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ANTIFUNGAL ACTIVITY OF AQUEOUS STEM EXTRACT OF *CYNANCHUM ACIDUM* (ROXB.)OKEN. AGAINST *ASPERGILLUS* AND *FUSARIUM* SPECIES ASSOCIATED WITH MAIZE

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ABSTRACT

The antifungal efficacy of the aqueous extract prepared from the stem of *Cynanchum acidum* (Roxb.) Oken. was evaluated *in vitro* against eight fungal pathogens associated with maize, namely *Aspergillus flavus*, *A. niger*, *A. candidus*, *A. flavipes*, *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, and *F. graminearum*. The extract was tested at concentrations of 10%, 20%, 30%, 40%, and 50%. The results revealed a concentration-dependent inhibition of fungal growth, with the maximum antifungal activity observed at 50% concentration. At this level, the extract exhibited the highest mycelial growth inhibition against *A. flavus* (100%), followed by *F. oxysporum* (90.2%), *F. solani* (89.5%), *F. moniliforme* (87.7%), *A. candidus* (78.9%), *A. niger* (78.0%), *A. flavipes* (73.2%), and *F. graminearum* (52.1%). Moderate inhibitory effects were recorded at 20–40% concentrations, whereas the least inhibition was observed at 10%. In comparison, the standard synthetic fungicides Bavistin and Thiram demonstrated complete (100%) inhibition of all test fungi. These findings indicate that the aqueous stem extract of *C. acidum* possesses significant antifungal potential, particularly at higher concentrations, and may serve as a promising natural alternative to synthetic fungicides in the management of maize-associated fungal pathogens.

KEYWORDS: *C. acidum*, *Fusarium*, *Aspergillus*, Anifungal activity.

INTRODUCTION

The use of medicinal plants as therapeutic agents dates back more than five millennia, as evidenced by ancient written records from early civilizations in China, India, and the Near East. Indeed, the use of plants for healing purposes is as old as humanity itself. Over the centuries, plants have served as an essential source of medicinal compounds, and numerous bioactive constituents with pharmacological potential have been isolated from natural plant sources. Many of these plants and their extracts have been employed in traditional medicine systems for their antibacterial, antifungal, and other therapeutic properties.

Medicinal plants continue to play a vital role in global healthcare, with approximately 80% of the world's population depending on traditional remedies that are predominantly plant-based. Herbal formulations, prepared from single or multiple plant ingredients, are often complex in composition and variable in their chemical nature. The vast plant kingdom still harbors a significant number of species with unexplored medicinal potential. The therapeutic value of these plants is primarily attributed to the presence of bioactive phytoconstituents such as alkaloids, tannins, flavonoids, and phenolic compounds, which exert specific physiological and pharmacological actions in the human body.

In recent years, the extensive and often indiscriminate use of antimicrobial drugs for the treatment of infectious diseases has led to the emergence of multiple drug-resistant pathogens. Despite the continuous development of new antibiotics by the pharmaceutical industry, resistance among microbial species continues to rise, thereby diminishing the efficacy of conventional chemotherapeutics. This growing concern has prompted renewed scientific interest in the screening of medicinal plants for their potential antimicrobial and antifungal activities.

India, recognized as one of the twelve mega-biodiversity hotspots of the world, possesses an extensive diversity of medicinal plants and serves as a rich repository of phytochemical resources. The exploration of these bioresources is crucial for identifying novel plant-derived antimicrobial agents with potential therapeutic and agricultural applications.

In this context, the present study was undertaken to evaluate the *in vitro* antifungal activity of the aqueous extract of the stem of *Cynanchum acidum* (Roxb.) Oken. (family: Apocynaceae),

commonly known as the cork tree, against seed-borne fungal pathogens of maize. The study aims to assess the potential of this plant extract as a natural alternative to synthetic fungicides in managing fungal infections associated with maize seeds.

