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In-Vitro Antifungal Activity of *Azadirachta indica*, *Ocimum tenuiflorum* & *Murraya paniculata* Leaf Extract against Three Phytopathogenic Fungi

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ABSTRACT

Fungal disease is one of the major problems in agriculture. Fungal pathogens are accountable for approximately 85% of plant diseases. Apart these, public health conditions are also influenced by consequential fungal infection as well as approximately 1.5 million killed and more than a billion people were affected by fungal disease. Our present exploration has been conducted to assess the antifungal efficiency of *Azadirachta indica, Ocimum tenuiflorum* and *Murraya paniculata* leaf extract against three phytopathogenic fungi *viz. Pichia kudriavzevii, Lasiodiplodia theobromae* and *Fusarium oxysporum,* at the concentration of 300 µg/disc by food poisoned technique. The result showed that all of these three extracts have significant antifungal efficiency against all of the tested fungus. Maximum antifungal activity was recorded in *Murraya paniculata* with an inhibition percentage of 100% (0.00±0.000 mm) against three fungi. In addition, *Lasiodiplodia theobromae* and *Fusarium oxysporum*, growth was totally suppressed in terms of *Ocimum tenuiflorum* and *Murraya paniculata* extract. The lowest antifungal effect was 47.18% (34.33±0.272 mm) revealed in *Azadirachta indica* extract against *Pichia kudriavzevii.* Among these three extracts the order of antifungal effect was *Murraya paniculata>Ocimum tenuiflorum>Azadirachta indica*. Amis of this screening to focus antifungal effects of three experimental medicinal plants. These findings indicate leaf of these three plants may be useful for the treatment of various diseases associated with these fungi and could be useful to develop novel, secure and fecund bio-fertilizer for pest control. Further phytochemicals analysis is required to evaluate the compounds responsible for their antifungal effects.

Keywords: Fungal diseases, *Azadirachta indica, Ocimum tenuiflorum,* and Antifungal activity.

INTRODUCTION:

Although asthma, AIDS, cancer, organ transplantation, and many other health conditions are influenced by consequential fungal infection as well as approximately 1.5 million killed and more than a billion people were affected by fungal disease yet it is a forlorn topic by public health specialists (Bongomin *et al.*, 2017). Apart from, the fungal pathogen is one of the biggest problems in agriculture. Fungus is the most devastating pathogen for plants and is responsible for 85% of plant's disease.

Pichia kudriavzevii infects immunodeficient patients considered as opportunistic pathogen induce mastitis in mammals (Hurst, 2016). Additionally, Trunk fungi *Lasiodiplodia theobromae* is responsible for Grapevine trunk diseases (GTDs) are the most destructive diseases of vineyards worldwide (Rusin *et al.*, 2020). Moreover, the pathogenic *Fusarium oxysporum* strains are liable to cause *Fusarium* wilt disease, conducted a major threat for agriculture, and ranked 10th most demolishing plant pathogenic fungus (Dean *et al.*, 2012; Fisher *et al.*, 2012).

The drugs are frequently used as antifungal have a side effect, hence there is exclusive need for the discovery of novel, secure and fecund antifungal drugs, for instance, medicinal plants could play a significant role in the field of drug discovery. *Azadirachta indica* extracts inhibited the growth of some fungus, bacteria, viruses, insecticides and also exposed antioxidant, anti-ulcer, anti-carcinogenic, and anti-malarial properties (Subapriya and Nagini, 2005). Alongside, extract from neem pledges and significantly abate fungal conidial germination (Jabeen *et al.*, 2013). Because of these antimicrobials and pharmaethological properties, *Azadirachta indica* could be used for pest control along with health benefits (Rony *et al*., 2019). *Ocimum tenuiflorum* belonging to the family of Lamiaceae also known as *Ocimum sanctum*, Tulsi or Holy Basil. Eugenol, one of the key phytochemical of *Ocimum tenuiflorum* oil act against fungi leading to bio-deterioration of foodstuff during storage (Subramanian, 2014).

