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Antifungal activity of moringa (*Moringa oleifera*) leaf extracts against major plant pathogens

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Moringa (*Moringa oleifera* Lam.) belonging to family Moringaceae; popularly known as a miracle plant has several medicinal uses due to its antimicrobial, antihelminitic, anti-inflammatory, antiarthritic, antipyretic, antihepatotoxic and antidiabetic properties (Varmani and Garg 2014). Essential oils and plant extracts obtained from the moringa plant are even effective against phytopathogenic fungi with no side effects on humans and animals. The bioactive compound can be obtained from moringa leaves, stem, fruits as well as roots. Moringa leaves are a very good source of vitamin A, B, C and minerals (Tahiliani *et al.* 2000, Yadav *et al.* 2024). Besides various medicinal properties, leaves, flowers and seeds of the moringa plant are said to possess various antimicrobial properties. The antimicrobial property (against bacteria, fungus and virus) is attributed due to the presence of benzyl isothiocyanate, benzyl carbamate, benzyl thiocarboxamide, water-solublelectine protein, cationic polypeptides, niaziminin, niazinin, niazimicin, glycosides, phenylacetonitrile, moringine, proanthocyanidins, kaempferol, rhamnetin, kaempferitin, isoquercitrin, pterygospermin, spirochin and anthonine in different moringa plant part extracts including leaves, bark, flower, seed and root (Dhakad *et al.* 2019). Different solvent has been used for the extraction of bioactive compound conferring antimicrobial property from the moringa plant. Presence of benzoic acid, gallic acid and beta benzaldehyde in methanolic extract and niazirin, niazirinin, niazininins A and B in ethanolic extract of moringa leaves has been reported by researchers (Faizi *et al.* 1994, Manguro and Lemmen 2007). Their findings suggest that extraction using different solvents widen the array of active compounds obtained from leaf and seed extracts. They obtained significantly higher inhibition of bacterial growth when methanol water was used for extraction followed by methanol and ethyl acetate. The findings imply that the extraction solvent has a significant effect on the recovery of phenolic components, which in turn affects the antibacterial, antioxidant, and enzymatic properties of the moringa leaf extract. Furthermore, Singh *et al*. (2013) have demonstrated a direct positive link between the total phenol and flavonoid content in plant extracts and antimicrobial activity, indicating that the extraction solvent plays a critical role in the production of bioactive chemicals.

Fungal diseases damage crop and cause huge economic loss. About 10–40% loss in crop production is caused by pathogens, animals and weeds (Savary *et al.* 2012). *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. (syn., *Sclerotium rolfsii* Sacc.), *Fusarium* spp. and *Rhizoctonia solani* are important soil borne pathogens and cause huge economic loss to both cereal and vegetable crops (Bastakoti *et al.* 2017). Use of plant extract with antimicrobial properties is a feasible alternative to costly, non-ecofriendly chemical pesticides. Hence, the research was conducted to study the effect of different solvents used in the preparation of plant extracts on the extraction of bioactive antimicrobial compounds from moringa leaf.

The study was carried out during 2021–22 at ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh. Organic solvents, viz. water; hexane; acetone and ethyl acetate were used for the extraction of bioactive antimicrobial compounds from dried moringa leaves. Plant extracts obtained from moringa leaves were tested against three major soil pathogens, viz. *Athelia rolfsii; Fusarium* spp.; and *Rhizoctonia solani*. Negative effect of seed extracts and oil of moringa plant on linear growth, spore germination and dry growth weight of major soil pathogens including *Athelia rolfsii, Fusarium* spp. and *Rhizoctonia solani* has been recorded by El- Mohamedy and Abdallah (2014). Use of neem-based products for control of various plant diseases is immensely popular (Campos *et al.* 2016). Through this research, we wish to explore the possibility of using moringa based products for plant disease management.

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Preparation of moringa leaves extract: Moringa leaves were collected from the research farm of ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India. Leaf extract was prepared according to the protocols of Al husnan and Alkahtani (2016) with minor modifications. The fresh leaves were air-dried at room temperature to constant weight. The dried leaves were ground into a powder with a mortar and pestle. Leaf extracts were prepared using different solvents like distilled water, hexane, acetone and ethyl acetate. 25 g of the powdered leaves were extracted in 100 ml of the solvent in a conical flask. The leaf powder was soaked in the solvent overnight and later placed on a shaker at 120 rpm for 30 min. The suspension was filtered using a membrane filter (pore size 0.45 µm) before use.

Preparation of media: The bio-efficacy experiment was conducted in potato dextrose agar (PDA) medium supplemented with different concentrations of moringa leaf extract. The medium was prepared in a way that each flask contained 3.9 g of PDA medium (Hi-media) suspended in a total volume of 100 ml that includes the varying concentration of extracts and distilled water.

