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In Vitro ANTIFUNGAL ACTIVITIES OF SOME HIGHER PLANTS AGAINST *Fusarium oxysporum* F. *Lycopersici* (SACC.) SNYDER AND HANSEN CAUSING WILT OF TOMATO

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ABSTRACT

Ethanolic extract of 32 samples (different available parts) of 25 plant species were screened for antifungal activity against *Fusarium oxysporum f. lycopersici* causing wilt of tomato by "Modified disc technique". The leaf extract of *Madhuca indica* J.F. Gmel. was found to exhibit maximum fungitoxicity against test pathogen. The leaf extract was further fractionated in different organic solvents, and antifungal activity was recorded. Only petroleum ether fraction inhibited the test pathogen. To obtain Minimum Inhibitory concentration of Petroleum ether fraction was further evaluate, and MIC was found 2.5$\mu g/ml$. At this MIC, the fraction was found to be fungicidal nature.

KEYWORDS: Higher Plant Extract, Antifungal Activity, *Fusarium oxysporum f. lycopersici*

Tomato crop is a important vegetable grown in very large scale in Azamgarh belonging eastern Uttar Pradesh. The tomato crop suffers a great loss due to Wilt disease. Our biosphere has a charming world of microorganisms which affect our daily life and also natural resources. Losses due to fungal disease have sometimes created a great history (Yadav and Beg, 2026). The use of synthetic pesticides for control of crop diseases sometimes damages the ecosystem, and damages the beneficiary microflora and contaminates water and soil, finally disturbs the food chain of ecosystem. Synthetic chemicals and fungicides have been found to carcinogenic, phytotoxic and residual effects (Bajaj and Ghose, 1975). The use of resistant varieties has been found to be the safest method for preventing plant diseases, but it has its own limitations. All diseases of major food and plantation crops may not be controlled solely by using resistant varieties (Prasad, 1964). Further, the pathogen may develop resistance to a drug or may develop new physiological races. Due to this reason, many of the resistant varieties of crops and fungicides soon become out of date. Therefore, there is a pressing need to investigate new sources of eco-friendly effective and innocuous fungicides (Brandes, 1967). Hooda and Srivastava (1998) have reported that natural fungicides are free from environmental toxicity. In comparison to synthetic pesticidal compounds, the natural compounds provide less phytotoxic, and easily biodegradable fungitoxic compounds; Kumar *et al.*, 1995; Saxena *et al.*, 2002). Yadav *et al.*, (2010) and Yadav *et al.*, (2026) have mentioned that the green plants appear to be reservoir of bio toxicants and constitute inexhaustible source of innocuous pesticides. In the present study, *Fusarium oxysporum f. Lycopersici* has been chosen as the test pathogen as it causes serious wilt disease in tomato crops, incurring heavy loss to the agriculturists of the eastern region of U.P. to which Azamgarh belongs. Therefore it was considered desirable to find antifungal activity in the plants of this locality. Various available higher plant parts were screened against the test fungus *Fusarium oxysporum f. lycopersici* causing wilt of tomato crops.

MATERIALS AND METHODS

Locally available plant parts of higher plants were collected randomly from various places of the Azamgarh city belongs eastern U.P. of India. The collected parts were surface washed with 70% ethanol and finally with distilled water. 5.0g (fresh weight) of each part was crushed by a warring blender. The pulp was kept immersed in 25 ml of 50% ethanol (v/v) overnight at room temperature and finally filtered through filter paper. Maximum constituents of the plant parts dissolved in the solvent due to the maximum polarity of ethanol and water. 2.0 ml of the filtered extract was soaked 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 impregnated in the disk. These were designated as "treatment disks. "Control disks" were also prepared by impregnating 2.0 ml of 50% (v/v) ethanol only onto the Whatman paper No. 1 disks. Czapek Dox agar culture medium was prepared and sterilized. In order to prevent bacterial contamination, the antibiotic Streptopencilin was added to the culture medium at the rate of 30 mg/litre and mixed thoroughly (Saxena *et al.* 2002). 10 ml. molten

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culture medium was poured into pre sterilized petri dishes, (3 inch diameter). 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 treatment disks were aseptically placed in the center of culture plates. One treatment disk was placed on each plate. Control plates were prepared similarly using control disks on the Czapek dox agar medium in the center of culture plate. Both treatment and control plates were inoculated with the test fungus *Fusarium oxysporum f .lycopersici.* For this purpose, a mycelial disk (5mm 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 (fig.2) by measuring the diameter of the area of grown fungus. The colony diameter of the test fungus in treatment and control sets was measured in mutual perpendicular directions, and fungitoxicity was recorded in terms of percent mycelial inhibition, calculated as per the formula (Saxana *et al.* 2002):

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

Where:

- $dc = Mean\ colony\ diameter\ of\ control$
- $dt = Mean\ colony\ diameter\ of treatment$

RESULTS

Different available plants and their parts were screened for their antifungal activity. The results have been recorded in Table 1**.** Out of 32 samples, the leaf extracts of *Madhuca indica* J.F. Gmel. showed maximum antifungal activity (90% mycelial inhibition) against the pathogen (Fig.1). Next to it were the leaf extracts of *Heliotropium indicum* Linn, *Lawsonia inermis* Linn, *Typha angustata* Bory & chaub. 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% in the experiments.

