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In vitro Antifungal Activity of Weed Plants of Eastern Uttar Pradesh against *Fusarium oxysporum f. lycopersici* (Sacc.) Snyder & Hansen

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Abstract
Today, global agriculture relies heavily on synthetic pesticides, with over 3.5 billion kilograms used annually. This widespread use has been linked to various health risks, ranging from acute poisoning to chronic illnesses, including multiple forms of cancer, Alzheimer's, Parkinson's, nervous disorders, infertility, leukemia etc. As a safer alternative, plants produce a different armory of secondary metabolites such as flavonoids, alkaloids, and terpenoids that possess fungicidal properties and biological activity against phytopathogenic fungi. These natural compounds show a sustainable and eco-friendly option for disease management. *Fusarium oxysporum* f. *lycopersici*, the causal organism of tomato wilt, also affects other crops like potatoes, peppers, brinjals, and legumes. Interestingly, many weed plants are rich sources of bioactive secondary metabolites, such as flavonoids, terpenoids, and phenolics, which are both effective against diseases and environmentally safe. The present study investigated the antifungal activity of aqueous extracts obtained from various parts of 40 weed plant species, belonging to 22 families. Among the tested samples, the inflorescence extract of *Hyptis suaveolens* of the family Lamiaceae showed the maximum antifungal efficacy, inhibiting 98.19% of mycelial growth of *F. oxysporum* f. *lycopersici*. The stem extract of the same plant also shows strong activity, with a 94.31% inhibition. Other significant results were the stem of *Spilanthes acmella,* whole plant of *Lathyrus aphaca*, and leaf of *Amaranthus gracilis* also exhibited notable antifungal properties with 85.60, 81.35 and 81.26 percent, respectively.

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INTRODUCTION

Fusarium oxysporum f. *Lycopersici* is a fungus that primarily causes the wilt disease in tomatoes. However, it can also infect other crop plants such as potato, chili, brinjal and legumes. In India, tomatoes are the third most important vegetable crop after potatoes and onions. They are mainly grown in the Rabi season in plain and during the summer and rainy seasons in hilly areas. Tomato crops are unsafe for many diseases caused by fungi, bacteria, viruses and nematodes. Among them, Fusarium wilt is one of the most destructive disease, causing serious economic

losses wherever tomatoes are grown (Sudhamoy *et al.,* 2009). Some *Fusarium* species produce harmful mycotoxins in food and agricultural products, making the problem worse (Nayaka *et al*., 2008; Mudili *et a*l., 2014). Therefore, farmers use cultural practices, chemical treatments, and biological methods to control Fusarium wilt disease in tomatoes (Singh *et al*. 2015).

Today, more than 3.5 billion kilograms of synthetic pesticides are used to control the various diseases of plants world-wide, which is overuse of chemical pesticides (Pretty and Zareen (2015). It has raised serious health and environmental concerns. Improper pesticide use has been linked to various health issues, such as different types of cancer (brain, breast, prostate, bladder, colon etc.) (Rani *et al*., 2021), Alzheimer's (Frisoni *et al*., 2022), Parkinson's (Perrin *et al*., 2021), nervous system damage (Sanborn *et al*., 2007), infertility (Bhardwaj *et al*., 2018), leukemia (Rafeeinia, 2022), and diabetes (Hernández-Mariano, 2022). Additionally, excessive pesticide use can harm the beneficial micro-flora in the soil, reducing soil quality and productivity (Pretty and Zareen, 2015).

As a safer and extra sustainable opportunity, scientists are exploring the use of herbal compounds produced by plants. These include flavonoids, alkaloids, and terpenoids, which are secondary metabolites that have antimicrobial properties. These plant based compounds are environmentally friendly, easily biodegradable into non-toxic materials, and can be used as natural pesticides in integrated pest management (IPM) programs (Salgado-Garciglia *et al*., 2008; Ribera and Zuniga, 2012). Many flowering plants have proven strong antifungal properties towards phytopathogenic fungi (Rates, 2001), making them a promising solution for disease management strategies in agriculture.

Weeds, defined as plants considered undesirable in a specific context, also cause negative impact on crop plants due to their high consumption of space and water (Holm *et al.* 1979) estimated that around 8,000 species of weeds are harmful to crops, reducing crop yield. However, weeds have gained attention for their potential role in sustainable plant disease management. Many weed species are rich in bioactive secondary metabolites along with flavonoids, terpenoids, and phenolic compounds. These herbal substances inhibit the growth of large number of fungi and are taken into consideration environmentally secure alternatives to synthetic fungicides (Nwachukure and Umechuruba, 2001).

