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In Vitro EVALUATION OF ANTIFUNGAL ACTIVITY OF SOME HIGHER PLANTS AGAINST PHYTOPATHOGENIC FUNGI *Helminthosporium oryzae*

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ABSTRACT

Ethanolic extract of 31 samples of 25 higher plants were screened against the test Pathogen *Helminthosporium oryzae*. The leaf extract of *Madhuca indica* Linn showed maximum antifungal potential (90% mycelial inhibition) against the test pathogen , Next to it were the leaf extracts of *Heliotropium indicum* Linn, *Lowsonia inermis* Linn, *Typha angustata* Linn. and stem bark extracts of *Terminalia arjuna* Roxb. Which showed moderate antifungal activity (80% mycelial inhibition) against the test pathogen. Other plant extracts inhibited the growth of fungus less than 80% during our experimentation. The MIC(Minimum Inhibitory concentration) of Petroleum ether fraction of active plant extract was worked out, and it was found 200 ppm. At this 200 ppm (MIC), the fraction of *Madhuca indica* was found to be natural fungicidal activity in our experiment.

KEYWORDS: Higher Plants, Antifungal Activity, *Helminthosporium oryzae*

Global environment has a charming world of microorganisms which not only affect our health and daily life but also our wealth as well as natural resources. Disease control has undergone cyclic changes from very early times (Yadav *et al.*, 2026). Excessive use of synthetic chemicals sometimes irreversibly damages the ecosystem, contaminates soil surface and ground water, and finally disturbs the food chain, (Hooda *et al.*, 1998). Such chemicals have been found to exhibit teratogenicity, carcinogenicity, phytotoxicity, and residual effects (Beg *et al.* (2025). In comparison to synthetic pesticides the natural compounds provide less toxic. more systemic and easily biodegradable, free from environmental toxicity and have little or no side effect in human beings (Dixit *et al.*, 1976). The Rice crops is infected by a number of fungal pathogens namely *Alternaria podwickii, Helminthosporium oryzae, Cercospora oryzae, Phoma glumarum, Fusarium sp., Pyricularia oryzae* and *Ustilaginoidea virens*, (Sintoo *et al.*, 2023). Some workers also reported Brown spot of rice are damage great extent of Rice crops in eastern UP belongs Azamgarh. Losses due to fungal diseases have sometimes created history (Thera *et al.*, 2021). We cannot forget the famous Great Bengal Famine that occurred in the Bengal province of India during 1943, due to the Brown spot disease of rice caused by *Helminthosporium oryzae*. It is estimated that 2-3 million people died from starvation, malnutrition, and disease (Ghose *et al.*, 1960). Fungi adversely affect the quality as well as quantity of agricultural products. Brown spot of rice is caused by *Helminthosporium oryzae*, a global disease of rice causing great economic loss up to 90% and reducing grain quality and market value (Chakravarty *et al.*, 1977; David *et al.*, 2018.) recorded a reduction in yield ranging from 26-52%. This facts inspired us to investigate the antifungal potential in higher plants against *Helminthosporium oryzae* causing Brown leaf spot in Rice.

MATERIALS AND METHODS

Collection of Plant

Locally available parts (31 samples) of 25 higher plants were collected randomly from rural areas of Azamgarh and identified by departmental herbarium (listed in Table 1) and screened against test pathogen *Helminthosporium oryzae*.

Culture Medium and Plant Mother Extract

Potato Dextrose Agar medium was prepared for test pathogen *Helminthosporium oryzae*. The sterilized Potato Dextrose Agar medium was poured into pre-sterilized petri plates (3 mm diameter) to cover the base of the plate. The collected plant parts were thoroughly washed with 70 percent ethanol and after it sterilized distilled water, and excess water were removed with the help of cheese cloth. 5.0g (fresh weight) of each part was crushed to prepare pulp with the help of pestle and mortar. The pulp was kept immersed in 25 ml of 50% ethanol (v/v) overnight at room temperature, (Maximum constituents of the plant parts dissolved in the solvent due to the maximum polarity of ethanol and water (Yadav *et al.* 2026) and finally filtered through cheese cloth and obtained extract were re-filtered through filter paper (Mother extract, as 100% congratulations).The mother

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extract further diluted obtained 50% concentration. These two fraction, 50% and 100% were used for preparation of treatment disks.