MATERIALS AND METHODS

Test plant: Fresh and healthy stems of *C. acidum*. were collected from the Mysore region, Karnataka, India. The plant material was initially washed thoroughly two to three times with running tap water to remove adhering debris and then rinsed once with sterile distilled water to ensure surface cleanliness. The stems were shade-dried under ambient laboratory conditions and subsequently air-dried at room temperature on sterile blotting paper. The dried material was then used for the preparation of aqueous extracts as described previously.[7]

Extraction

Aqueous extract: One hundred grams of thoroughly washed and air-dried healthy stems of *C. acidum*. were macerated with 100 mL of sterile distilled water using a Waring blender (Waring International, New Hartford, CT, USA) for 5 minutes. The resulting macerate was filtered through a double layer of sterile muslin cloth and subsequently centrifuged at 4000 × *g* for 30 minutes. The supernatant was then filtered through Whatman No. 1 filter paper and sterilized at 120 °C for 10 minutes. This filtrate served as the 100% aqueous mother extract. The extract was stored aseptically in a sterile brown bottle at 5 °C until further use.[8]

Test fungi: Eight fungal species isolated from maize (*Zea mays* L.) seeds were used as test organisms to evaluate the antifungal activity of the plant extract. These included four species of *Fusarium* viz, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, and *F. solani* and four species of *Aspergillus* viz, *A. niger*, *A. flavus*, *A. flavipes*, and *A. candidus*. All fungal isolates were purified, maintained on Potato Dextrose Agar (PDA) medium, and sub-cultured periodically to ensure viability and purity prior to use in the antifungal assays.

Antifungal activity assay by poisoned food technique

Antifungal Activity Assay

The antifungal activity of the aqueous stem extract of *C. acidum* was evaluated using the poisoned food technique. Czapek Dox Agar (CDA) medium was amended with different concentrations (10%, 20%, 30%, 40%, and 50%) of the aqueous extract. The amended media were poured into sterile Petri plates and allowed to cool and solidify under aseptic conditions. Mycelial discs (5 mm in diameter) from seven-day-old cultures of *Fusarium* and *Aspergillus*

species were aseptically placed at the center of each plate. The plates were incubated at 25 ± 1 °C for seven days. CDA medium without the plant extract but containing an equivalent volume of sterile distilled water served as the control. Each treatment was replicated three times. The radial mycelial growth (colony diameter) was measured in millimeters, and the percentage inhibition of mycelial growth, if any, was determined by the formula PI = C-T/ C x 100, where C = diameter of control colony and T = diameter of treated colony.[9] The minimal inhibitory concentration (MIC) for each test fungus was determined following the procedure described by.[10] The data obtained were subjected to statistical analysis using Analysis of Variance (ANOVA), and mean separation was performed using Tukey's Honest Significant Difference (HSD) test to determine significant differences among treatments.

Chemical Fungicides (Standards)

Two commercial synthetic fungicides, Bavistin and Thiram, were evaluated for their antifungal efficacy using the same poisoned food technique and served as reference standards for comparison with the plant extract.

STATISTICAL ANALYSIS: he experimental data were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects. Percentage values were first transformed to their corresponding arcsine square root values prior to analysis to stabilize variance and normalize the data distribution. Mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test at a significance level of $P \leq 0.05$ to identify statistically significant differences among treatments.

RESULT

The aqueous stem extract of *C. acidum*. exhibited varying degrees of antifungal activity against the eight test fungi, with the percentage of mycelial growth inhibition increasing proportionally with extract concentration (Table 1).

Among the tested species, *Aspergillus flavus* showed the highest susceptibility to the extract, recording 93.4% inhibition at 50% concentration. The inhibition decreased progressively with lower concentrations, showing 81.3%, 68.9%, 39.2%, and 19.2% inhibition at 40%, 30%, 20%, and 10% concentrations, respectively. *Fusarium oxysporum* exhibited the next highest inhibition, with 90.2% at 50% concentration, followed by 72.9%, 51.3%, 38.0%, and 18.1% inhibition at 40%, 30%, 20%, and 10% concentrations, respectively.

Fusarium solani showed 89.5% inhibition at 50% concentration and 16.2% inhibition at 10%, with moderate activity observed at intermediate concentrations (20–40%). Similarly, *F. moniliforme* recorded 87.7% inhibition at 50% concentration and 16.2% at 10%. *Aspergillus candidus* exhibited moderate inhibition, showing 78.9% at 50% and 11.2% at 10% concentration, while *A. niger* demonstrated 78.0% and 10.0% inhibition at the same respective concentrations.

The least antifungal activity was observed in *A. flavipes* and *F. graminearum*, with 73.2% and 52.1% inhibition, respectively, at 50% concentration. In general, the percentage inhibition increased progressively with the concentration of the aqueous extract.