Murraya paniculata L family of Rutaceae, commonly used as herbal therapy in India, China, Indonesia, and Bangladesh (Zhang *et al.*, 2011). It contains an exuberant source of phyto compounds, liable for antifungal, antibacterial, analgesic, antispasmodic, bronchodilator, vasodilating actions, anti-thyperglycemic and antioxidant action(Fazal-ur-Rehman *et al.*, 2014; P *et al.*, 2015; Saqib *et al.*, 2015). The above plants are widely used as medicinal plants in Bangladesh. Our present investigation deals with the evaluation antifungal efficiency of *Azadirachta indica, Ocimum tenuiflorum,* and *Murraya paniculata* leaf extract against *Pichia kudriavzevii, Lasiodiplodia theobromae* and *Fusarium oxysporum* to find out effective agents that could be useful to develop new, secure and effective bio-fertilizer to pest control and as well as treatment of various disease associated with these fungi.

MATERIALS AND METHODS:

Plant materials - Plant leaves of *Azadirachata indica, Ocimum teniflorum,* and *Murraya paniculata* were collected from Rajshahi University Campus, Rajshahi-6205, Bangladesh. These plant leaves were used as plant material in our current study.

Extract preparation - The samples were washed individually under running tap water to remove any traces of soil particles and other dirt, cut into small pieces and air-dried for seven days. 20 gm of powdered dried leaves soaked with 60 mL methanol and allowed to macerate at room temperature for 15 days with an orbital shaker. Then, strained through sterile mark in cloth and finally filtered through sterile Whatman No.1 filter paper. The filtrates were then set for 3 days to evaporate the methanol and afford a blackish mass. The output extracts were collected to glass vials and preserved separately in a refrigerator at 4°C.

Test microorganisms - Methanol leaf extract of *Azadirachta indica, Ocimum tenuiflorum, and Murraya paniculata* were investigated for their antifungal efficiency against three fungi including *Pichia kudriavzevii, Lasiodiplodia theobromae,* and *Fusarium oxysporum.* Investigated fungi were collected from Prof. Joarder DNA and Chromosome Research Lab. Dept. of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.

Determination of antifungal activity - In-vitro antifungal activity was accomplished by food poisoned technique against three pathogenic fungi *Pichia kudriavzevii, Lasiodiplodia theobromae* and *Fusarium oxysporum* at the concentration of 300 µg/ml. 20 ml quantities of potato dextrose agar (PDA) media were plated in the petri dish with fungal culture. In this investigation, 300 µg/ml concentrations of plant extracts were spread with a sterile spreader on test organism-seeded plates. A plate containing only fungal culture was used as a control. The activity of the plant extract was determined after seven days of incubation at 37ºC temperature. After 7 days solid dry weight of the fungus was taken using an oven. The antifungal activity of these plants extract was assessed on the

basis of inhibition of mycelial growth (%) and calculated using the formula (Habib *et al*., 2019).

Inhibition of mycelial growth (%) = $[(G_c-G_t)/G_c]*100$

Where, 'C' is the average diameter of the fungal colony in control plates and, 'T' is the average diameter of the fungal colony in poisoned plates (Gupta and Tripathi, 2011).

RESULTS AND DISCUSSION:

In-vitro antifungal efficiency of three medicinal plants *Azadirachta indica*, *Ocimum tenuiflorum and Murraya paniculata* methanol leaf extract were exhibited strong antifungal activity against *Pichia kudriavzevii, Lasiodiplodia theobromae* and *Fusarium oxysporum* at the concentration of 300 µg/disc (**Fig 1**). The diameter zone of inhibition of *Azadirachta indica*, *Ocimum tenuiflorum and Murraya paniculata* leaf extract are presented in **Table 1**.

Table 1: Zone of inhibition (mm) of *Azadirachata indica*, *Ocimum teniflorum* and *Murraya paniulata* against *Pichia kudriavzevii*, *Lasiodpladia theobromae and Fusarium oxysporum.* Values are represented by mean±SE of three biological replicates.

Zone of Inhibition (mm)			
Plant extract	***Pichia kudriavzevii***	***Lasiodpladia theobromae***	***Fusarium oxysporum***
Control	65.00±0.00	44.00±0.00	51.00±0.00
Azadirachta indica	34.33±0.27	11.66±0.27	20.00±0.47
Ocimum teniflorum	0.00±0.00	0.00±0.00	15.33±0.54
Murraya paniulata	0.00±0.00	0.00±0.00	0.00±0.00

