Efficacy of plant formulations of *Andrographis paniculata* against soil-borne *Fusarium verticilloides*

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ABSTRACT

Most fungi show mutual association with the plants; some survive on dead and decaying residues of plants and animals. On the other hand, a few of the soil-borne fungi show pathogenicity to plants and act as plant pathogens. These fungi show destruction and spoilage of crop plants, which ultimately reduces crop production. Fungi cause critical damage to crops during germination and growth. Soil-borne fungus *Fusarium verticilloides* is a fungal pathogen of crop plants. The rot and wilt diseases caused by *Fusarium sp.* significantly reduce the production of crops. Therefore, it is a necessity to search for sustainable strategies to overcome the pollution of soil and water bodies by synthetic chemical fungicides for the control of fungal pathogens. Dressing of seeds or seedlings of crop plants with extracts of *Andrographis paniculata* is strategised against fungal infections. The fungi were isolated from the infected ginger crop from agricultural fields, identified by morphological characters and 18S rRNA sequencing. The plant was identified and specimen was deposited. Plant extraction was done followed by bioactive compound separation. In order to determine the efficacy of plant extracts against fungi, the *in vitro* effect of leaf extracts by agar agar-based antifungal techniques was performed in the present study. The ethanolic extracts and individual andrographolide extracted from *A. paniculata* significantly reduced the mycelial growth of *Fusarium verticilloides*. The present study would help to increase the productivity of crops by inhibiting the infections by soil-borne fungal pathogens during the germination and growth stages of crops without adverse effects on the environment.

Keywords: *Andrographis paniculata,* soil-borne fungal pathogens, *Fusarium verticilloides,* 18S rRNA, antifungal potential, sustainable fungicide

1. INTRODUCTION

An increasing population leading to increased demand for food, and to fulfil the demand, agricultural producers' use of numerous synthetic chemical fertilizers and pesticides in agricultural fields has also increased. The crops are significantly affected by the insects and pests. And to control insects and pests, strategies are used. Overuse of chemical pesticides is done by agriculturists. Overall, the use of all kinds of pesticides in agricultural pest control involves harsh chemicals. The improper applications of these chemical pesticides lead to numerous disorders in humans, wildlife [1], and aquatic organisms. Among these, fungal pest management is one of the challenges faced by farmers. And the chemical fungicides get directly exposed to the soil by means of seed treatments before seeding the crop seeds. These fungicides inhibit fungi, but at the same time, they contaminate soil and groundwater, which ultimately affects humans, livestock, the aquatic and soil ecosystems. So, to provide a safe alternative, it is necessary to find new solutions to the synthetic chemical fungicides. Herbal fungicides because of their biodegradability could be an alternative to synthetic chemical pesticides. *Andrographis paniculata,* a plant belonging to the family Acanthaceae, possesses hepatoprotective [2], antifungal, anticancer, antihyperglycemic [3], antioxidant and antimicrobial potential [4]. In Asia, the plant *A. paniculata* has been used in medicinal preparations from ancient times. Leaf aqueous extracts of *A. paniculata* significantly inhibited *M. nanum*, *Curvularia* sp., and *F. dimerium* beside *A. paniculata* leaf leachate significantly inhibited *Curvularia sp*. The extract also found significantly effective on the spore sediment height of the fungi [5]. Extraction of *A. paniculata* in aqueous and ethanolic solvents dissolves flavonoids, terpenoids and phenolic acids in extracts and significantly inhibiting soil-borne pathogenic fungi, including *Fusarium oxysporum* and *Fusarium solani* [6]. Extraction of leaves of *A. paniculata* using methanol as solvent found to exhibit effective action as a natural pest control; along with the fungicidal activity, it also supports and promotes germination and growth of crops like *O. sativa* [7]. The use of this plant in crop rotation and intercropping patterns would be helpful in disease management. Andrographolide is a major bioactive component from the plant, exhibiting antifungal actions by restricting growth of mycelia and inhibiting of germination of spores of *Fusarium solani* and *Alternaria solani,* respectively [8]. Extracts of *A. paniculata* prepared using hexane, methanol and chloroform exhibit a broad spectrum of antibacterial and antifungal activity against crop pathogens with significant inhibition of the growth of bacteria and fungi found in the methanolic extracts, followed by chloroform and hexane extracts [9].

