinal properties of the plant. But a few information is available regarding the fungal diseases and its management of the fruits and plant in home and abroad[6]. In Libya El-Gali and Ibrahim (2014) worked on lemon and found *Alternaria alternata* (Fr.) Keissler pathogenic[7]. So far, there is no report available regarding the control of fungal diseases of this lemon fruits[8]. Present work is an attempt to screen the efficacy

of common fungicides and botanicals against the fungi associated with lemon fruits *in vitro*.

METHODS AND MATERIALS

Fresh lemon fruit samples were collected from different markets of Dhaka city and stored in a refrigerator at 4°C. After 5 to 7 days of storage the fruits showed symptoms of fungal infestation. The fungi associated with the infected fruits were isolated following tissue plating method on potato dextrose agar (PDA) medium[8]. The pH of the medium was adjusted to 6.0. Infected tissues of the fruits were placed on PDA in Petri plates and incubated at 25-28°C. Fungi grew from the tissues were transferred to fresh PDA plates. Pure culture of the isolated fungi were prepared and preserved in a refrigerator at 4°C for further use. The fungal isolates were identified based on morphological characteristics recorded under a compound light microscope using standard literature [9-13].

Pathogenicity of the isolates was determined following detached fruit inoculation technique[14].

Healthy and matured citrus fruits were collected, surface sterilized with 1.0% chlorox (Sodium hypochlorite is a chemical compound with the formula NaOCl) and rinsed with sterilized distilled water. Two holes were made on the sterilized surface of fruits with a sharp cork borer (5mm diameter) at a depth of 4 mm.

To prepare inocula, 5 mm mycelium blocks were cut from the young culture of the isolated fungi with a 5 mm cork borer. For inoculation, the blocks of each fungus were placed inside the hole with a sterilized scalpel at one block per hole. In the control sets, 5 mm fresh PDA blocks were placed inside the holes of lemon. Three replications were maintained for each treatment as well as control. Ten fruits were used per replications. Inoculated fruits were incubated at 25°C. Inoculated fruits were observed regularly. Characteristic symptoms of fruit rot appeared on inoculated fruits after 5-7 days of incubation. The fungi were isolated from artificially inoculated fruits and compared with naturally infected fruits[15].

The common and locally available plants with antifungal activity viz., *Azadirachta indica*, *Citrus limon*, *Mangifera indica*, *Polyalthia longifolia* and *Tagetes erecta* were selected for the experiment. The 100 gm of leaves per plant material was washed in distilled water. Then the leaves were chopped into small pieces. Stock solution of an extract of each plant was individually prepared using sterilized water and chopped leaf material (1:1 w/v) in a clean blender. The mass of a plant part was squeezed through three folds of fine cloth. The supernatants were filtered through Whatman filter paper No.1 and the filtrate was collected in 250 ml Erlenmeyer flasks. Ethanol leaf extracts at 5%, 10% and 20% concentrations was prepared from stock solution and evaluated against test fungi following poison food techniques[16].

Fifteen ml of autoclaved PDA medium supplemented with 5, 10 and 20% ethanol plant was separately poured into Petri plates from test tubes, allowed to cool and solidify. The Petri dishes containing medium devoid of the extract but with same amount of distilled water served as control. After complete solidification of the medium, 5 mm disc of seven day old culture of each test pathogen was inoculated in the centre of the Petri dishes with solidified PDA medium. The plates were incubated at 25±2°C for seven days. After incubation the colony diameter was measured in mm. For each treatment three replications were maintained. The efficacy of the plant extract in terms of percent inhibition of mycelial growth (I) was calculated using the formula: Percent inhibition = C - T / C × 100, where C = Average increase in mycelial growth in control plate and T = Average increase in mycelial growth in treatment plate.

