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NOTE

Antifungal Investigation of the Constituents of *Moringa oleifera lamk*. Root Bark Extract

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Antifungal activity of *Moringa oleifera* root bark extract was treated against *Aspergillus niger* and *Neurospora crassa*. Per cent mycelial inhibition of aqueous, methanolic and ethanolic extract were determined. Significant inhibition especially of ethanolic extract was seen against *Neurospora*. Comparatively the extracts were less inhibitory against *Aspergillus* sps.

Key Words: Antifungal activity, *Moringa olifera*, *Aspergillus niger*, *Neurospora crassa*.

The global consensus to reduce inputs of chemical pesticides which are perceived as being hazardous has provided opportunities for developing or screening some novel biocides of plant origin. Plant Kingdoms literally have thousands of compounds and metabolites in it. Their role and function regarding its antifungal or antibacterial nature is still a subject of investigations, over years much effect has been devoted to the search for new antifungal materials from natural sources[1,2]. Hooda and Srivastava[3] have mentioned that natural fungicides are free from environmental toxicity. In comparison to synthetic compounds the natural compounds provided less phytotoxic, more systematic and easily biodegradable fungitoxic compounds[4]. Effect of various plant extract in disease therapy has encouraged researchers to look for the extract of roots, seeds, leaves, stems and barks of many plants with a view to harness their constituents for the treatment of various disease[5]. *Moringa oleifera lamk*. is a member of the Moringaceae family. It has high potential pharmaceutical activities such as antibacterial, antifungal and antispasmodic[6].

The fungus selected as research organism were *Aspergillus niger* and *Neurospora crassa*. *Aspergillus* sp the common contaminant of starchy foods and food stuffs. It produces mycotoxins and growth of fungi may cause spoilage and quantity of foods[7]. *Neurospora crassa* the other pathogen selected is quickly reproducing and easy to culture and able to survive on minimal media. The aim of the present study is to evaluate the antifungal activity of methanolic, ethanolic and aqueous extract of *Moringa olifera* root bark against *Aspergillus niger* and *Neurospora crassa*.

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***Moringa* samples and preparation of extract:** *Moringa oliefera* (root bark) specimens were collected from Purnia, district of Bihar. The root barks were shade dried at room temperature and powdered. Active compounds were extracted using the Soxhlet apparatus for 3 to 4 d. Two solvents *viz.*, ethanol, methanol and distilled water were used in extraction process. 0.5 kg powdered roots were macerated with 1 L of each solvent. The extracts were concentrated by distillation processes.

Microrganism: Fungal cultures of *Aspergillus niger* strain and *Neurospora strain* were procured from Plant Pathology and Microbiology laboratory, Patna University, Patna and cultured on potato dextrose agar medium (PDA). Seven days old cultured were used.

Preparation of inoculum: Ethanolic, methanolic and aqueous 10 mL of extract were mixed with 100 mL of molten potato dextrose agar medium cooled to 45 ºC and sterilized in Autoclave. Test fungi *Aspergillus* and *Neurospora* sps were cultured on above media separately on different treated Agar plates. Each treatment was replicated thrice with appropriate untreated control. There were incubated for 4 d at 28 ± 1 ºC before recording the radial mycelial growth of test fungus.

Antifungal activity test: To determine the antifungal activity of plant extract the fungi, colony diameter in natural perpendicular direction at 2-3 radii of each treatment as well as control sets of each test fungus were measured after 4 d.

Per cent inhibition of mycelial growth was calculated on mean values of colony diameter by following Vincent's formula

$$\text{Per cent inhibition of mycelial growth} = [d_c\text{-}d_t/d_c] \times 100$$

where d_c = Average diameter of fungal colony in control set. d_t = Average diameter of fungal colony in treatment set.

The extracts prepared in all the three solvent including distilled water showed varied antifungal activity against both the test fungi used. Change in concentration of extract was directly proportional to per cent mycelial inhibition. The measurement of colony diameter of each isolate on 96 h, after incubation an presented in Tables 1 and 2 below and photographically represented too.

According to the results (Table-1), the ethanolic extract of *Moringa oleifera* was found to be highly significant in reducing radial growth of *Neurospora* somewhere about 89 %. The other extract in order of superiority were methanolic and distilled water in 84.2 and 78.8 %, respectively. The inhibitory effect can be attributed to the presence of some antifungal toxicants.

In case of *Aspergillus* sp the different solvent extraction in order of superiority were methanolic > ethanolic > distilled water, whereas in *Neurospora* sps it were ethanolic > methanolic > distilled water. A perusal of the data in the Tables 1 and 2 reveals that. *Aspergillus* sp is more resistant than Neurospora towards the root bark extract of *Moringa oleifera*.

It is concluded from the results that different solvent extracts of *Moringa oleifera* can inhibit the two test fungi mycelial growth. It was also investigated that the effectiveness of extract was only upto 96 h. After that the mycelial colony resumed

TABLE-1
PER CENT MYCELIAL INHIBITION OF *Neurospora* BY *M. oliefera*

Extract	Concentratiton of solvent (v/v) (%)	Mean colony diameter test pathogen (mm)	Per cent mycelial Inhibition of fungal colony (%)
Control (no treatment)	–	76.0	0.00
Ethanol	50	10.0	86.00
	100	8.0	89.00
Methanol	50	13.5	82.00
	100	12.0	84.21
Distilled water	50	17.0	76.63
	100	16.0	78.90

TABLE-2
PER CENT MYCELIAL INHIBITION OF *Aspergillus* sp BY *M. oleifera*

Extract	Concentration of solvent (v/v) (%)	Mean colony diameter of test pathogen (mm)	Mycelial inhibition of fungal colony (%)
Control	–	76	0.00
Ethanol	50	38	50.00
	100	35	53.94
Methanol	50	24	68.40
	100	20	73.68
Distill water	50	46	39.40
	100	40	47.00

its original growth. This feature depicts that its efficacy is only up to a limited period. A intermitted treatment is required for further research. Moreover, because of the water soluble nature of its toxic principle, it is ideal for developing into herbal pesticides.

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