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ABSTRACT

Mondol MAM (2020) Clove and cinnamon are the promising sources of bioactive compounds for preventing food spoilage and food poisoning caused by *Penicillium* spp. *Int. J. Sustain. Crop Prod.* 15(2), 22-26.

Synthetic chemicals are generally used to prevent food spoilage and food poisoning opportunistic fungi. The use of synthetic chemicals in food preservation and crop protection has enormous negative impacts due to chemical residues in food and feed including hazardous effects on human health, environment and emerging resistant microbes. Due to such concerns, the demand for safer and natural chemicals to prevent food spoilage and food poisoning has increased. Herbs and spices have a long history of preventing crop diseases, food spoilage and preservation. This study investigated the antifungal properties of methanol extracts of 17 spices against *Penicillium* sp. Antifungal activity of spice extracts were determined by disc diffusion method. Clove and cinnamon extracts at a concentration of 5 mg/disc exhibited marked antifungal activity against tested pathogen (25 mm and 20 mm zone of inhibition, respectively) when compared to positive control iprodione (20 mm zone of inhibition). Other spice extracts did not show any activity at applied concentration (5 mg/disc) against tested pathogen. The antifungal property of clove and cinnamon extracts make these spices of potential interest for the control of food spoilage and food poisoning opportunistic fungi in a natural and safer ways.

Key words: *activity, extracts, fungicides, spices, pathogen, zone of inhibition*

INTRODUCTION

Collectively, fungi and fungal-like organisms (FLOs) cause more plant diseases than any other group of plant pest with over 8,000 species shown to cause diseases. Pathogenic fungi cause plant diseases such as anthracnose, leaf spot, rust, wilt, blight, coils, scab, gall, canker, damping-off, root rot, mildew, and dieback. Systemic foliar pathogens are major causes for yield and commercial crop losses and diminished crop quality (Iqbal *et al.* 2018). Today, crop-destroying fungi account for yield losses of ~20% worldwide, with a further 10% loss postharvest. Currently fungal diseases of human are spiraling, and the global mortality rate now exceeds that for malaria or breast cancer and is comparable to those for tuberculosis and HIV (Brown *et al.* 2012).

Penicillium species are considered to be ubiquitous and opportunistic saprophites, most of them primarily found in soil and decaying vegetation, but also associated with human food supplies. This group of fungi causes losses in high value crop products through deterioration and decomposition especially citrus fruits (lemon, orange, grapefruit, mandarin etc), date palm, apple, kiwifruit, black plum, guava, stored grains, apple etc (Holmes and Eckert 1999 and Eckert and Eaks 1989). The fungi gain entry if fruit is damaged during handling and storage, and then decay can spread from fruit to fruit. These species grow rapidly at 20-25°C but very slowly below 5°C or above 30°C.

Symptoms of attack on citrus fruits by different *Penicillium* spp. include blue mold, green mold, whisker mold, soft lesions covered with light bluish-green colored spore masses that later expanded to cause fruit rot, bulb rot, wet core rot etc (Moosa *et al*. 2019). This pathogen not only is responsible for weight loss, softening, pigmentation, discoloration, rotting, off-odors and off-flavors of fruit products but also produce and accumulate harmful mycotoxins and carcinogenic compounds, such as ochratoxin A, citrinine, patulin, penicillic acid and others in processed fruit products (e.g. apple sauce, butter, jams, juices) (Perrone and Susca 2017). Postharvest decay speed up deterioration of fruits and vegetables by the fungi such as *Rhizopus*, *Aspergillus* and *Penicillium* species which affects the quality and shortens the shelf-life (Lichter *et al*. 2002; Wanchaitanawong *et al*. 2005). Management of postharvest decaying fungi is accomplished by several synthetic fungicides (benomyl, thiabendazole, imazalil, guazatine, sodium ortho-phenylphenate, pyrimethanil, azoles). Due to long time prophylactic uses, major plant pathogenic fungi have become resistant to each main class of fungicides (Fisher *et al.* 2018). On the other hand, these synthetic fungicides are persistent organic compounds and their residues in the foods and environment are imposing enormous threat to human health and ecosystem. To address these issues related with synthetic fungicides and sustainable agriculture practices, we need effective and safe alternative.