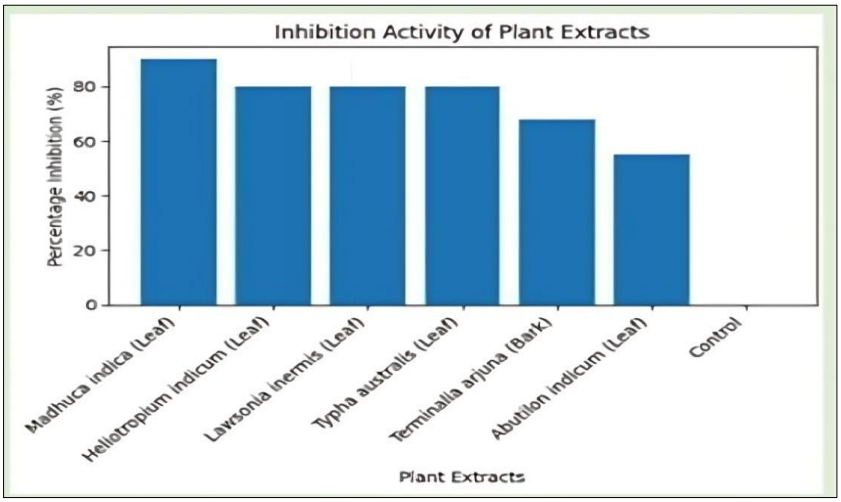

Figure 1: Antifungal activity of some higher plants against *F.oxysporum f. lycopersici* showing 90% Inhibition by extracts of *Madhuca indica* as compared to control

Figure 2: Control plate showing no mycelial inhibition and treatment plate showing maximum. Inhibition of mycelium against *Fusarium oxysporum f.lycopersici*

Table 1: Antifungal activity of different plant extracts screened against test pathogen *Fusarium oxysporum f. Lycopersici.*

S.No.	Name of plants	Families	Part used	%Mycelial Inhibition
1	*Abutilon indicum* Linn.	Malvaceae	Leaf	55.00
2	*Achyranthes aspera* Linn.	Amaranthaceae	Leaf	33.00
3	*Adhatoda vasica* Nees.	Acanthaceae	Leaf	55.00
4	*Anthocephalus cadamba* (Roxb)Mig.	Rubiaceae	Leaf	67.00
5	*Argemone mexicana* Linn.	Papaveraceae	Leaf	32.00
6	*Emblica officinalis* Gaertn	Euphorbiaceae	Leaf	65.00
7	*Euphorbia hirta* Linn.	Euphorbiaceae	Leaf	37.00
8	*Heliotropium indicum* Linn.	Boraginaceae	Leaf	80.00
9	*Ipomoea fistulosa* Mart.	Convolvulaceae	Leaf	33.00
10	*Jatropha gossypifolia* Linn	Euphorbiaceae	Leaf	43.00
11	*Lawsonia inermis* Linn.	Lythraceae	Leaf	80.00
12	*Madhuca indica* J.F. Gmel	Sapotaceae	Leaf Stem bark	90.00 55.00
13	*Melia azadirach* Linn	Meliaceae	Leaf Stem bark	50.00 56.00
14	*Mentha piperita* Linn.	Lamiaceae	Leaf	62.00
15	*Nicotiana plumbaginifolia* Viv.	Solanaceae	Leaf	37.00
16	*Pongamia pinnata* Linn.	Fabaceae	Leaf Stem bark	65.00 80.00
17	*Punica granatum* Linn.	Punicaceae	Leaf	47.00
18	*Rumex dentatus* Linn.	Polygonaceae	Leaf	47.00
19	*Saraca indica* Linn.	Caesalpiniaceae	Leaf Stem bark	68.00 72.00
20	*Solanum nigrum* Linn.	Solanaceae	Leaf	43.00
21	*Tamarindus indica* Linn.	Caesalpiniaceae	Leaf Stem bark	36.00 28.00
22	*Terminalia arjuna* Roxb.	Combretaceae	Leaf Stem bark	35.00 68.00
23	*Typha angustata* Bory & Chaub.	Typhaceae	Leaf	80.00
24	*Nerium indicum.* Linn.	Apocynaceae	Leaf	36.00
25	*Xanthium strumarium*Linn.	Compositae	Leaf Root	40.00 35.00

Fraction of the Ethanolic Extracts

Minimum Inhibitory concentration of some extracts has been studied by different workers and they have found considerable variation. (Yadav *et al.*, 2026). Likewise in present Studies the ethanolic extract of active plant extract was also fractionated by differential solubility as followed by Dixit *et al.*, 1976. It is a general principle that different organic solvents have different polarities, i.e. Efficiency to dissolve specific chemicals and compounds into them. (Saxena *et al.*, 2002). The ethanolic extracts of *Madhuca indica* was fractionated by different organic solvents one by one in sequence from non- polar to polar. All the fraction were tested against the test pathogen only petroleum ether fraction of active plant was found antifungal against the test pathogen. During our experimentation Minimum Inhibitory concentration of Petroleum ether fraction was found antifungal at the concentration of $2.5 \mu g/mL$. However to fix the exact dose requires further investigations.

DISCUSSION

The leaf extract of *Madhuca indica* J.F. Gmel. can be exploited as a good source of herbal fungicides against wilt disease of tomato crops. Various workers have screened a large number of plants belonging to angiosperms for their antimicrobial potential. Mostly, aqueous, crude extracts or expressed juices of herbs have been used to evaluate antimicrobial activity (Srivastava and Yadav, 2008; Beg *et al.*, 2025; Yadav *et al.*, 2026). However, some workers have used organic extractives

(Lopez *et al.*, 2002). Aqueous extracts of herbs or crude juices may lose their potency due to degradation of active constituents by continued enzymatic activity (Srivastava *et al.*, 2008; Yadav and Beg, 2026). During the course of our experiment, 50% ethanol was used as an extractive and also ethanol ensures that the extraction of maximum compounds as well as facilitates further purification of active fraction(s) (Dhar *et al.*, 1973; Saxena *et al.*, 2002). In the present study tomato wilt incurring heavy loss of the farmers of the eastern region of Uttar Pradesh to which Azamgarh belongs.

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