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Evaluation of antifungal property of certain plant extracts on *Curvularia* sp and *Meloidogyne incognita*

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Abstract
Two experiments were performed using extracts of different botanicals possessing antimicrobial and antifungal properties. The plants used are viz. *Mangifera indica, Olea europaea, Phyllantus emblica, Populus deltoides, Musa acuminata, Azadirachta indica, Terminalia arjuna, Bixa orellana, Sterculia alata.* The effects of different leaf extracts was examined and observed against *Curvularia* and *Meloidogyne incognita* in *In-vitro* condition. All the botanicals tested against *Curvularia* sp showed strong inhibition property in suppressing the mycelia growth. When tested against *Meloidogyne incognita* it significantly increased the mortality on hatching of the J_2 juvenile. *Populus* (T_3) showed the strongest inhibition property against *Curvularia* sp while *Bixa orellana* (T_8) was most effective against M. incognita. The knowledge on efficacy of fungicides and nematicides bioagents in suppressing pathogen growth will help in implementing effective management strategy against *Curvularia* sp and *Meloidogyne incognita*.

Keywords: Antimicrobial, *Curvularia* sp, *Meloidogyne incognita*, plant extracts

Introduction
In recent years botanicals have become an alternative to pesticides and some botanicals are already being used commercially in insect and pest management (Agnihotri *et al*., 1999) [1] as they contain a rich source of antimicrobial properties. Natural plant products are important source of new agrochemical for the control of plant diseases (Kagale *et al*. 2004) [16]. Numerous plants have been found to possess phytochemical properties which are toxic to several plant pathogenic fungi (Goussous *et al*. 2010) [13]. They not only provide effective control but also they are limited hazard to soil environment (Noling and Becker, 1994) [18]. Hence, the aim of this present study evaluates the effect of different plant extract against *Azadirachta indica, Terminalia arjuna, Populus, Phyllantus emblica, Musa acuminata, Bixa orellana.*

Curvularia is a phytopathogenic species with a worldwide distribution and wide host range, particularly cereals and grasses (Poaceae) (Ellis 1971; Sivanesan 1987; Manamgoda *et al*. 2015) [10, 26, 19]. Most *Curvularia* sp are found in tropical regions, though a few are found in temperate zones. Plant diseases range from seedling failure to leaf blight. The growth of *Curvularia* on stored grain, thatch, or other dead plant material appears to be smudges of blackish dust.

Root knot nematode known as *Meloidogyne incognita* is a major plant parasitic nematodes affecting quantity and quality of the crop production in many annual and perennial crops. Infected plants shows typical symptoms including root galling stunting and nutrient deficiency, particularly nitrogen deficiency (Siddiqui *et al.*, 2010) [27]. Plant parasitic nematodes have widely infected plant species and have reduced crop yield by 5%, mainly thought root-knot gall formation and nutritional deprivation (Sasser, 1977) [25]. Toxicity of root extract of different plants against nematodes have been reported by many researchers (Onifade, and Egunjobi 1994, Goswami, and Vijayalakshmi, 1986, Egunjobi. and Afolami, 1976) [20, 14, 11]. Root- knot nematodes are one of the most important nematode pests of crop plants and have a diverse host
range. RKN (*Meloidogyne spp*.) are sedentary root endoparasites and are involved in the development of specialized feeding structures known as giant cells.

Materials and methods
Isolation of *Curvularia* sp.
Infected samples of leaves of *Bambusa ventricosa* was collected from nursery of College of

Forestry SHUATS, Allahabad and brought to the laboratory (Plate 1). Infected sample of leaf was cut into pieces. These were then surface sterilized for 1 min in 10% solution of sodium hypochlorite, rinsed in sterile distilled water and dried on sterile paper towel before inoculating on potato dextrose agar (PDA) in test tube slants and were later sub-cultured for pure culture. This was all performed in the laminar air flow chamber and the cultures were incubated for three days.

Identification of fungi

The infected samples were identified by using of 10x and 40x magnification in the microscope. Morphological characters such as hyphae, conidia, conidiophores were observed and identified based on standard books of mycology (Elliot, 1971; Barnett *et al*., 1972) [6]. The specimen was found to be *Curvularia* sp (Plate 2).

