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Antifungal assay of some common plants against *Colletotrichum falcatum* (Went) Butler

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Abstract

Sugarcane is an important commercial crop that is frequently affected by a wide range of plant pathogens, leading to significant losses in productivity and quality. Among these diseases, red rot caused by *Colletotrichum falcatum* (Went) Butler is one of the most destructive and widely distributed diseases worldwide. Although synthetic pesticides are commonly used for the management of plant pathogens, their prolonged and excessive use poses serious concerns, including environmental pollution, toxicity to non-target organisms, and ecological imbalance.

In recent years, attention has shifted toward the use of plant-based natural products as safer and eco-friendly alternatives. The present study was conducted to evaluate the in vitro antifungal activity of ethanolic extracts obtained from different parts of 42 plant species against *Colletotrichum falcatum*.

The results demonstrated that most of the plant extracts exhibited varying degrees of antifungal activity. Among them, the ethanolic leaf extract of *Oxalis* Linn. (Family: Oxalidaceae) showed the highest inhibition (99.0%) of mycelial growth of the test pathogen. The next highest inhibition (97%) was recorded with the leaf extract of *Ageratum conyzoides* L. (Family: Asteraceae).

The findings of the present investigation suggest that plant extracts possess significant fungitoxic properties, which can be effectively utilized as natural and eco-friendly alternatives to synthetic fungicides. Further studies on the isolation and characterization of active compounds are required to develop sustainable strategies for plant disease management.

Key words : Antifungal assay. *Colletotrichum falcatum, Saccharum afficinarum,* Antifungal activity.

Agriculture plays a vital role in the global economy; however, plant diseases cause significant losses, estimated to be around 21% worldwide in crop production[1]. These diseases not only reduce the yield but also adversely affect the quality of agricultural produce, thereby impacting both farmers and the agrobased industries.

Sugarcane (*Saccharum officinarum* L.) is one of the most important commercial crops of India, and the country is among the leading producers of sugar globally. However, sugarcane cultivation is severely affected by several diseases, among which red rot disease, caused by *Colletotrichum falcatum* (Went) Butler, is considered one of the most destructive. The pathogen produces toxic metabolites (phytotoxins), such as destruxins, which contribute to disease development and crop damage.

Various methods are currently employed to manage *C. falcatum*, including chemical control (fungicides), cultural practices, nutrient management, and biological control strategies. Among these, synthetic fungicides are widely used due to their immediate effectiveness. However, their long-term application poses serious concerns, including environmental pollution, health hazards, toxicity to non-target organisms, residual effects, and the development of resistance in pathogens[9]. Moreover, many synthetic chemicals have been reported to exhibit carcinogenic, mutagenic, and teratogenic effects, posing risks to human health and ecosystems.

In contrast, plant-derived products have been used traditionally for the management of plant diseases and are gaining renewed attention due to their eco-friendly and biodegradable nature. These natural compounds are generally considered safer, with minimal or no harmful side effects compared to synthetic pesticides[5,8]. Most plant-based antimicrobial compounds degrade rapidly in the environment, thereby reducing pesticide pollution[3].

Figure 1. *Oxalis corniculata* L.

Figure 2. *Ageratum conyzoides* L.

Figure 3. Infected stem of sugarcane showing acervuli

The concept of Integrated Pest Management (IPM) emphasizes the use of environmentally sustainable approaches, including resistant cultivars, biological control agents, and plant-based products, to maintain pest populations below damaging levels[11]. Plants produce a wide range of secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, many of which possess strong antimicrobial properties. These compounds can play a significant role in controlling phytopathogens and may also have applications in treating microbial infections in humans and animals[13].

In recent years, there has been an increasing interest in exploring natural plant products as safe and effective alternatives to synthetic fungicides for controlling plant diseases. The district of Azamgarh (Eastern Uttar Pradesh) is rich in plant biodiversity, and the traditional knowledge of indigenous plant species has been well documented[2,4,12].

Therefore, the present study was undertaken to evaluate the in vitro antifungal potential of selected plant extracts against *Colletotrichum falcatum* (Went) Butler, the causal agent of red rot disease in sugarcane. The findings of this study may contribute to the development of eco-friendly and sustainable disease management strategies.

Collection of Plant Material :

A total of forty-two plant samples representing 42 different species belonging to 24 families were collected from various locations in the Azamgarh district of Eastern Uttar Pradesh, India. The fresh aerial parts (leaves, stems, and other available parts) of selected plants were collected during the study period.