Preparation of test concentrations: 0.25, 0.50, 0.75 and 1.00 ml of moringa leaf extract were added to PDA medium as to obtain the final volume of 100 ml with the resulting formulation of 250, 500, 750 and 1000 mg/litre concentration. Media of each concentration from the conical flask was poured into sterilized petri-plates under aseptic conditions in a laminar flow chamber.

Inoculation and incubation: The efficacy of the moringa leaf extract was tested against three plant pathogens *Athelia rolfsii, Rhizoctonia solani* and *Fusarium oxysporum*. Pure culture of the pathogen grown on a PDA medium for a week was used for inoculation. A 5 mm thick disc of the fungus (spores and mycelium), uniformly cut from the plate was inoculated aseptically to the centre of the petri-plate. Inoculation of each pathogen deployed on petri-plates containing varying concentration of moringa leaf extract in different solvents. In the control plates, plant pathogens were inoculated into PDA medium without any plant extract. After inoculation, the plates were incubated in a BOD incubator at 28±1°C till the pathogens could completely cover the medium surface in the control plates.

Antifungal activity: Antifungal activity was evaluated as per the method reported in the literature (Majumder *et al.* 2020). The mycelial growth in the fungi (mm) both in treated (T) and control (C) was measured diametrically in three different directions and growth inhibition (I) was calculated as:

$$I\ (\%) = (C\text{-}T)/C \times 100$$

ED_{50} (Effective dose required for 50% inhibition of fungus growth) and ED_{50} (mg/litre) values (Effective dose for 50% inhibition) were calculated using SPSS Package (Probit Analysis) (Majumder *et al.* 2020).

Inhibitory effect against Athelia rolfsii: The moringa leaf extract possesses antifungal activity as it could significantly inhibit the mycelial growth of *Athelia rolfsii*. The antifungal activity varied with the change in the solvent used for the extraction of active compounds. The inhibition percentage in the water extract of moringa leaf was significantly higher when compared to other solvents used for extraction (Fig. 1). A gradient increase in the concentration of leaf extract in the PDA medium led to a gradual increase in antifungal activity as 30.74, 39.26, 64.44 and 88.89% mycelial inhibition was observed at 250, 500, 750 and 1000 mg/kg of leaf extract in water. The supplementation of leaf extract at 1000 mg/kg could inhibit mycelial growth by 88.89, 74.81, 69.26 and 12.22% when water, acetone, ethyl acetate and hexane, respectively were used as a solvent for the extraction of antifungal compounds. Significantly higher inhibition was observed when water was used for extraction followed by acetone, ethyl acetate and hexane. Antifungal activity

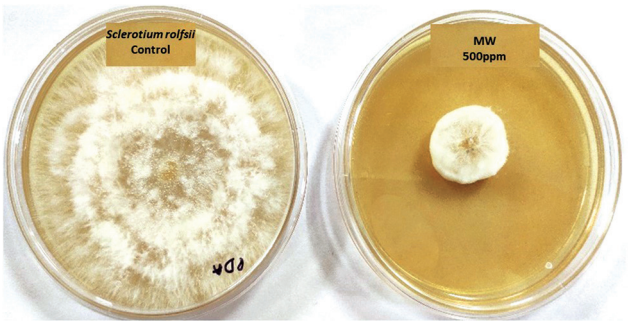

Fig. 1 Inhibition of mycelial growth of *Athelia rolfsii* in water extract (500 ppm) as compared to control.

Table 1 Fungicidal activity of the different solvent extracts against *Athelia rolfsii*

Concentration (mg/kg)	Inhibition percentage	SD	ED_{50} in mg/litre	Chi-square
		Water extract		
250	30.74	2.80	546.51	4.09
500	39.26	1.70		
750	64.44	2.22		
1000	88.89	1.11		
		Acetone extract		
250	21.85	1.70	624.35	6.87
500	31.85	1.70		
750	55.19	1.70		
1000	74.81	2.80		
		Ethyl acetate extract		
250	18.52	1.70	680.75	2.31
500	34.81	3.57		
750	50.00	3.33		
1000	69.26	2.80		
		Hexane extract		
250	1.11	1.11	3.81E+03	0.93
500	2.22	1.11		
750	5.93	1.70		
1000	12.22	1.11		

Table 2 Fungicidal activity of the different solvent extracts against *Rhizoctonia solani*

Concentration (mg/kg)	Inhibition percentage	SD	ED_{50} in mg/litre	Chi-square
		Water extract		
250	21.85	1.70	620.61	12.57
500	28.15	1.70		
750	55.56	1.11		
1000	78.15	1.70		
		Acetone extract		
250	12.59	2.31	670.57	8.05
500	26.30	1.70		
750	51.85	1.70		
1000	62.96	1.70		
		Ethyl acetate extract		
250	11.11	1.11	885.70	5.21
500	20.37	2.31		
750	38.89	1.11		
1000	61.48	1.70		
		Hexane extract		
250	1.11	1.11	1.27E+04	0.47
500	2.22	1.11		
750	3.33	1.11		
1000	7.04	3.57		

Table 3 Fungicidal activity of the different solvent extracts against *Fusarium* spp.