Extraction of the plant materials

Six important plants leaves viz. *Azadirachta indica*, *Terminalia arjuna*, *Populus*, *Phyllantus emblica*, *Musa acuminata*, *Bixa orellana* were washed with tap water and dried. Surface sterilization was done with 1% sodium hypochlorite and thereafter washed with distilled water. 10g of the dried leave was weighed and then ground in mortar and pistil. Sterile distilled water of 10ml was added to it. The content was filtered using a piece of muslin cloth and centrifuged for 10 minutes each.

Preparation of plant extract and screening

The plant extracts bioassay was evaluated at recommended concentration by poison food technique (Dhingra and Sinclair, 1995) [9] at lab condition. 10 ml of plant extracts were first poured into petri dishes then, 90ml of molten PDA at 45 – 50°C was poured aseptically on the plant extract in the petri dishes. After cooling down, with the help of a 5mm cork borer, *Curvuralia* sp were introduced into the sterilized petri-plates. The treatments were replicated four times, incubated at room temperature of 28 – 30°C for 24, 48, 72 and 96 hours in the BOD. Suitable control plates were also maintained. Measurement of the mycelia growth extension of the fungus was taken at every 24h of incubation. The percent inhibition of mycelial growth over control was calculated using the formula of Vincent (1947).

$$I = \frac{\quad}{\quad} 100$$

Where,

I = Inhibition of mycelial growth.

C = radial growth of fungus in control

T = Radial growth of fungus in treatment.

Collection of *Meloidogyne spp.* egg and screening

Roots infested with root-knot nematode were washed and kept in fresh water in room temperature. Roots were then cut into pieces and after cooling to normal temperature, the egg masses of *Meloidogyne incognita* (J_2) were collected from the galls using dissecting needle and forceps (Plate 4). The eggs masses of J_2 were kept in a glass cavity block and 50% concentration of different extracts of botanicals were prepared by diluting standard solution with distilled water placing it in glass cavity block and kept at room temperature to allow egg hatch for 24, 42 and 72h hours respectively. While glass cavity block containing 20% egg masses with 80% of distilled water were served as control (Alam, 1985). The egg masses of J_2 were then transferred to a nematode counting plate and counted every 24 hours, using a stereoscopic microscope.

Table 1: Botanicals and its uses

Botanical Name	Family	Common name	Parts used	Uses
Magnifera indica	*Anacardiaceae*	Mango	Leaves	*Magnifera indica* is one of the most tropical fruits in the world. It is an important herb in Ayurvedic medicine. Various parts of the plant are used as a stomachic, cough, asthma, laxative, piles, liver disorders etc. It is also used in UTI and diabetes.
Olea europhaea	*Oleaceae*	Olive	Leaves	It is an evergreen shrub or tree. It is used traditionally as skin cleanser, hypotensive, laxative and also used for the treatment of urinary infections, asthma, diarrhea and colon cancer.
Phyllantus emblica	*Phyllanthaceae*	Gooseberry	Leaves	*It* is one of the most important plants in various traditional systems of medicine in India. It is used to enhance digestion, treat diabetes, jaundice, stomachache, gynecological disorder. It is also considered as an effective remedy for heart
Populus deltoides	*Salicaceae*	Poplar	Leaves	The bark tincture can be used to teeat rheumatism, gout, scury and infections of the chest, kidneys and stomach. It is also used to relieve the pain of menstrual cramps.
Musa acuminata	*Musaceae*	Banana	Leaves	It can be used as an anthelmintic, as dressings on wounds and blistered skin surfaces, and as a tonic to get relief from joint pains as well as to improve blood circulations. Flowers can be cooked and eaten by diabetics.
Azadirachta indica	*Meliaceae*	Neem	Leaves	All parts of neem tree are used for different medicinal purposes. Neem is a good remedy for cardiac problems.it is also used as an insecticide.
Terminalia arjuna	*Combretaceae*	Arjun	Leaves	It is a good source of minerals, which helps to prevent bone loss and improve bone mineral density. It is also used for ulcer treatment.
Bixa orellana	*Bixaceae*	Sindoor	Leaves	It is used to treat snake bites. The leaves and seeds are used to soothe an irritated stomach. The infusion of the leaves has appeared to be effective against bronchitis, sore throat, and eye inflammation.
Sterculia alata	*Malvaceae*	Buddha coconut	Leaves	It is used as a food thickener and emulsifier. Mostly used for skin disease treatment, fever and minor wounds.