Four fungicides namely, Bavistin 50 WP (500 ppm carbendazim), Green gel (64% Mancozeb + 8% Metalexil), Ridomil MZ Gold (Mancozeb 64 %) and Tall 25 EC (propiconazole 25% EC (250 g/L)with different ingredients were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. For each fungicide, a stock solution having the concentration of 10,000 ppm was prepared. The calculated amount of the stock solution of a fungicide was supplemented with sterilized PDA medium to get the concentrations of 100 ppm, 200 ppm and 400 ppm. The concentrations of fungicides are expressed in term of its active ingredients. Twenty ml of the supplemented medium of a particular concentration was poured in a sterilized Petri plate and allowed to solidify. In the control set, requisite amount of sterilized water instead of fungicides solution was added to the PDA medium. Five mm mycelia agar disc cut from the margin of actively growing culture of test fungi for each treatment and was inoculated at the center of the plate. Three replications were maintained in each case. The inoculated plates were incubated at 25±2°C. The radial growth of colonies was measured at the 5th day of inoculation.The fungitoxicity of the selected fungicides in terms of percentage inhibition of mycelial growth (I) was calculated using the formula: Percent inhibition = (C - T / C × 100, where C = Average increase.

RESULT AND DISCUSSION

A total of six species of fungi wee isolated from *Citrus lemon*. The isolated fungi were *Aspergillus niger*, *Candida krusei*, *Fusarium* sp., *Rhizopus stolonifer*, *Penicillium digitatum* and *Trichoderma* sp. All the isolated fungi were found to be pathhogenic to lemon fruit. Table 1. Shows the frequency of association of *P. digitatum* and *Trichoderma* sp. with lemon during storage were highest (100 %) followed by *C. krusei* (66.6%), *Fusarium* sp. (53.3%) and *A. niger* (46.66%). Wheareas *Rhizopus stolonifer* showed lowest frequency of association (13.33%). Ethanol extract of five angiospermic plant at 5,10 and 20% concentrations were used in this experiment. Among the selected plants *A.*

indica showed complete inhibition of radial growth of all the fungi at all concentrations used. *Citrus limon* completely inhibited the radial growth of *Fusarium* sp., *P. digitatum* and *R. stolonifer* at 20% concentration. *Mangifera indica* completely inhibited the growth of *A. niger*, *Fusarium* sp. and *Trichoderma* sp. at the same concentration. *Polyalthia longifolia* completely inhibited the growth of six fungi at 20% concentration. *Tagetes erecta* exclusively inhibited the radial growth of *P. digitatum* at the same concentration.

Table 1: Frequency of association of fungi with lemon in storage.

Name of fungi	frequency of association (%)
Aspergillus niger	46.66
Candida krusei	66.6
Fusarium sp.	53.3
Penicillium digitatum	100
Rhizopus stolonifer	13.33
Trichoderma sp.	100

Polyalthia longifolia also capable of complete inhibition of radial growth of *A. niger*, *C. krusei* and *Trichoderma* sp. at 10% concentration. *Citrus lemon* showed maximum 89.94% inhibition of *Fusarium* sp. at the same concentration. *Mangifera indica* exhibited 81.05% growth inhibition of *A. niger*. *Tagetes erecta* inhibited 69.23% radial growth of *P. digitatum* at 10% concentration. *Citrus lemon* showed maximum 76% radial growth inhibition of *Fusarium* sp. at 5% (Figs.1-5).

Tall 25 EC completely inhibited the radial growth of the test fungi at all the concentration used. Bavistin completely inhibited the radial growth of *C. krusei*, *Fusarium* sp., *P. digitatum* and *R. stolonifer* at 400 ppm concentration. Ridomil completely inhibited radial growth of *C. krusei*, *Fusarium* sp. and *R. stolonifer* at the same concentration. Green gel completely inhibited radial growth of *C. krusei* and *P. digitatum* at 400 ppm concentration.

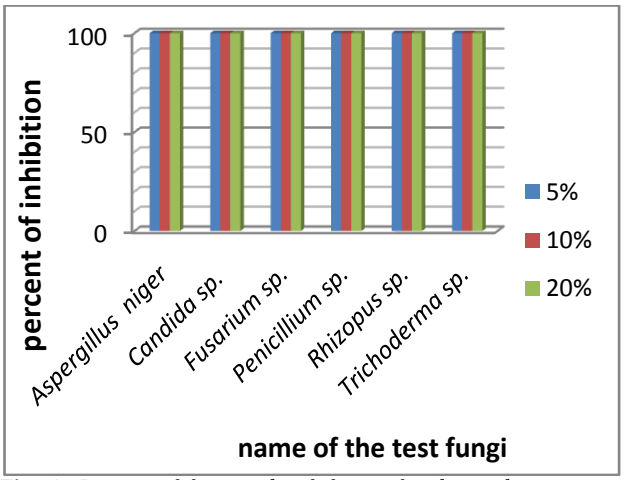

Fig. 1. Percent inhibition of radial growth of test fungi against *Azadirachta indica* at different concentrations.