Disc Preparation and Efficacy Testing

2.0 ml of the filtered extract of plant (pre - prepared 50% concentration and 100% concentration of mother extract) was soaked separately into a disc (diameter 15 mm) of Whatman paper No. 1 with the help of a hair drier. The process was repeated with sterilized forceps until the whole extract was completely impregnate in the disk. These both disk were designated as "treatment disks. Similarly Side by side, "control disks" were also prepared by impregnating 2.0 ml of 50% (v/v) ethanol only onto the Whatman paper No. 1 disks., for comparison mycelium growth in treatment and control culture plate. Potato dextrose agar medium was prepared and sterilized. The sterilized medium was poured into presterilized petri plates (3`` diameter), aseptically (Yadav *et al.*, 2026). In order to prevent bacterial contamination, the antibiotic Streptopencillin was added to the medium at the rate of 30 mg/litre and mixed thorough. The treatment and control disks were assayed for antifungal activity against the test pathogen by the "Modified paper disc technique" (Saxena *et al.*, 2002). The impregnated treatment disks were aseptically placed on the medium in the center of culture plates. One treatment disk was placed on each plate. Control plates were prepared by placing control disks on the agar medium in the center. Both treatment and control plates were inoculated with the test fungus *Helminthosporium oryzae*. For this purpose, a mycelial disk (5 mm in diameter), cut from the periphery of a 7-day old culture of the test fungus was aseptically inoculated. The plates were incubated at $30^{\circ}(\pm 1^{\circ}C)$ for six days, and observations were recorded on the seventh day. The experiments were kept in three replicates and repeated twice to confirm the result. Results were obtained by comparing the activity in treatment disks and control disks, by measuring the diameter of the area of grown fungus mycelium (Table 1).

The colony diameter of the test fungus in treatment and control sets was measured in mutually perpendicular directions, and fungitoxicity was recorded in terms of percent mycelial inhibition, calculated as per the formula (Saxana *et al.*, 2002):

$$\%myceliial\ inhibition = \frac{dc - dt}{dc} \times 100$$

Where:

- dc = Mean colony diameter of control
- dt = Men colony diameter of treatment

RESULTS

Different available plants and their parts were screened for antifungal activity. The results have been recorded in Table 1. Out of 31 samples of 25 higher plants, the leaf extracts of *Madhuca indica* Linn. showed maximum antifungal activity (90% mycelial inhibition) against the test pathogen (Figure 1). Next to it were the leaf extracts of *Heliotropium indicum* Linn, *Lawsonia inermis* Linn, *Typha angustata* Linn. and stem bark extracts of *Terminalia arjuna* Roxb. Which showed moderate antifungal activity (80% mycelial inhibition) against the test pathogen (Figure 2). Other plant extract showed less or no antifungal activities during our experimentation.

Figure 1: Comparision of Control and Treatment Plate

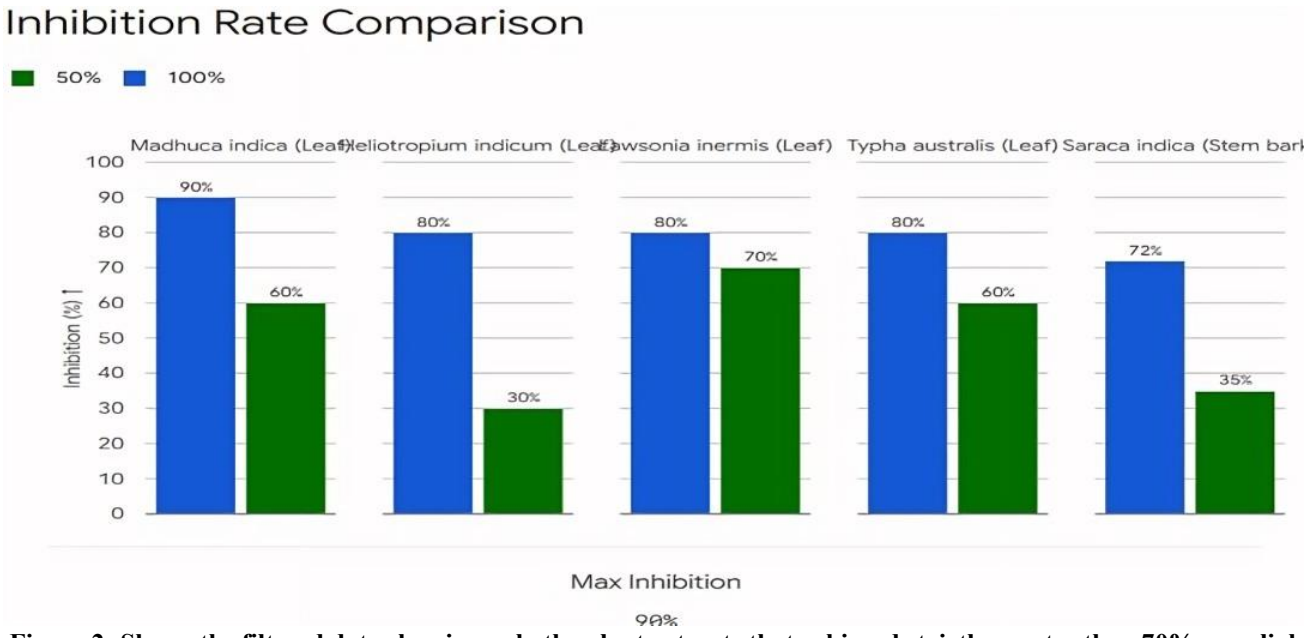

Figure 2: Shows the filtered data showing only the plant extracts that achieved strictly greater than 70% mycelial inhibition, along with an interactive bar chart comparing their effectiveness at both 50% and 100% concentrations.