From the above, it is far clear that there is a demand to research new fungitoxicants which are without problems biodegradable and provide inexhaustible resources (Beye, 1978). The district of Azamgarh in eastern U.P. has a wealthy flora, and knowledge of indigenous plants is nicely documented (Srivastava, 1986; Chandra 1984; Beg *et al*. 2006). Therefore, the present work was carried out to analyze the *in vitro* potential antifungal activity of few common weed plants towards *the Fusarium oxysporum* f. *lycopersici* (Sacc.) Synder & Hansen, the causal organism of tomato wilt.

MATERIALS AND METHODS

A total of forty weed plant species from twenty-two different plant families, were collected from various locations across the Azamgarh district in Eastern Uttar Pradesh (Table 1). Fresh Plant species of collected weeds were taxonomically identified in the department of Botany, Shibli National College, Azamgarh, using *Duthie's Flora* (1903–1929). For each samples, 20 grams of plant material were surface sterilized using 70% ethanol and subsequently rinsed with sterilized distilled water. The plant tissues were then crushed by using a sterile mortar and pestle, and extracted in 20 ml of sterilized distilled water. The resulting mixture was filtered aseptically through double-layered muslin cloth to obtain the aqueous plant extract. The antifungal activity of the extracts was evaluated using the poisoned food technique described by Grover and Moore (1962). Five milliliters of each extract were mixed with 5 ml of molten, sterilized Czapek's Dox Agar medium in pre-sterilized Petri dishes and swirled to ensure uniform mixing. For the control group, the same medium was supplemented with an equal volume of sterilized distilled water. A 4 mm diameter mycelial disc, taken from the edge of a 7-day-old culture of *Fusarium oxysporum* f. *lycopersici*, was aseptically placed at the center of each Petri dish. Each treatment, including the

control, was maintained in triplicate. The fungal toxicity of the extract was assessed by calculating the following formula used earlier (Mohana and Raveesha, 2007).

Percent inhibition of mycelial growth = $\frac{C-T}{C}$x100

Where

C = Average mycelial growth in control petri dish,

T = Average mycelial growth in treatment petri dish

RESULTS AND DISCUSSION

Table 1: Screening of various parts of weed plant extracts on mycelial inhibition (%) of *Fusarium oxysporum f. lycopersici* (Sacc.) Snyder & Hansen.

Name of the Plants	Family	Part Used	Mycelial inhibition (%)
*Abutilon indicum (*Linn.) Sweet	Malvaceae	Leaf	29.41 ±0.5550
*Abutilon indicum (*Linn.) Sweet	Malvaceae	Stem	26.38±0.8047
*Abutilon indicum (*Linn.) Sweet	Malvaceae	Fruit	23.35±1.8113
Acalypha indica Linn.	Euphorbiaceae	Stem	41.62±1.6300
Achyranthes aspera Linn.	Amaranthaceae	Leaf	8.00±0.5488
Ageratum conyzoides Linn.	Asteraceae	Leaf	23.14±0.8327
Amaranthus gracilis Desf.	Amaranthaceae	Leaf	81.26±1.1431
Amaranthus spinosus Linn.	Amaranthaceae	Leaf	78.12±1.0158
Ammannia senegalensis Lamk.	Lythraceae	Leaf	27.61±1.4901
Antigonon leptopus Hook. & Arn.	Polygonaceae	Leaf	45.07±0.9997
Antigonon leptopus Hook. & Arn.	Polygonaceae	Flower	38.43±1.3544
Argemone mexicana Linn.	Papaveraceae	Leaf	37.54±1.3607
Avena sterilis Linn.	Poaceae	Leaf	42.30±0.7582
Blumea obliqua Druce	Asteraceae	Leaf	73.10±1.0880
Chenopodium album Linn.	Chenopodiaceae	Leaf	46.23±1.2100
Chenopodium album Linn.	Chenopodiaceae	Inflorescence	40.26±1.2054
Chenopodium murala Linn.	Chenopodiaceae	Leaf	69.27±1.2745
Clerodendrum indicum (Linn.) Kuntze	Verbenaceae	Leaf	41.97±1.3691
Clerodendrum indicum (Linn.) Kuntze	Verbenaceae	Flower	30.38±1.3335
Clitoria ternatea Linn.	Fabaceae	Leaf	39.28±1.2838
Coccinia cordifolia (Linn.) Cogn.	Cucurbitaceae	Leaf	20.65±1.1789
*Cocculus hirsutus (*Linn.) Diels	Menispermaceae	Leaf	69.29±1.5852
Convolvulus arvensis (Linn.) Diels	Convolvulaceae	Leaf	19.04±1.0145
*Coronopus didymus (*Linn.) Sm.	Brassicaceae	Whole Plant	23.17±1.0130
Chrozophora verbascifolia A. Juss.	Euphorbiaceae	Leaf	61.41±1.0772
Cynodon dactylon (Linn.) Pers.	Poaceae	Whole Plant	49.00±0.9766
Cyperus compressus Linn.	Cyperaceae	Leaf	49.16±0.7643
Cyperus rotundus Linn.	Cyperaceae	Leaf	43.26±0.6397
Desmodium gangeticum (Linn.) DC.	Fabaceae	Leaf	66.14±0.8756
Euphorbia dracunculoides Lamk.	Euphorbiaceae	Leaf	80.06±0.8877
Euphorbia hirta Linn.	Euphorbiaceae	Leaf	51.98±0.6987
Euphorbia hirta Linn.	Euphorbiaceae	Stem	50.11±0.9710
Evolvulus alsinoides Linn.	Convolvulaceae	Leaf	29.11±0.7924
Gnaphalium indicum Linn.	Asteraceae	Leaf	27.47±0.6496
Hyptis suaveolens Piot.	Lamiaceae	Leaf	50.83±0.5914
Hyptis suaveolens Piot.	Lamiaceae	Stem	94.31±0.4230
Hyptis suaveolens Piot.	Lamiaceae	Inflorescence	98.19±0.7486