Extraction of leaves of *Centella asiatica* and *Andrographis paniculata* in methanol as solvent strongly inhibited germination of spores of fourteen plant-pathogenic fungi. Several *Fusarium* and *Drechslera* species were highly sensitive, and mixed extracts enhanced antifungal efficacy, indicating promising field application [10].

As synthetic chemical fungicides cause soil pollution, the plant formulations can be used instead of chemicals, and sustainable development can be achieved with this green approach. Use of plant materials as botanical pesticides for biocontrol strategies in agricultural pest management might provide an eco-friendly and natural alternative to chemical fungicides. The study would help increase the productivity of crops without adverse effects on soil microbiota by inhibiting the infection by fungi during germination and growth.

2. MATERIALS & METHODS

2.1 Isolation of fungus from infected ginger crop

Infected ginger was identified and the sample was collected from agricultural field. The tissues of the ginger were softened and dried out due to fungal infection. The diseased ginger sample was brought to the laboratory in sampling bag. At laboratory careful washing was done to remove mud and soil particles without removing the traces of fungus over it followed by inoculation fungal specimen over potato dextrose agar (PDA). After allowing the complete growth of fungi for 4 days at 27ºC. Fungal isolates were sub-cultured on fresh PDA media so as to obtain the pure fungal cultures. The isolated cultures were preserved for further use.

2.2 Characterization of isolated fungus

A. Morphological characterization of isolated fungi

Morphological observations of the isolated fungi were performed by means of staining of mycelium with lactophenol cotton blue. A small mycelial sample was placed on a glass slide, stained with lactophenol cotton blue solution, and examined microscopically to observe hyphal morphology, conidial structures, and septation patterns for preliminary species identification.

B. Molecular characterization using 18S rRNA sequencing

The genomic DNA of the pure fungal isolate was isolated, and the internal transcribed spacer (ITS) region was partially amplified using the universal primers ITS1 and ITS4. On a 1.3% agarose gel, PCR amplification produced a single, identifiable band. Following the removal

and purification of the amplified product, forward and reverse primers were utilised for bidirectional sequencing. The NCBI GenBank database was compared to the acquired ITS sequence using BLAST software. Closely connected sequences were selected based on alignment quality and percentage similarity. ClustalW was used to align various sequences, and a distance matrix was subsequently generated. The isolate's taxonomic placement was subsequently determined by constructing a phylogenetic tree with MEGA 11 [11].

2.3 Herbarium preparation and deposition

A specimen with a flowering or fruiting body, along with vegetative parts, was collected and mounted carefully on blotting paper; the mounted specimen was then kept in wooden blocks for proper drying, maintaining of and pressing of the specimen, followed by poisoning process with 4-6% mercuric chloride in 90% ethanol for prevention of pests and mounted on a herbarium sheet. The prepared herbarium was then labelled with details like botanical name, collection data and location [12]. After the complete preparation of herbarium, the specimen was deposited at the Botanical Survey of India (BSI), Pune.

2.4 Collection of plant leaves

Healthy plants were selected for the collection of leaves, without disrupting or causing disruption to habitat of the plant. After collection, the leaves were thoroughly washed to remove dirt and soil particles and dried in shade at normal temperature. After drying the leaves grounded to powder and stored.

2.5 Preparation of crude extract

Extraction of leaf powder with 95% ethanol was done by cold maceration method. Approximately 50 g leaf powder combined with ethanol, and for 72 hrs kept on a rotary shaker. Obtained extract filtered with Whatman's filter paper, the filtrate collected in a bowl and dried in a hot air oven below 50°C. Obtained dried extract was stored at 4°C for activities.

2.6 Extraction of Andrographolide

Washing of dried crude extract was done for several times with toluene until all the coloring pigments was removed; in this process, removal of pigments in the extracts followed by filtration was done. The residues were collected washed with warm methanol and freeze drying was performed.

2.7 Characterization of extracted Andrographolides

Preliminary Color test

The color tests were performed to confirm, extracted compound as andrographolide. Around 0.5g extracted compound dissolved in 5mL distilled water, and filtered, the filtrate was used for the following color tests [13].