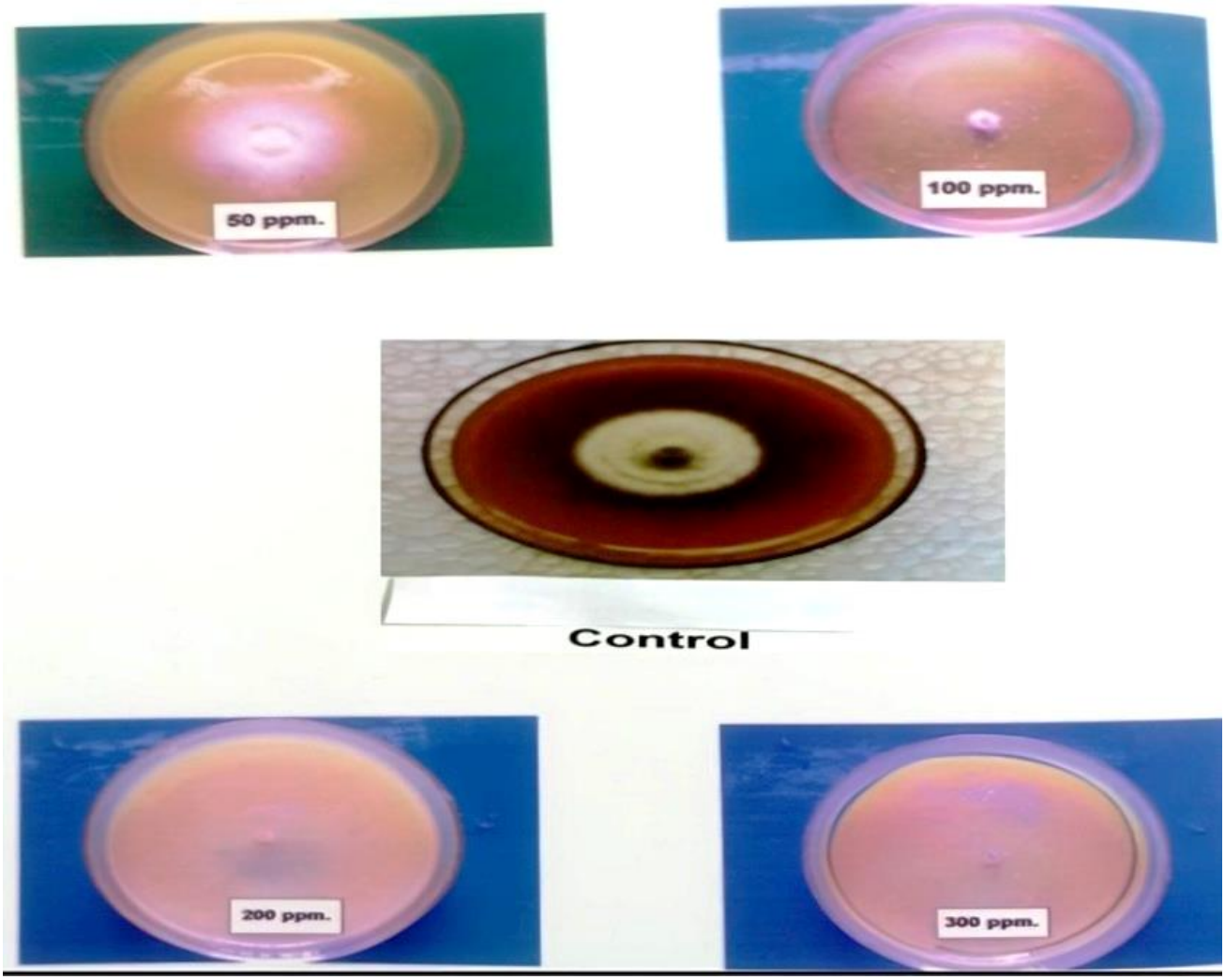

Figure 3: Photo plate showing MIC of Active fraction.