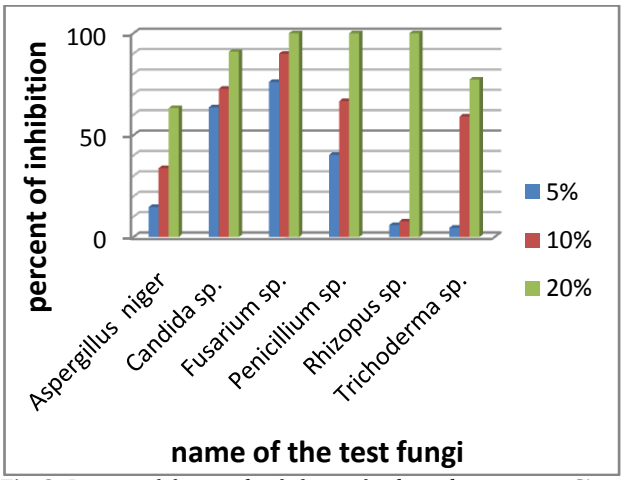

Fig. 2. Percent inhibition of radial growth of test fungi against *Citrus limon* at different concentrations.

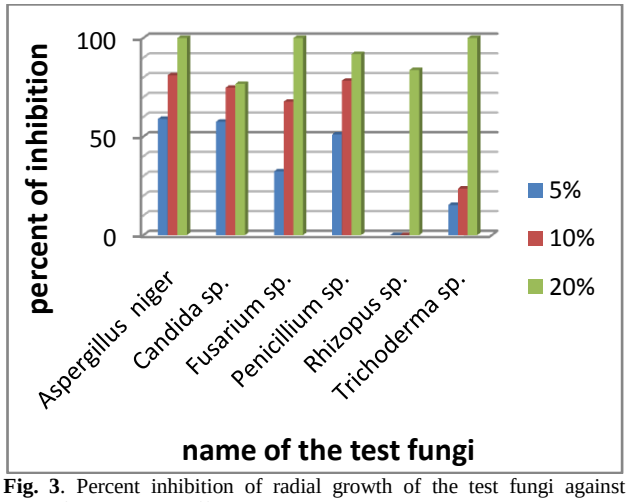

Fig. 3. Percent inhibition of radial growth of the test fungi against *Mangifera indica* at different concentrations.

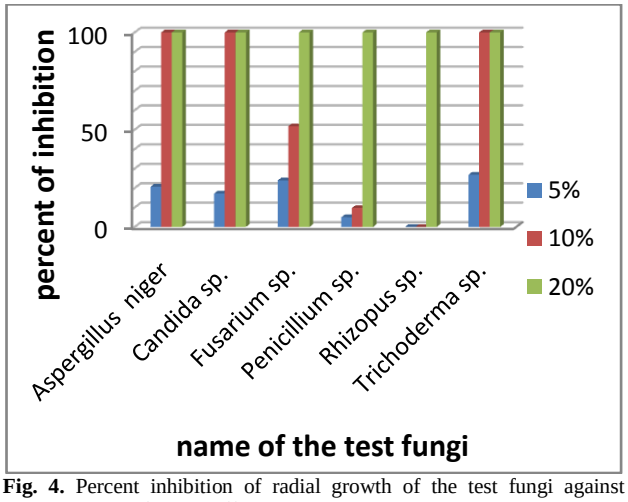

Fig. 4. Percent inhibition of radial growth of the test fungi against *Polyalthia longifolia* at different concentrations.