Lantana camara Linn.	Verbenaceae	Leaf	42.41±0.9051
Lantana camara Linn.	Verbenaceae	Stem	27.04±0.9016
Lantana camara Linn.	Verbenaceae	Flower	21.25±1.0534
Lathyrus aphaca Linn	Fabaceae	Whole Plant	81.35±0.8351
Launaea asplenifolia Hook. f.	Asteraceae	Leaf	32.14±0.6795
Lindenbergia indica (Linn.) Kuntze	Scrophulariaceae	Leaf	71.20±1.0372
Mazus japonicus (Thunb.) Kuntze	Scrophulariaceae	Whole Plant	41.49±1.3194
Malvastrum coromandelianum (Linn.) Garcke.	Malvaceae	Leaf	76.02±0.9317
Malvastrum coromandelianum (Linn.) Garcke.	Malvaceae	Stem	54.69±0.6503
Nepeta hindostana (Roth.) Haines.	Lamiaceae	Leaf	43.18±0.4406
Nepeta hindostana (Roth.) Haines.	Lamiaceae	Stem	32.20±0.9803
Nicotiana plumbaginifolia Viv.	Solanaceae	Leaf	40.47±0.9641
Nicotiana plumbaginifolia Viv.	Solanaceae	Stem	39.00±0.9893
Parthenium hysterophorus Linn.	Asteraceae	Leaf	17.61±0.6070
Peristrophe bicalyculata (Retz.) Nees.	Acanthaceae	Leaf	22.57±0.6206
Peristrophe bicalyculata (Retz.) Nees.	Acanthaceae	Stem	12.71±0.9289
Polygonum plebeium R. Br.	Polygonaceae	Whole Plant	78.72±0.6563
Portulaca oleracea Linn.	Portulacaceae	Leaf	8.78±0.6648
Ranunculus sceleratus Linn.	Ranunculaceae	Leaf	61.97±0.8180
Rumex dentatus Linn.	Polygonaceae	Leaf	61.37±0.9984
Spilanthes acmella Linn.	Asteraceae	Stem	85.60±0.6051

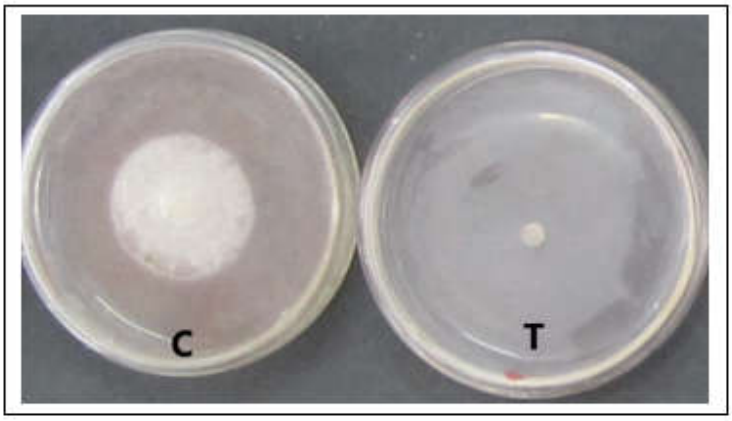

Figure 1: Showing mycelial inhibition of *Fusarium oxysporum f. lycopersici* (Sacc.) Snyder & Hansen by Inflorescence of *Hyptis suaveolens* Piot.