Table 1: Antifungal activity of different concentrations of plant extracts screened against test pathogen *Helminthosporium oryzae*

S.No.	Plant & their family	Plant used Part	% Mycelial Inhibition at 50% conc	% Mycelial Inhibition at 100% conc.
1	*Abutilon indicum* Linn (Malvaceae)	Leaf	10.00	35.00
2	*Achyranthes aspera* Linn. (Amaranthaceae)	Leaf	15.00	33.00
3	*Adhatoda vasica* Nees. (Acanthaceae)	Leaf	30.00	55.00
4	*Anthocephalus cadamba* (Roxb.) (Rubiaceae)	Leaf	32.00	67.00
5	*Argemone mexicana* Linn. (Papaveraceae	Leaf	26.00	32.00
6	*Emblica officinalis* Gaertn. (Euphorbiaceae)	Leaf	20.00	65.00
7	*Euphorbia hirta* Linn. (Euphorbiaceae)	Leaf	00.00	37.00
8	*Heliotropium indicum* Linn. (Boraginaceae)	Leaf	30.00	80.00
9	*Ipomoea fistula* Mart. (Convolvulaceae)	Leaf	20.00	31.00
10	*Jatropha gossypifolia* Linn. (Euphorbiaceae)	Leaf	20.00	43.00
11	*Lawsonia inermis* Linn (Lythraceae)	Leaf	70.00	80.00
12	*Madhuca indica* Linn. (Sapotaceae)	Leaf Stem bark	60.00 00.00	90.00 14.00
13	*Melia azadirach* Linn. (Meliaceae)	Leaf Stem bark	40.00 50.00	50.10 56.00
14	*Mentha piperita* Linn. (Lamiaceae)	Leaf	50.00	62.00
15	*Nicotiana plumbaginifolia* Viv. (Solanaceae)	Leaf	00.00	37.00
16	*Pongamia pinnata* forst.(Leguminaceae)	Leaf	50.00	65.00
17	*Punica granatum* Linn. (Punicaceae)	Leaf	00.00	47.00
18	*Rumex dentatus* Linn. (Polygonaceae)	Leaf	00.00	47.00
19	*Saraca indica* Linn. (Caesalpinaceae)	Leaf Stem bark	30.00 35.00	68.00 72.00
20	*Solanum nigrum* Linn. (Solanaceae)	Leaf	00.00	43.00
21	*Tamarindus india* Linn.(Caesalpinaceae)	Leaf Stem bark	20.00 00.00	36.00 16.00
22	*Terminalia arjuna* Roxb.(Combretaceae)	Leaf Stem bark	00.00 20.00	35.00 68.00
23	*Typha australis* Schum.(Typhaceae)	Leaf	60.00	80.00
24	*Thevetia peruviana* Pers. (Apocynaceae	Leaf	00.00	35.00
25	*Xanthium strumarium* Linn (Compositae)	Leaf Root	20.00 20.00	40.00 35.00

Fraction of the Ethanolic Extracts

It is a general principle that different organic solvents have different polarities, i.e. efficiency to dissolve specific compounds into them (Saxena *et al.*, 2002). On the basis of this principle the hydroethanolic extracts of active plants were treated with different organic solvents one by one non- polar to polar salvent (Sahoo *et al.*, 2010). The petroleum ether fraction was found antifungal against the test pathogen. Minimum Inhibitory concentration (MIC) of Petroleum ether fraction of active plant extract was found antifungal against the test pathogen and MIC was found at 200 ppm. At this MIC (200 ppm) the fraction of active plant extract was found topossess fungicidal activity during our experiments (Figure 3).

DISCUSSION

Thus, the leaf extract of *Madhuca indica* Linn can be used as a good source of herbal fungicides against *Helminthosporium oryzae* causing Brown leaf spot of Rice. Various workers have screened a large number of plants belonging to angiosperms for their antimicrobial properties. (Dhar *et al.*, 1973; Hooda *et al.*, 1998; Yadav *et al.*, 2009; Yadav *et al.*, 2026). Mostly, crude extracts or expressed juices of plants have been used to evaluate antifungal and antibacterial potential (David *et al.*, 1918; Beg *et al.*, 2025). However, some workers have used

organic extractives (Lopez *et al.*, 2002). During the course of the present assay, 50% ethanol was used as an extractive. The use of 50% ethanol ensures extraction of maximum compounds as well as facilitates further purification of active fraction (s) (Dhar *et al.*, 1973; Saxena *et al.*, 2002). Aqueous extracts of expressed juices may lose their efficacy due to degradation of active constituents by continued enzymatic activity, (Saxena *et al.*, 2002). MIC (Minimum Inhibitory concentration) of some compounds has been studied by several workers and found to vary considerably. (Harish *et al.*, 2008; Sahoo *et al.*, 2010; Yadav *et al.*, 2026). The findings thus suggest that the leaf of *Madhuca indica* Linn. having strong fungicidal can be exploited as potent fungicide against test pathogen.

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DECLARATION

The authors declare that the manuscript is an original work, and has not been previously published. All sources and literature used during this research have been appropriately cited and acknowledge and no financial aid from any funding agency. The entire work was completely self funded by the author.

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