Spices have been used since ancient times to give taste, color and flavor of food and also as food preservatives and disease remedies. The use of spices in foods is an integral part of Bangladeshi cuisine but little is known about their fungicides potential. The chemical compounds present in spices are nontoxic, easily biodegradable and eco-friendly. The present work was designed to assess *in vitro* the fungicides properties of methanol extracts obtained from 17 spices used in Bangladeshi cuisine against plant pathogenic fungus *Penicillium* sp. This is an approach to identify antifungal compounds in natural sources that may be used for controlling fungal diseases of foods.

MATERIALS AND METHODS

General experimental procedures

Sterile filter paper disk (BioMaxima S.A., Poland), potato dextrose agar (PDA) (Scharlau, Spain), filter paper (Whatman Int. Ltd, Maid Stone, England), heavy duty blender (Havells, India), methanol (Merck, Germany), iprodione (Auto Crop Care Ltd, Dhaka, Bangladesh), colorimeter (Model-S 9121, Systonic, India) and vortex machine (VM-10, witeg, Germany) were bought from local suppliers. Sterilization, aseptic works and solvent evaporation were done using vertical autoclave machine (Model: LVA-202, Labocon, UK), horizontal laminar airflow cabinet (Model: LLFH-204, Labocon, UK) and rotary evaporator (Model: HS-2005S-N, Hahnshin S&T Co., Ltd, Korea).

Preparation of spices extracts

A total of 17 fresh spices were collected from local Charagali market, Tongi, Gazipur. For extraction, the freshly collected plant parts were thoroughly washed with sterilized distilled water. The material was dried in an oven at 45°C for 48 hrs followed by grinding into a fine powder. 5 g of each powdered spice material was dissolved in enough methanol to make 100 ml (5% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterilized flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. The filtrates were evaporated and dried at 40°C under reduced pressure using rotary vacuum evaporator. The extract yield was weighted and yield percentage was calculated using the following formula: Extract yield (g/100 g) = $(W_1 \times 100)/W_2$ where W_1 is the weight of the extract residue obtained after solvent removal and W_2 is the powder weight of spices.

Antibacterial activity of the spice extracts

Collection and identification of test pathogen

The test pathogen was collected from Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh and identified as *Penicillium* sp. based on 16S rDNA sequence analysis which had similarity 99.82% for *Penicillium citrinum* and 99.81% for *Penicillium brevicompactum*.

Seed culture of test pathogen

To prepare the seed culture, test pathogen was streaked on the sterilized potato dextrose agar medium (PDA) (prepared according to the manufacturer instruction) (3.9% w/v) from stock culture and then incubated at 30°C for 3 days. This culture was used for antifungal activity screening of the selected 17 spice extracts.

Antibacterial activity test of the spice extracts

To prepare the activity assay plate, PDA medium was sterilized at 121°C for 15 min by autoclave machine. The sterilized PDA medium was poured into the sterilized Petri dish (120 mm) and left to solidify in laminar airflow cabinet. This layer of PDA medium is used as bed. The spores from seed culture plate of *Penicillium* sp. was removed by sterilized cotton swab and dissolved in a sterilized colorimeter test tube containing 1 ml sterilized distilled water and adjusted optical density to 0.1 which is equivalent to approximately 0.4×10^4 CFU/mL. These dissolved spores were poured into a 100 ml conical flask containing sterilized PDA medium at around 37°C, mixed well and poured on the previously solidified PDA medium layer containing Petri dish and left for solidification. Similarly, activity test plate for positive (standard) and negative controls was prepared but here 70 mm Petri dish was used.

Antifungal activity against *Penicillium* sp. of the spice extracts was determined by agar disk diffusion assay method (Bauer *et al*. 1966). In brief, each spice extract was diluted in a combination of methanol and ethyl acetate in such way that 20 µl contained 5 mg extract. 20 µl of each diluted extract was taken out by the micropipette and impregnated in the sterile microbial susceptibility testing paper disk (6 mm). After drying, all the disks containing test samples were transferred about 2 cm apart by a sterilized forcep on the surface of the previously spread *Penicillium* sp. spores agar plate. Then this plate was incubated at 28°C for 4 days. After incubation, zone of growth inhibition for each extract was measured in mm. Iprodione (5 mg/disk) and one sterile empty paper disk (6 mm) were used as positive (standard) and negative controls in this experiment, respectively.

RESULTS AND DISCUSSION

The percentage yield of methanol extract obtained from 17 spices (Fig. 1) is shown in Table 1. The extract of 5 g of dried spices yielded residues ranging from 0.18 to 1.44 g. The highest yield of plant extract was obtained from *Syzygium aromaticum* (28.8%) followed by *Myristica fragrans* (20.0%) while *Coriandrum sativum* gave the lowest extract yield (3.6%).