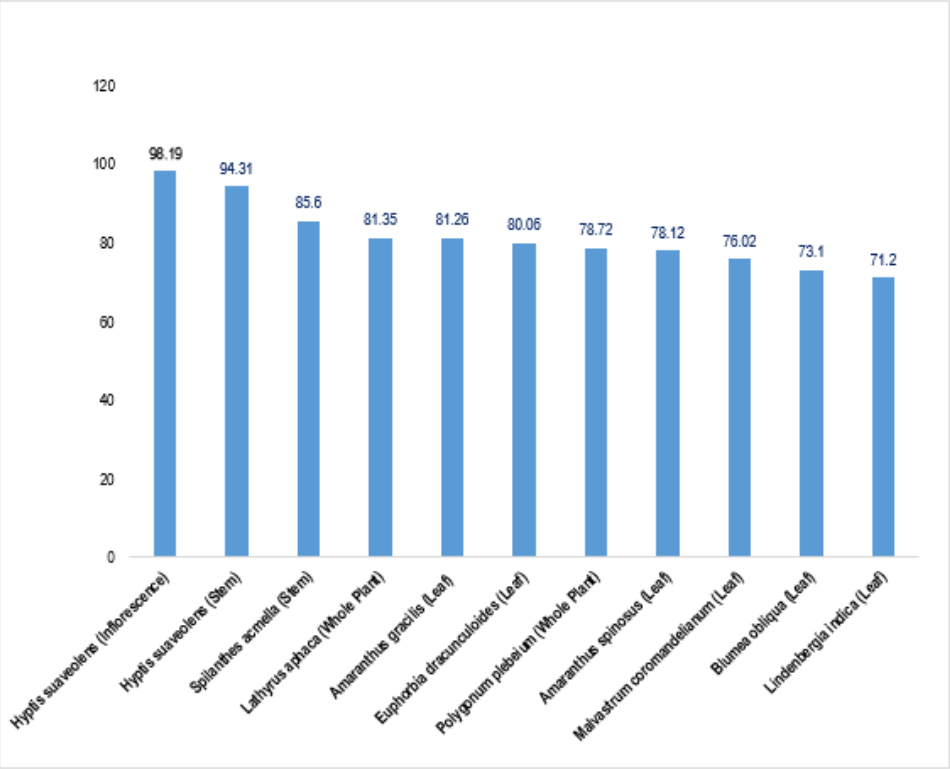

Figure 2: Samples of ten plant species showing highest antifungal activity

A total of 58 aqueous extracts prepared from 40 various weed plant species, representing 22 plant families, were tested for their efficacy against *Fusarium oxysporum* f. *lycopersici*. The results revealed notable variation in the effectiveness of the extracts, with all tested plants exhibiting some degree of mycelial growth inhibition compared to the untreated control. Among the tested extracts, the inflorescence of *Hyptis suaveolens* belonging to the family Lamiaceae showed the highest antifungal activity, suppressing 98.19% of mycelial growth. This was followed by the stem extract of the same species, which showed 94.31% inhibition. Other noteworthy extracts included the stem of *Spilanthes acmella* (85.60% inhibition), the whole plant of *Lathyrus aphaca* (81.35%), and the leaf of *Amaranthus gracilis* (81.26%). The degree of mycelial inhibition varied across different species and families, suggesting that the fungitoxic potential is species-specific. Similar inter-family differences in antifungal efficacy were previously reported by Hajek (1961), who noted that members of the family Fabaceae exhibited stronger antifungal activity compared to grasses (Gramineae). The antifungal properties observed in these samples are likely due to the presence of various bioactive phytochemicals. These compounds, which may act independently or synergistically, have been shown in previous studies to inhibit fungal growth (Field *et al*., 2006; Giordani *et al*., 2008). Many such substances are classified as phytoalexins. Plant produced compounds that are synthesized in response to pathogen attack, known as phytoalexins include- oligosaccharides, isoflavonoids, terpenoids, and acetylenic acids, all of which have demonstrated potent antimicrobial activity. Importantly, most of these plant-derived compounds are fully biodegradable and leave no toxic residues in the environment, making them a promising alternative to synthetic fungicides and a valuable addition to sustainable plant disease management strategies.

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