Result and Discussion

Curvularia sp

Data presented in Table 2 and depicted in Figure 1 reveals antifungal activity of six plant extracts were observed against the radial growth of *Curvularia* sp, where some of the plant extract showed great inhibition property against *Curvularia* sp (Plate 3*)*.

Among the plant extracts examined, T_3 (*Populus*) at 24 and 48 hours was found to be significantly reduced form T_1 (*Azadirachta indica*), T_0 (Control) and T_4 (*Musa acuminata*), whereas it was found non- significant form T_6 (*Phyllantus emblica*) and T_5 (*Bixa orellana*). At 72 and 96 hours T_3 was found to be significantly reduced form T_0, T_2, T_1, T_6, T_4 and T_5.

The result of this study shows that differential activities of plant extracts on the mycelial growth of *Curvularia* sp beacause many of these extracts have shown significant and aggressive inhibition against the mycelium growth of test fungi.

Curvularia sp can cause severe diseases of different plant taxa and are often tend to cause danger to agricultural production by reducing yield and quality. Thus, it is evident from the above study that the plant extracts of all the tested plants are found to be effective against tested fungi in their different concentrations. In some similar studies, several reports have shown that the acqueous of medicinal plants plays a significant role in controlling several phytopathogenic fungi (Jacob and Sivaprakasan, 1994; Arya *et al*., 1995; Lin *et al*., 2001; Okemo *et al.*, 2003 Choi *et al*., 2004) [15, 4, 17, 21, 7].

Table 2: Radial mycelial growth of *Curvularia* sp as affected by treatments.

Botanicals	Extract Concentration	Mycelium growth after Inoculation				Percent inhibition
		24h	48h	72h	96h	
Control (T_0)	10%	0.65	1.17	1.7	1.94	
Azadirachta indica (T_1)	10%	0.72	1.22	1.4	1.63	9.49%
Terminalia arjuna (T_2)	10%	0.58	1.04	1.59	1.88	7.29%
Populus (T_3)	10%	0	0	0	0	0
Musa acuminata (T_4)	10%	0.62	0.95	1.05	1.25	29.19%
Bixa orellana (T_5)	10%	0.133	0.23	0.65	0.93	35.45%
Phyllanthus emblica (T_6)	10%	0.33	0.8	1.35	1.52	27%
C.D		0.309	0.418	0.606	0.805	