Table 1. Vernacular, common and botanical names of the spices used in activity test against *Penicillium* sp. with their percentage of methanol extract yield

SL	Vernacular name	Common name	Botanical name	Parts used	Yield (%)
1.	Ada	Ginger	*Zingiber officinale*	Rhizome	10.8
2.	Holud	Turmeric	*Curcuma longa*	Rhizome	9.0
3.	Rosun	Garlic	*Allium sativum*	Clove	11.4
4.	Jayatri	Mace	*Myristica fragrans*	Aril of fruit	20.0
5.	Golmorich	Black pepper	*Piper nigrum*	Seed	8.6
6.	Kalozira	Black cumin	*Nigella sativa*	Seed	7.5
7.	Methi	Fenugreek	*T. foenum-graecum*	Fruit	9.8
8.	Donia	Coriander	*Coriandrum sativum*	Fruit	3.6
9.	Mori	Fennel	*Foeniculum vulgare*	Seed	9.4
10.	Daruchini	Cinnamon	*Cinnamomum verum*	Bark	14.3
11.	Zera	Cumin	*Cuminum cyminum*	Seed	11.4
12.	Choto elach	Green cardamom	*E. cardamomum*	Fruit	5.7
13.	Jayfal	Nutmeg	*Myristica fragrans*	Fruit	11.4
14.	Tespata	Bay Leaf	*Laurus nobilis*	Leaf	13.0
15.	Kalo elach	Black cardamom	*Amomum subulatum*	Fruit	11.2
16.	Lobonggo	Clove	*Syzygium aromaticum*	Flower buds	28.8
17.	Star masla	Star aniseed	*Pimpinella anisum*	Seed pods	18.6

Fig. 1. Pictures of 17 spices used in antifungal activity (serial no. 1 to 17 of Table 1) against *Penicillium* sp.

In vitro activity of methanol extracts of 17 spices (Table 1 and Fig. 2) was evaluated against opportunistic plant pathogenic fungus *Penicillium* sp. The zone of inhibition (mm) exhibited by spice extracts are listed in Table 2. Among the studied spices, extract obtained from clove (*S. aromaticum)* showed potent activity with inhibition zone 25 mm followed by cinnamon extract (*C. verum*) (zone of inhibition 20 mm) when compared with positive control, iprodioone (zone of inhibition 20 mm). Unfortunately, other spice extracts did not show any activity at an applied concentration (5 mg/disc) against tested pathogen including negative control (Table 2).

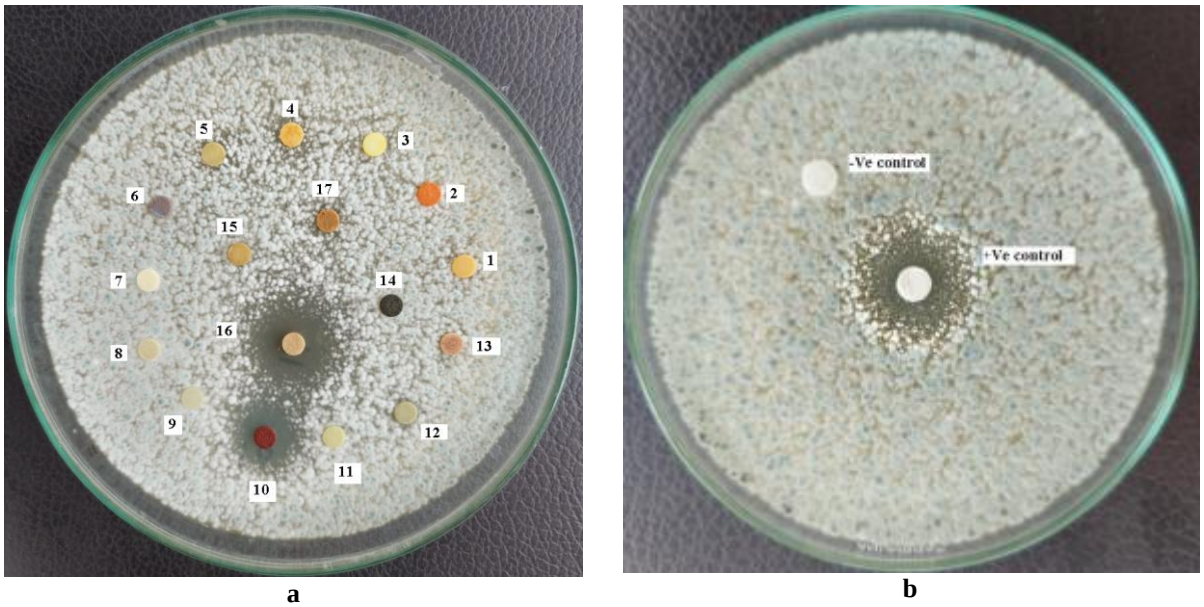
Several earlier reports indicated that clove extracts showed antifungal activity against a wide range of genera such as *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Fusarium* and *Saccharomyces* which is consistence with the present study (Chattopadhyay and Bhattacharyya, 2007). Major parts of clove consist of two bioactive compounds, eugenol (71.56%) and eugenol acetate (8.99%) (Nassar *et al.* 2007). Molds, yeast and bacterial growth could be inhibited by the application of clove essential oil (Chattopadhyay and Bhattacharyya 2007 and Burt 2004).