The taxonomic identification of all plant species was carried out in the Department of Botany, Shibli National P. G. College, Azamgarh, with the help of standard floristic literature, particularly Duthie's[6] Flora of the Upper Gangetic Plain.

Preparation of Plant Extracts :

The collected plant materials were thoroughly washed with sterile distilled water to remove dust and contaminants. About 5.0 g of fresh plant material from each sample was crushed into fine pulp using a sterile mortar and pestle or a Waring blender.

The obtained pulp was transferred into sterile containers and mixed with 2.5 ml of 50% ethanol (v/v). The mixture was kept overnight at room temperature for proper extraction of bioactive compounds. After incubation, the extract was filtered through Whatman No. 1 filter paper to obtain a clear filtrate.

Preparation of Discs :

Sterile Whatman No. 1 filter paper discs (10 mm diameter) were prepared. Each disc was impregnated with 2.0 ml of the plant extract using sterile forceps and allowed to dry with the help of a hair dryer under aseptic conditions. These were designated as treatment discs.

Similarly, control discs were prepared by impregnating filter paper discs with 2.0 ml of 50% ethanol (v/v) only.

Preparation of Culture medium :

Czapek Dox Agar (CDA) medium was prepared according to standard protocol and sterilized by autoclaving. The sterilized medium was poured into pre-sterilized Petri plates (approximately 5 mm depth) and allowed to solidify.

Figure 4. Pure culture media of *Colletotrichum falcatum*

Test Pathogen :

The fungal pathogen *Colletotrichum falcatum* (Went) Butler was used as the test organism. A 7-day-old culture of the fungus was maintained on suitable agar medium.

Antifungal Assay (Modified Paper Disc Technique) :

The antifungal activity of plant extracts was evaluated using the modified paper disc method. A 5 mm diameter mycelial disc taken from the actively growing margin of a 7-day-old fungal culture was aseptically placed at the center of each Petri plate containing solidified medium.

The treatment and control discs were then aseptically placed on the medium surface in separate plates. Each plate contained only one disc. All plates were incubated at 37 ± 2°C for 48 hours.

During incubation, the bioactive compounds present in the treatment discs diffused into the medium. If antifungal compounds were present, they inhibited the growth of the test pathogen, resulting in a clear zone of inhibition around the disc.

Each treatment and control was performed in triplicate to ensure accuracy and reproducibility.

Measurement of Antifungal Activity :

The antifungal activity was determined by measuring the diameter of fungal colony growth in both treatment and control plates. The percentage inhibition of mycelial growth was calculated using the formula given by Mohana and Raveesha[10]:

Percent inhibition of mycelial growth $= \frac{DC - DT}{DC} \times 100$

Where:

- DC = Mean colony diameter in control
- DT = Mean colony diameter in treatment

Observations :

Table-1. Screening of plants for their antifungal activity against Colletotrichum falcatum by modified paper disc technique.

S. no.	Botanical name	Family	Plant parts used	Mycelial inhibition (%)
1.	*Adhatoda vasica* Linn.	Acanthaceae	Leaf	72%
2.	*Allium sativum* Linn.	Amaryllidaceae	Leaf	57%
3.	*Amaranthus viridis* Linn.	Amaranthaceae	Leaf	55%
4.	*Chenopodium album* Linn.	Amaranthaceae	Leaf	34%
5.	*Achyranthus aspera* Linn.	Amaranthaceae	Leaf	45%
6.	*Catharanthus roseus* (L.) G.Don	Apocynaceae	Leaf	53%
7.	*Calotropis gigantea* Linn.	Apocynaceae	Leaf	60%
8.	*Carissa carandas* Linn.	Apocynaceae	Leaf	41%
9.	*Coriandrum sativum* Linn.	Apiaceae	Leaf	36%
10.	*Colocasia esculenta* Linn.	Araceae	Leaf	45%
11.	*Ageratum conyzoides* Linn.	Asteraceae	Leaf	97%
12.	*Acmella paniculata* (Wall. ex DC.) R.K. Jansen	Asteraceae	Leaf	53%
13.	*Xanthium strumarium* Linn.	Asteraceae	Leaf	60%
14.	*Tagetes erecta* Linn.	Asteraceae	Leaf	33%
15.	*Eclipta alba* Linn.	Asteraceae	Leaf	70%
16.	*Parthenium hysterophorus* Linn.	Asteraceae	Leaf	70%
17.	*Tecoma stans* Linn.	Bignoniaceae	Leaf	59%
18.	*Cannabis sativa* Linn.	Cannabaceae	Leaf	70%
19.	*Carica papaya* Linn.	Caricaeae	Leaf	44%
20.	*Acalypha indica* Linn.	Euphorbiaceae	Leaf	67%
21.	*Ricinus communis* Linn.	Euphorbiaceae	Leaf	50%
22.	*Euphorbia hirta* Linn.	Euphorbiaceae	Leaf	50%
23.	*Trigonella foenum-graecum* Linn.	Fabaceae	Leaf	47%
24.	*Vachellia nilotica* (L.) P.J.H. Hurter & Mabb.	Fabaceae	Leaf	63%
25.	*Clerodendrum infortunatum* Linn.	Lamiaceae	Leaf	80%
26.	*Ocimum sanctum* Linn.	Lamiaceae	Leaf	60%
27.	*Punica granatum* Linn.	Lythraceae	Leaf	35%
28.	*Morus alba* Linn.	Moraceae	Leaf	41%
29.	*Moringa oleifera* Lam.	Moringaceae	Leaf	42%