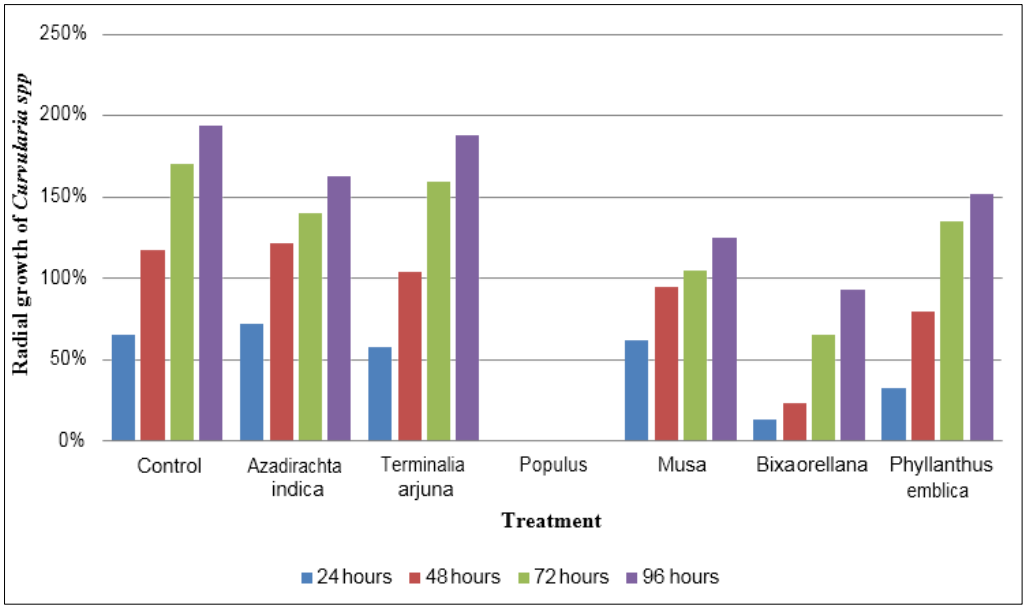

Fig 1: Effect of botanicals on the radial growth of *Curvularia* sp.

Plate 1: Leaf spot caused by *Curvularia* sp on *Bamboo ventricosa*

Plate 2: Conidia of *Curvularia* sp. (40x)

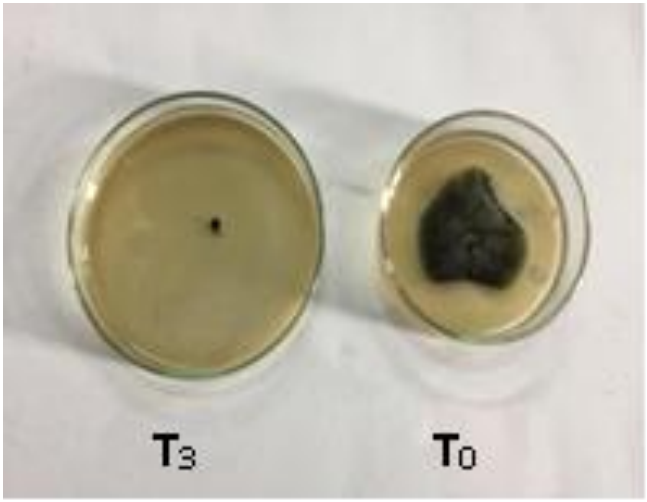

Plate 3: Mycelial growth of *Curvularia* sp on untreated Control (T_0) and *Populus* (T_3) treated PDA.

Meloidogyne incognita

Data presented in table 3 and depicted in figure 2 reveals that all the planlet extracts examined showed different inhibition in the hatching of M. incognita juveniles (Plate 5). Among the plant extract examined, T_8 (*Bixa orellana*) at 24 and 48hours was found to be significantly reduced from T_0 (Control), T_2 (*Olea europaea*), T_1 (*Magnifera indica*), T_9 (*Sterculia alata*), T_5 (*Musa acuminata*), T_3 (*Phyllantus emblica*) and T_4 *(Populus*), whereas it was found non-significant from T_6 (*Azadirachta indica*) and T_7 (*Terminalia arjuna*). At 72 hours T_8 was found to be significantly reduced from T_1, T_2, T_9, T_5, T_4 and T_0 while T_8 (*Bixa orellana*), T_7 and T_6 were found to be non-significant from each other but they were significantly reduced from T_1, T_2, T_9, T_5 and T_4.

All the plant extract showed different antimicrobial property. Some of the plants observed have shown strong inhibition in the hatching of J_2. Several authors (Okeniyi *et al*., 2010; Abbasi *et al*., 2008; Orisajo *et al*., 2007; Adegbite and Adesiyan, 2005 and Opereke *et al*., 2005) [22, 3, 23, 2, 24] had observed the potential of using plant extracts against the control of plant parasitic nematodes. The inhibitory effect observed in egg hatching might be due to the chemicals present in the extracts that possesses ovicidal and larvicidal properties (Adegbite and Adesiyan, 2005) [2]. Chitwood (2002) [8] recommended that the nemacidal properties of different plant species varies with plant species and cultivar, the plant tissue used, plant growth stage, application method and the nematode species tested.