In comparison, the commercial synthetic fungicides Bavistin and Thiram, when tested at their recommended concentration of 2.0%, showed complete inhibition (100%) of mycelial growth in all test fungi, indicating their superior efficacy relative to the plant extract.

Table 1: Antifungal activity of aqueous extract of seeds of stem of *C. acidum* (Roxb.) Oken. against seed borne fungi of maize.

Fungi	Mycelial Growth Inhibition(%)						
	Concentration of Aqueous Extract					Bavistin	Thiram
	10%	20%	30%	40%	50%	2%	2%
F. graminearum	10.0^{a} ±0.1	18.9^{b} ±0.1	28.3^{c} ±0.1	36.1^{d} ±0.1	52.1^{e} ±0.1	100.0^{f} ±0.0	100.0^{f} ±0.0
F. moniliforme	16.2^{a} ±0.0	29.3^{b} ±0.1	42.7^{c} ±0.2	65.2^{d} ±0.1	87.7^{e} ±0.0	100.0^{f} ±0.0	100.0^{f} ±0.0
F. oxysporum	18.1^{a} ±0.1	38.0^{b} ±0.0	51.3^{c} ±0.0	72.9^{d} ±0.0	90.2^{e} ±0.1	100.0^{f} ±0.1	100.0^{f} ±0.1
F. solani	16.2^{a} ±0.0	28.9^{b} ±0.0	45.9^{c} ±0.1	68.9^{d} ±0.0	89.5^{e} ±0.2	100.0^{f} ±0.0	100.0^{f} ±0.2
A.niger	10.0^{a} ±0.2	23.9^{b} ±0.0	43.9^{c} ±0.0	61.1^{d} ±0.0	78.0^{e} ±0.1	100.0^{f} ±0.2	100.0^{f} ±0.1
A.flavus	19.2^{a} ±0.0	39.2^{b} ±0.0	68.9^{c} ±0.0	81.3^{d} ±0.0	93.4^{e} ±0.0	100.0^{f} ±0.0	100.0^{f} ±0.0
A. flavipes	12.4^{a} ±0.0	21.0^{b} ±0.0	38.4^{c} ±0.0	56.8^{d} ±0.1	73.2^{e} ±0.1	100.0^{f} ±0.0	100.0^{f} ±0.0
A.candidus	11.2^{a} ±0.0	23.9^{b} ±0.0	43.1^{c} ±0.0	60.1^{d} ±0.0	78.9^{e} ±0.1	100.0^{f} ±0.1	100.0^{f} ±0.1

- Values are the mean of three replicates, ±standard error
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey‟s HSD
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

DISCUSSION

Post-harvest fungal contamination of food commodities is commonly controlled through the application of synthetic chemical fungicides. However, the extensive use of these synthetic agents has raised significant concerns due to their potential adverse effects, including carcinogenicity, teratogenicity, and residual toxicity. Moreover, many of these fungicides exhibit undesirable characteristics such as high acute toxicity, long environmental persistence, bioaccumulation in the food chain, and non-selective action that adversely affects both target and beneficial organisms.[11–14] It has been reported that less than 0.1% of the pesticides applied to crops actually reach the target pests, while the remaining 99.9% disperses into the environment, leading to widespread ecological contamination.[15]

To mitigate these harmful impacts, recent research has focused on identifying alternative, eco-friendly, and sustainable strategies for pest and pathogen control. Biological and botanical approaches are gaining prominence as they offer safer, non-polluting, and biodegradable options for crop protection.[16] Plant-derived products, in particular, have attracted global attention as potential sources of novel chemotherapeutic agents in plant disease management. Their advantages include non-phytotoxicity, systemic action, easy biodegradability, and the ability to stimulate host plant metabolism.[17]

In the present study, the aqueous stem extract of *C. acidum*. demonstrated strong antifungal activity against all tested fungal species of *Fusarium* and *Aspergillus*, indicating its potential as a natural antifungal agent. The observed inhibition was concentration-dependent, with maximum efficacy at 50% extract concentration. These findings suggest that *C. acidum* contains bioactive compounds capable of suppressing the growth of pathogenic fungi associated with maize seeds.

Further studies are warranted to evaluate the antifungal potential of extracts prepared using different solvents and to isolate, purify, and characterize the specific bioactive constituents responsible for the observed antifungal activity. Such investigations could contribute to the development of plant-based fungicides that are both effective and environmentally safe alternatives to conventional synthetic chemicals.

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