30.	*Oxalis corniculata* Linn.	Oxalidaceae	Leaf	99%
31.	*Phyllanthus niruri* Linn.	Phyllanthaceae	Leaf	52%
32.	*Anagallis arvensis* Linn.	Primulaceae	Leaf	58%
33.	*Rumex crispus* Linn.	Polygonaceae	Leaf	47%
34.	*Portulaca oleracea* Linn.	Portulaceae	Leaf	67%
35.	*Nigella sativa* Linn.	Ranunculaceae	Leaf	42%
36.	*Citrus limonum* Linn.	Rutaceae	Leaf	34%
37.	*Murraya koenigii* Linn.	Rutaceae	Leaf	54%
38.	*Verbascum phlomoides* Linn.	Scorphulariaceae	Leaf	33%
39.	*Datura stramonium* Linn.	Solanaceae	Leaf	57%
40.	*Solanum nigrum* Linn.	Solanaceae	Leaf	53%
41.	*Solanum lycopersicum* Linn.	Solanaceae	Leaf	58%
42.	*Laportea aestuans* (L.) Chew	Urticaceae	Leaf	45%

A total of 42 ethanolic extracts obtained from 42 different plant species belonging to 24 families were evaluated for their antifungal activity against *Colletotrichum falcatum* (Went) Butler. The results revealed a significant impact of various plant extracts on the mycelial growth of the test pathogen when compared with the untreated control.

A considerable variation in antifungal activity was observed among the tested plant species (Table-1). However, all the plant extracts exhibited inhibitory effect, indicating the presence of fungitoxic constituents.

Among all the tested samples, the ethanolic leaf extract of *Oxalis cornicutata* Linn. (Family: Oxalidaceae) showed the highest antifungal activity, with approximately 99% inhibition of mycelial growth. The next most effective extract was obtained from the leaves of *Ageratum conyzoides* L. (Family: Asteraceae), which exhibited 97% inhibition.

Significant antifungal activity was also recorded in the leaf extracts of *Adhatoda vasica* and *Clerodendrum infortunatum*, showing 72% and 80% inhibition, respectively. The degree of mycelial inhibition varied considerably among different plant families as well as species, suggesting differential distribution of bioactive compounds.

Such variation in fungitoxicity among plant families has also been reported by Hajek[7], who observed that members of the Leguminosae family exhibited higher antifungal activity compared to Poaceae.

The antifungal potential of these ethanolic plant extracts may be attributed to the presence of various phytochemicals, which can act either individually or synergistically. Compounds that inhibit the establishment and growth of plant pathogens are commonly referred to as phytoalexins. Several plant-derived substances, including oligosaccharides, flavonoids, terpenoids, and acetylenic acids, are known to act as strong elicitors of phytoalexin production.

An important advantage of plant-based antifungal agents is that they are biodegradable and eco-friendly, leaving minimal or no harmful residues, thereby reducing environmental pollution caused by synthetic pesticides.

Based on the present investigation, it can be concluded that most of the tested plants possess fungitoxic compounds capable of inhibiting the mycelial growth of *Colletotrichum falcatum*. Further research focusing on the antifungal spectrum, isolation, and chemical characterization of active compounds is necessary. Such studies may provide a promising alternative to synthetic fungicides and contribute to sustainable plant disease management.

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