Several studies reported that cinnamon extract exhibited activity against *Aspergillus flavus* and *A. parasiticus* (Bullerman 1974, Viollon and Chaumont 1994). The most important constituents of cinnamon are cinnamaldehyde and *trans*-cinnamaldehyde which contribute to the various biological activities (Yeh *et al.* 2013). In addition to being used as a spice and flavoring agent, cinnamon is used as coagulant, antimicrobial, antifungal, antioxidant, antidiabetic, anti-inflammatory, antitermitic, nematicidal, mosquito larvicidal, insecticidal, antimycotic, and anticancer agent (Rao and Gan 2014). Traditionally, cinnamon is also used as tooth powder to treat toothaches, dental problems and bad breath.

Table 2. Antibacterial activity of spice extracts (1-17) and standard against *Penicillium* sp.

SL	Vernacular name	Common name	Botanical name	Inhibition zone
1.	Ada	Ginger	*Zingiber officinale*	-
2.	Holud	Turmeric	*Curcuma longa*	-
3.	Rosun	Garlic	*Allium sativum*	-
4.	Jayatri	Mace	*Myristica fragrans*	-
5.	Golmorich	Black pepper	*Piper nigrum*	-
6.	Kalozira	Black cumin	*Nigella sativa*	-
7.	Methi	Fenugreek	*T. foenum-graecum*	-
8.	Donia	Coriander	*Coriandrum sativum*	-
9.	Mori	Fennel	*Foeniculum vulgare*	-
10.	Daruchini	Cinnamon	*Cinnamomum verum*	20
11.	Zera	Cumin	*Cuminum cyminum*	-
12.	Choto elach	Green cardamom	*E. cardamomum*	-
13.	Jayfal	Nutmeg	*Myristica fragrans*	-
14.	Tespata	Bay Leaf	*Laurus nobilis*	-
15.	Kalo elach	Black cardamom	*Amomum subulatum*	-
16.	Lobonggo	Clove	*Syzygium aromaticum*	25
17.	Star masla	Star aniseed	*Pimpinella anisum*	-
18.	Iprodione (+ve control)			20
19.	Blank disk (-ve control)			-
	'-' indicate not active; inhibition zone was measured in millimeters			

In addition to human diseases, fungal pathogens destroy a third of all food crops annually causing huge economical loss and impacting global poverty (Fisher *et al.* 2012). Nowadays, consumers are worried about synthetic chemicals used in crop protection and food preservation due to adverse health effects caused by residues of these chemicals. It may be suggested from the findings that the clove and cinnamon methanol extracts can be used as a potential source of natural antifungal agents in place of synthetic chemical as fungicides and preservatives.



a **b**

Fig. 2. Antifungal activity of 17 spice extracts (serial numbers 1 to 17 of Table 2) (a) and iprodione (standard) (b) against *Penicillium* sp.

CONCLUSION

Spoilage of crops and foods is often caused by the growth of opportunistic fungi which is prevented mainly by the application of synthetic chemicals. The adverse effects of these chemicals on human health and ecosystem demand to search for natural, effective and eco-friendly fungicides and preservatives. Among methanol extracts of 17 spices, clove and cinnamon extracts exhibited potent antifungal activity against *Penicillium* sp. Bioassay guided isolation, structure determination and subsequently activity test of bioactive compounds of methanol extracts of clove and cinnamon against crop pathogenic and food spoilage opportunistic fungi can be helpful in developing natural, safer and eco-friendly alternatives to synthetic fungicides and preservatives.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

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