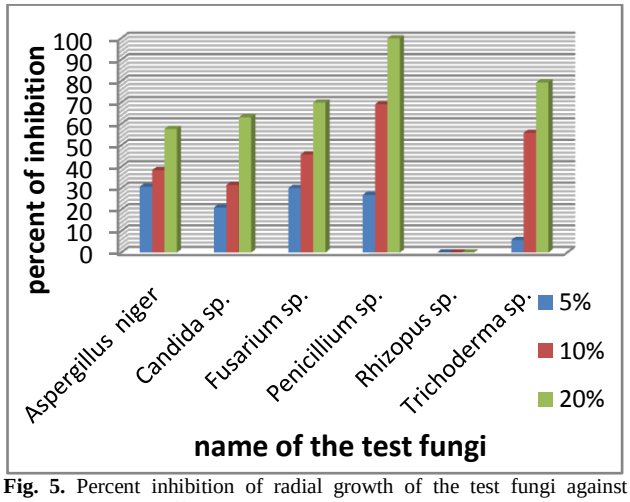

Fig. 5. Percent inhibition of radial growth of the test fungi against *Tagetes erecta* at different concentrations.

At 200 ppm concentration Bavistin completely inhibited growth of *C. krusei*, *Fusarium* sp. and *R. stolonifer*. At the same concentration Ridomil also completely inhibited growth of *R. stolonifer*. Green gel showed maximum inhibition of *P. digitatum* 89.37% at the same concentration. Bavistin also was capable of complete inhibition of *Fusarium* sp and *R. stolonifer* at 100 ppm concentration. At the same concentration Ridomil completely inhibited the radial growth of *R. stolonifer*. *Penicillium digitatum* exhibited maximum inhibition of 69.86% by Green gel (Figs. 6-9).

Present results indicate that Tall 25 EC is capable of inhibiting the radial growth of *A. niger*, C. *krusei*, *Fusarium* sp., *R. stolonifer*, *P. digitatum* and *Trichoderma* sp. at 100 ppm concentration. Bavistion and Ridomil also completely inhibited the radial growth of *R. stolonifer* at the same concentration. Wheareas Grenngel have no effect on *R. stolonifer*.

Present results indicate that Tall 25 EC is capable of inhibiting the radial growth of *A. niger*, C. *krusei*, *Fusarium* sp., *R. stolonifer*, *P. digitatum* and *Trichoderma* sp. at 100 ppm concentration. Bavistion and Ridomil also completely inhibited the radial growth of *R. stolonifer* at the same concentration. Wheareas Grenn gel have no effect on *R. stolonifer*.

Sandra (2004) reported Green mould caused by the fungus *P. digitatum* is typically the worst postharvest disease of lemon and lemon was treated with Bavistin to control green mould disease[17].

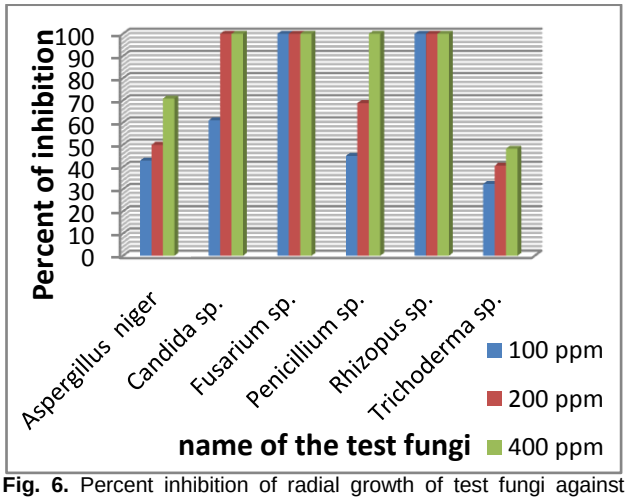

Fig. 6. Percent inhibition of radial growth of test fungi against Bavistin at different concentrations.