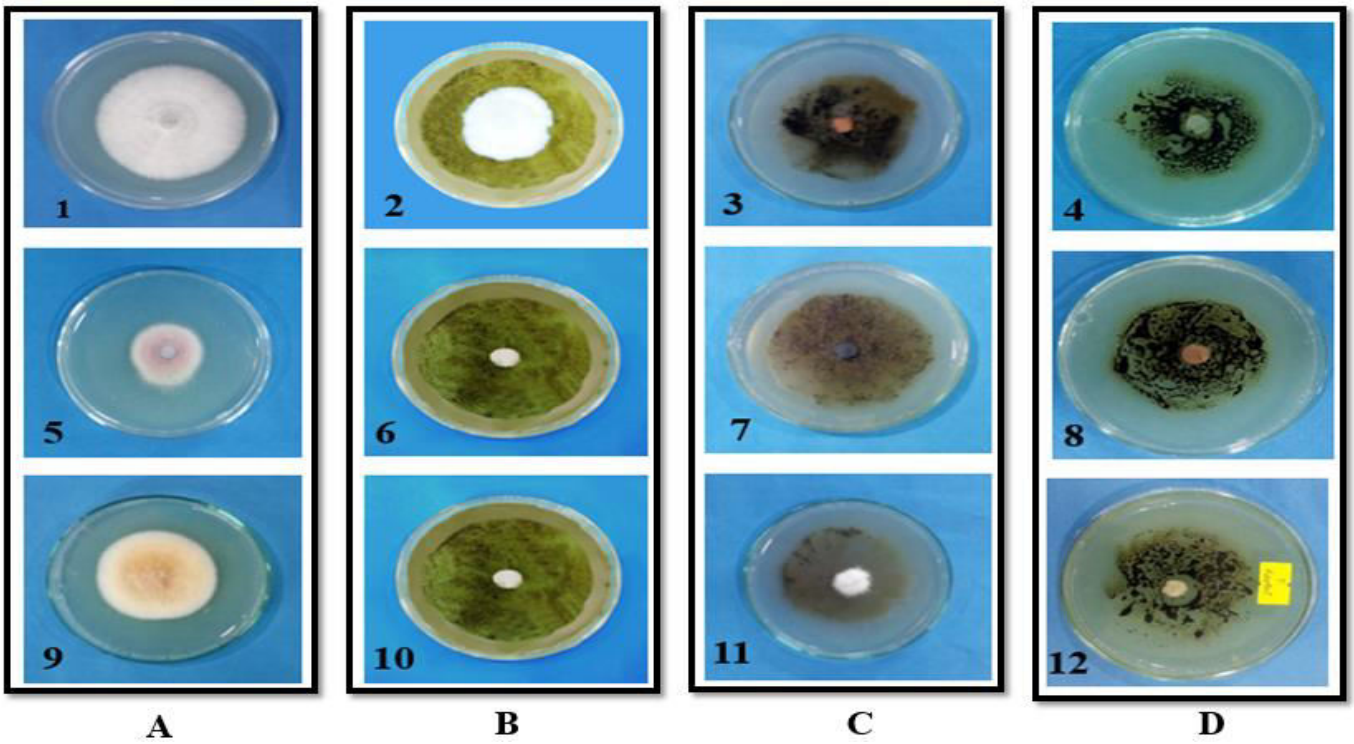

Fig 1: Antifungal activity of *Azadirachata indica*, *Ocimum teniflorum* and *Murraya paniulata* against three fungi. A) Control, B) Treated with *Azadirachata indica*, C) *Ocimum teniflorum*, D) *Murraya paniulata*; (1-4) *Pichia kudriavzevii*, (5-8) *Lasiodpladia theobromae and* (9-12) *Fusarium oxysporum.*

Among these three extracts, the most significant effect was found in *Murraya paniculata* leaf extract with an inhibition percentage of 100% (0.00±0.000 mm) against all tested fungus (**Fig 2**, **C**). Sundaram *et al*. observed that all the aqueous, hexane, and ethanol extract of *Murraya paniculata* showed antifungal

potency contrary to *Aspergillus niger* (Sundaram *et al.*, 2011). Our present investigation found that methanol leaf extract of *Azadirachta indica* showed antifungal activity against all tested fungal pathogens.

In *Azadirachta indica,* the most significant antifungal activity was found against *Lasiodiplodia theobromae* and *Fusarium oxysporum* with inhibition percentages of 73.48% (11.667±0.272 mm) and 60.78% (20.00± 0.471 mm) respectively (**Fig 2**, **A**).