Concentration (ppm)	Inhibition percentage	SD	ED_{50} in mg/litre	Chi-square
		Water extract		
250	21.85	1.70	583.104	12.803
500	31.48	1.70		
750	57.04	1.70		
1000	82.59	2.31		
		Acetone extract		
250	15.19	1.70	629.91	11.75
500	28.52	2.31		
750	53.33	1.11		
1000	64.81	1.70		
		Ethyl acetate extract		
250	12.59	1.70	864.77	5.15
500	21.85	2.31		
750	40.37	1.70		
1000	62.22	1.11		
		Hexane extract		
250	1.11	1.11	9.23E+03	0.37
500	2.22	1.11		
750	4.07	0.64		
1000	7.78	2.94		

ED_{50} is the dose of the drug at which 50% of the maximum potential effect is produced. 50% inhibition is obtained at which dose.

in the hexane extract of the moringa leaf was very poor as supplementation of 1000 mg/kg of the extract could only inhibit mycelial growth by 12.22%. The ED_{50} value of 546.51 mg/litre was significantly lower in the water extract due to stronger mycelial inhibition. When the active component from moringa leaves was extracted using water, acetone, or ethyl acetate, respectively, an ED_{50} value of 546.51, 624.35, and 680.75 mg/litre was noted (Table 1).

Inhibitory effect against Rhizoctonia solani: *R. solani* is an important soil-borne pathogen that causes damping-off disease in various vegetable crops. The moringa leaf extracts were effective against the pathogen and could significantly retard its growth (Husnan *et al*. 2016). The percent mycelial inhibition was significantly higher when leaf extracts were prepared in water followed by acetone, ethyl acetate and hexane. At 1000 mg/kg concentration an inhibition of 78.15, 62.96, 61.48 and 7.04% in mycelial growth was observed when water, acetone, ethyl acetate and hexane were used for the extraction of active compound, respectively. A significantly lower ED_{50} value of 620.61 was observed when water was used as a solvent followed by acetone (670.57) and ethyl acetate (885.70) (Table 2).

Inhibitory effect against Fusarium *spp.*: The moringa leaf extract did show a negative effect on the growth of *Fusarium* spp. The percent mycelial inhibition ranged from 21.85–82.59% at different concentrations of the leaf extract when water was used as a solvent. Significantly higher inhibition was observed in the water extract followed by acetone and ethyl acetate. The ED_{50} value was computed to be 583.10, 629.91, and 864.77 when water, acetone and ethyl acetate, respectively were used for the extraction of bioactive compounds. Greater percentage of *Fusarium* spp. inhibition resulted in comparatively lower ED50 values, indicating the polar nature of the antimicrobial agents (Table 3). The moringa leaf extract with water showed better fungicidal activity against all the tested fungi followed by acetone > ethyl acetate > hexane. Hexane extract showed the least fungicidal activity against all the tested fungi. The result indicates that, the compound(s) that are active in moringa leaves may be polar in nature, as hexane has the lowest activity and water extract has the highest. There is a rule in chemistry that "Polar like polar", means polar solvent extract the polar compound from the moringa leaf (Husnan *et al*. 2016). The polarity index of water is highest i.e. 10.2 followed by acetone (5.1) > ethyl acetate (4.4) > hexane (0.1). When working in more polar solvents, external solvation of the ion pairs presumably increases. Accordingly, the inter ionic distance in the ion pairs should increase, leading to higher rate constants and higher extraction of the polar compounds. The opposite is expected for less polar solvents. When applied to several plant diseases, moringa leaf extracts exhibited differing degrees of antifungal activity. Further work is required to identify and isolate the secondary metabolites and active compound.

SUMMARY

Widespread use of chemical pesticides leads to soil and water pollution with harvest detrimental to human health. A viable alternative is to use environment-friendly substitutes that do not possess residue problems. Moringa is popularly known as "the tree of life" as each part of the tree has some medicinal attributes. An attempt was made to explore the use of moringa extracts for plant disease management. Moringa leaves were extracted with different solvents (water, acetone, ethyl acetate and hexane) and tested against major soil-borne pathogens (*Athelia rolfsii, Rhizoctonia solani* and *Fusarium* spp.). The leaf extracts showed an inhibitory effect on the growth of all the pathogens. Significantly higher inhibition was observed in plates where water was used for the extraction of bioactive compounds. The ED_{50} value of 546.51 mg/litre was reported for the pathogen *Athelia rolfsii* when water was used for extraction followed by acetone (624.25 mg/litre) and ethyl acetate (680.75 mg/litre). The inhibition percentage and ED_{50} value varied significantly with the change in the solvent used for the extraction purpose. According to the results, moringa leaf extracts can be utilized as a good substitute for chemical pesticides since they are efficient against plant pathogens.

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