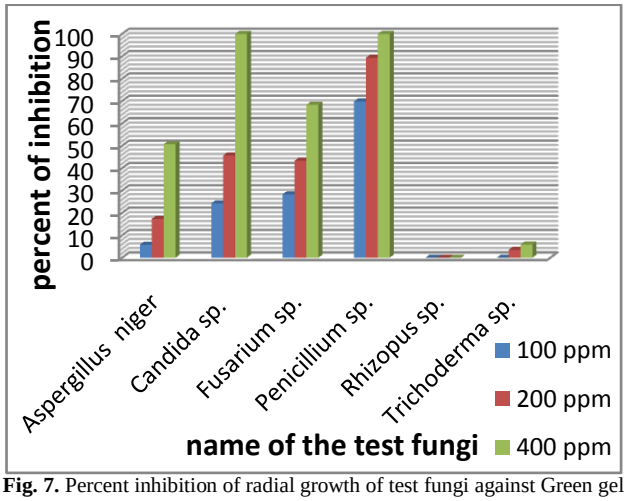

Fig. 7. Percent inhibition of radial growth of test fungi against Green gel at different concentrations.

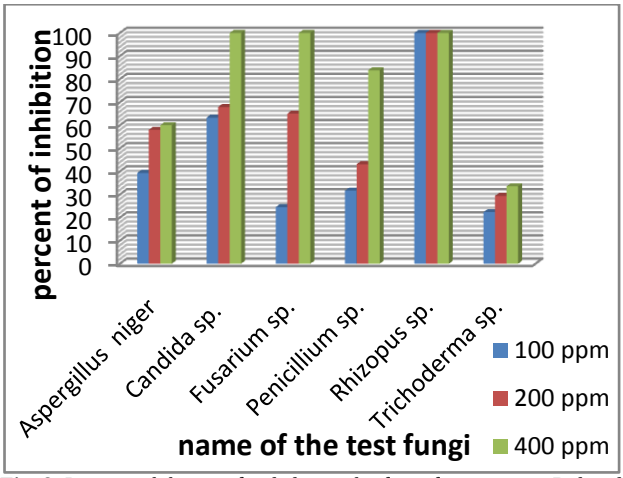

Fig. 8. Percent inhibition of radial growth of test fungi against Ridomil at different concentrations.

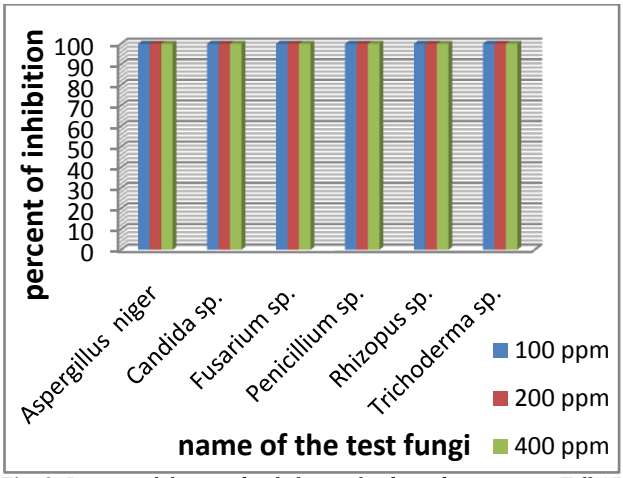

Fig. 9. Percent inhibition of radial growth of test fungi against Tall 25 EC at different concentrations.

The most important method of protecting the plants against the fungal attack is the use of fungicides. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently effect the humans through the food chain. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some eco-friendly measures for the management of diseases.

Research on plant-derived natural products for the use in agriculture went into decline for a number of years. But this trend is now reversed as it becomes evident that plant natural products still have enormous potential to inspire and influence the modern agrochemical research[18].

The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance[19]. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases [20].

There are many health benefits of lemons that have been known for centuries. Lemon contains antioxidants called bioflavonoids. Researchers think these bioflavonoids are responsible for the health benefits of lemon. Lemons' has strong antibacterial, antiviral, and immune-boosting powers. Lemon juice is a digestive aid, liver cleanser. Regular use of the lemon juice helps in weight loss. Lemons contain many substances-notably citric acid, calcium, magnesium, vitamin C, bioflavonoids, pectin, and limonene--that promote immunity and fight infection. Now it is essential to protect these valuable fruits from fungal attack. Present research is the first approach of controlling storage fungi of lemon fruits in Bangladesh. Report on the activities of selected plant extracts and fungicides used in this experiment is new addition to Plant Pathology.

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