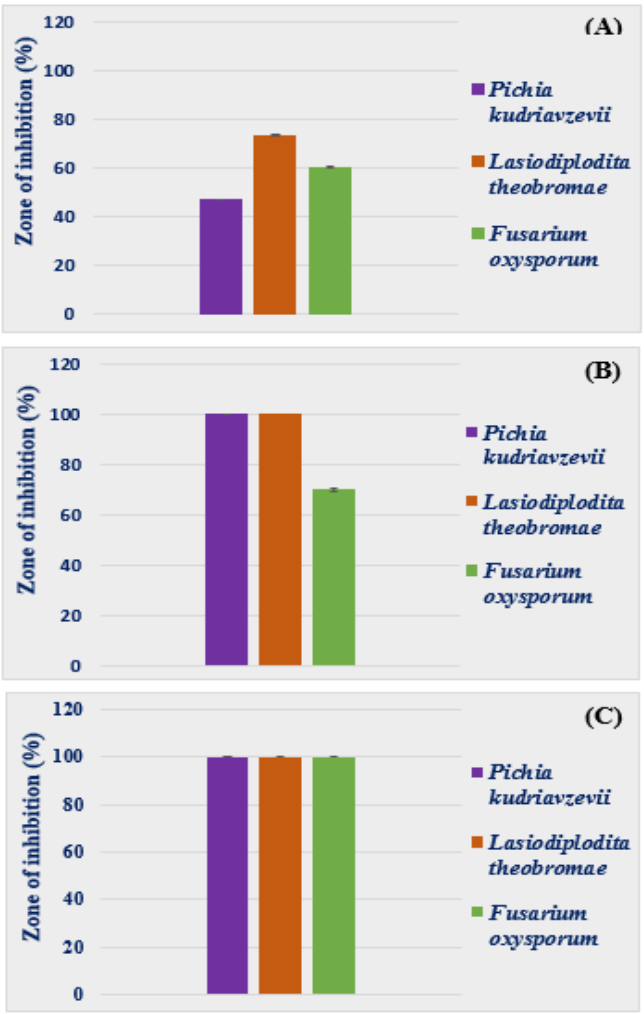

Fig 2: Inhibition percentage of *Azadirachta indica* (A), *Ocimum teniflorum* (B) and *Murraya paniulata* (C) methanol leaf extract against three phytopathogenic fungi *Pichia kudriavzevii*, *Lasiodpladia theobromae and Fusarium oxysporum* at the concentration of 300 µg/disc. Data are represented by mean±SE of three biological replicates.

Khan *et al*. explained that *Azadirachta indica* leaf extract had a characteristic effect on dermatophytes, leading to the exclusion in protease action of dermatophytes due to maybe it flavonoid quercetin in the extract (Matthews, 1989). Our present finding exhibited three fungi growth was significantly inhibited by our investigated extracts at 300µg/disc, whereas Shivpuri *et al*. found *Azadirachta indica* ethanol extract had antifungal efficiency to five pathogenic fungi at the concentration from 500 to 1000 µg/ml (Shivpuri *et al*., 1997). In addition, Kishore *et al*. also reported that *Azadirachta indica* inhibited about 90% *Phaeoisariopsis personata* conidial germination (Kishore *et al.*, 2001).

In addition, *Ocimum tenuiflorum* leaf extract revealed remarkable antifungal efficiency at 300 µg/disc. Among the three fungi, *Pichia kudriavzevii* and *Lasiodiplodia theobromae* growth were fully inhibited by *Ocimum tenuiflorum* methanol leaf extract with an inhibition percentage of 100% (0.00±0.00 mm) and 100% (0.00±0.00 mm) (**Fig 2**, **B**).

Moreover, *Ocimum tenuiflorum* also showed an antifungal effect on *Fusarium oxysporum* with an inhibition percentage of 69.93% (15.33±0.544 mm). Gupta *et al*. found that *Ocimum Sanctum Linn* aqueous extract reducing the fungal growth of *A. niger, Cladpsporium sp*. and *Rizopous,* at the concentration of 40% (Gupta *et al.*, 2013). Besides these many scientific studies indicated that *Ocimum sanctum* has antifungal activity and these studies validation our present investigation (Shahen *et al*., 2019)

CONCLUSION:

In this investigation, *Azadirachta indica, Ocimum tenuiflorum* and *Murraya paniculata* methanol leaf extract has shown potential antifungal properties. We also found the maximum antifungal effect in *Murraya paniculata* against all tested fungus. These findings indicate leaf of these three plants could be useful to treat various diseases associated with these fungi. Further phytochemicals analysis is required to assess the compounds responsible for their antifungal effects.

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CONFLICTS OF INTEREST:

The authors declared no potential conflicts of the interest with the present experiment.

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