T**able 3:** Effect of botanicals on the emergence *of Meloidogyne incognita* (J_2)

Plants	Extract Concentration	No. of eggs at 0 (zero) day	Release Juvenile after exposing to botanicals			Percent inhibition
			24 hours	48 hours	72 hours	
Control	50%	333	173	9.34	6.34	0%
Magnifera indica	50%	323	140	98.34	86	42.70%
Olea europaea	50%	754	152.67	75	63	48.65%
Phyllanthus emblica	50%	393	44.67	15	3.34	78.33%
Populus	50%	241	35	34	31	82.34%
Musa acuminata	50%	323	65.34	47	38.67	73.32%
Azadirachta indica	50%	451	4	2.34	2	95.58%
Terminalia arjuna	50%	316	3	2.34	0	97.17%
Bixa orellana	50%	226	2	0.34	0	98.76%
Sterculia alata	50%	229	95	82.34	60	58.07%
CD		N.S	6.614	3.694	4.308	

Plate 4: Galls of *Meloidogyne incognita*

Plate 5: *Meloidogyne incognita* (J_2) under microscope.

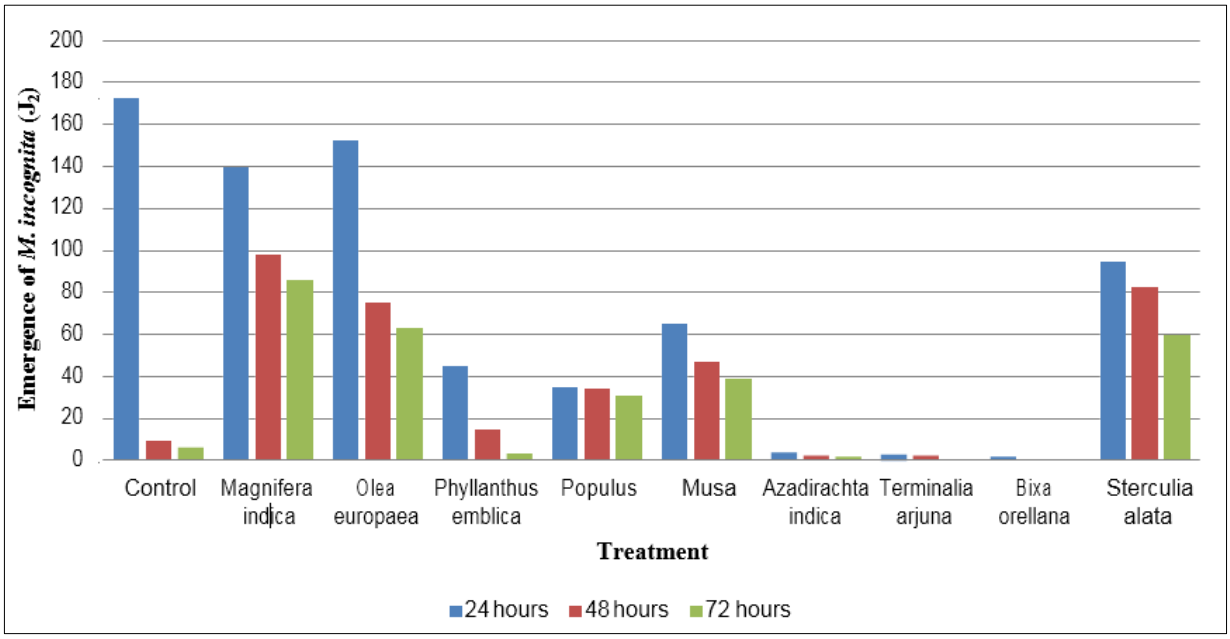

Fig 2: Effect of botanicals on the emergence *of Meloidogyne incognita* (J_2)

Conclusion

The study has demonstrated that medicinal plants namely *Magnifera indica*, *Olea europaea*, *Phyllantus emblica*, *Populus deltoides*, *Musa acuminata*, *Azadirachta indica*, *Terminalia arjuna*, *Bixa orellana*, *Sterculia alata* are very effective and suitable for inhibiting the *mycelial* growth of *Curvularia* sp. These plants could be utilized to field trials to improve their effectiveness in field condition. Thus it can be concluded that certain medicinal plant extracts can be a source of cost effective and effective nematicides of root knot nematodes.

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