2.7.1 Test 1: For this test, two solutions were prepared, first one 2% w/v solution of 3,5-dinitrobenzoic acid; prepared by dissolution of 3,5- dinitrobenzoic in ethanol and a second one 5.7% w/v solution of potassium hydroxide in ethanol. 2 Drops of both solutions were added to 0.5 mL of filtrate and change in color was observed.

2.7.2 Test 2: In this test 3-5 drops of the 5.7%, w/v solution of potassium hydroxide in ethanol were added to 0.5 mL of filtrate until appearance of red color. Filtrate was then set aside for 10 to 15 minutes and color change was observed.

2.8 Evaluation of antifungal activity by the poisoned food technique

Antifungal activity of extract and andrographolide extracted from *A. paniculata* was done by poisoned food technique. For this, different concentrations of crude ethanolic extract and andrographolide were prepared in 20% ethanol to check the antifungal activity of the plant extract. For control media was prepared containing only 20% ethanol. Concentrations of extracts and extracted andrographolide, viz., 10mg/mL, 20mg/mL, 30mg/mL, 40mg/mL, 50mg/mL, were made. Each concentration added to different petri plate, followed by adding melted PDA to make the concentration of media 1mg/mL, 2mg/mL, 3mg/mL, 4mg/mL and 5mg/mL respectively. After solidification of PDA, discs of 5mm diameter were made properly sterilized in hot air oven followed by UV sterilization and dipped in a 7-day-old fungal culture prepared in SDA broth and aseptically inoculated on PDA plates. The fungal isolate was taken in triplicate. Inoculation was done at 27°C for 5-7 days, and fungal growth or inhibition was observed and recorded.

3. RESULTS AND DISCUSSION

3.1 Isolation and morphological characterization of fungi

Infected ginger was collected, washed carefully to sustain the fungus on it and inoculated on PDA. After isolation on PDA plates, the fungal colonies appeared concentric brownish white

mycelial circles (refer to Fig. 1A). Lactophenol cotton blue staining of these isolates facilitated morphological characterization shown in Fig. 1B, revealing structures consistent with *Fusarium* species.

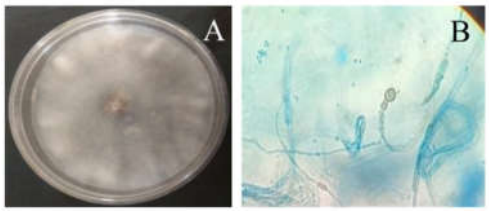

Fig. 1 A- Isolated fungi, B- Microscopic image of isolated fungi

3.2 Molecular characterization of fungal isolates by 18S rRNA sequencing

The 18S rRNA sequencing was followed by phylogenetic analysis for the identification of isolates (Fig. 2). The isolated fungus showed close similarity with *Fusarium verticilloides.* The sequencing data for the isolate submitted to GenBank, National Centre for Biotechnology Information (NCBI), and processed with accession number PV259701.

3.3 Herbarium preparation and deposition

The herbarium for the specimen of *A. paniculata* was prepared by following standard procedures. Authorized identification and deposition of the herbarium was done at BSI, Pune. Also, the certificate was received from BSI with specimen no. 01 and reference No. BSI/WRC/Tech./2025/JVD-196.

3.4 Collection and extraction of leaves of *Andrographis paniculata*

Andrographis paniculata leaves were collected from Melghat, Amravati, following approval from the Maharashtra State Biodiversity Board (MSBB), Nagpur. Healthy leaves were collected, washed and shade-dried to maintain phytochemical stability. After drying, leaves were grounded to a fine powder and strained through mesh sieve. Ethanol extraction of the powdered leaves was done with ethanol as solvent, the obtained extract employed for extraction of andrographolide, which was used for subsequent phytochemical and bioactivity analyses.

Fig. 2 *A. paniculata* plant

3.5 Characterization of extracted andrographolide

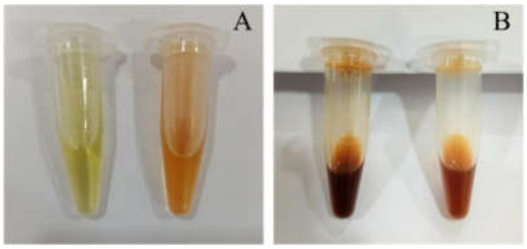

Fig. 3 A- Test 1, B- Test 2

3.5.1 Test 1: Color change to a yellowish orange is indication of sample contained bioactive compounds.

3.5.2 Test 2: The initially obtained dark red color changed to orange-red, indicating the presence of a diterpene lactone.

Both color tests confirmed that the extracted compound is andrographolide.

3.6 Evaluation of antifungal activity by the poisoned food technique

Antifungal activity of the ethanolic extract & andrographolide from *Andrographis paniculata* was evaluated by observing growth of fungal mycelium at different concentrations. Significant inhibition of mycelial growth was observed at concentrations 4mg/ml of extract and 3mg/ml of andrographolide, indicating significant antifungal activity, while concentrations of 1mg/ml to 2mg/ml of andrographolide and up to 3mg/ml of extract did not exhibit potent inhibitory effect on fungal growth (Fig. 4 and Fig. 5).

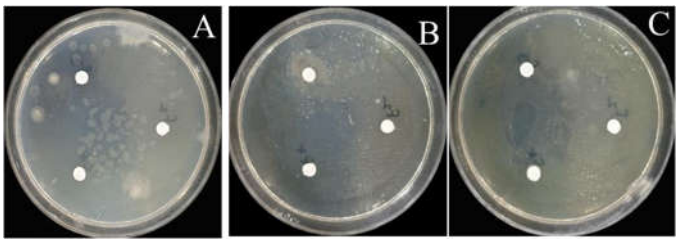

Fig. 4 Antifungal activity of ethanolic extract of *A. paniculata* in media plates
A- Control, B- 2mg, C- 4mg concentrations per mL

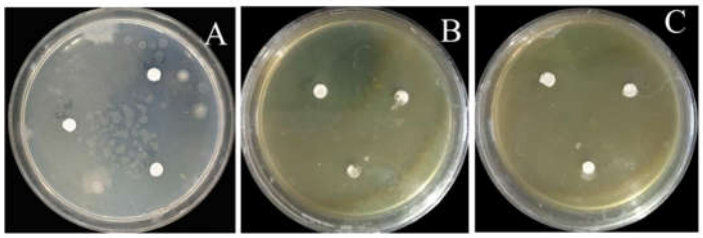

Fig. 5 Antifungal activity of andrographolide extracted from *A. paniculata* in media plates
A- Control B- 2mg, C- 3mg concentrations per mL

From these results it was found that extracted compound exhibited characteristics features of andrographolide by means of preliminary color tests. Plant extract and andrographolide exhibit potent antifungal activity against characterized *F. verticilloides.*

4. DISCUSSION

From the morphological, microscopical, and molecular examinations, it was determined that the isolated fungus was *Fusarium verticilloides,* which supports the findings of Munir [14], who investigated the morphological and molecular characteristics of *Fusarium* responsible for soft rot (rhizome rot) disease. The characterization of andrographolide, an active principle from *A. paniculata,* by color tests matches the findings of studies carried out to confirm the presence of andrographolide by Sharma & Sharma [13]. Ethanolic extracts of *Andrographis paniculata* inhibited fungal mycelium better than aqueous and methanolic extracts, with the maximum efficiency against fungal isolates from soft-rot-affected ginger studied by Nidiry [8].

5. CONCLUSION

From the results of the present investigation, *A. paniculata,* a potential reservoir of bioactive andrographolide with superior antifungal activities against *F. verticilloides*. Both, ethanolic extract and the extracted andrographolide had good inhibitory activity against soilborne phytopathogen, *F. verticilloides,* causing severe crop loss. These results also provide evidence indicating that *A. paniculata* formulations may constitute green and sustainable alternatives or supplements to synthetic chemical fungicides, which could alleviate the problems associated with fungal resistance. The application of these natural botanical fungicides in integrated pest management programmes could have potential to improve protection against fungi, as well as promote sustainable agricultural practices. Further research should focus on molecular mechanisms for antifungal effects of andrographolide and evaluation of its field applications, safety in diverse crop systems, to harness its potential as a biopesticide. Further studies should focus on field applications of the plant extracts and bioactive components, development of novel fungicides for commercial purposes to achieve goal of sustainable farming practices in the agricultural fields and minimizing load or applications of synthetic chemical pesticides responsible for soil, water and air pollution, disruption of environmental balance.

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