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An *in-vitro* Evaluation of Antifungal Potential of *Withania somnifera* and *Ageratum conyzoides* weed plants against *Alteneria solani*

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ABSTRACT
The weed plants also known as ethnomedicinal plants due to their medicinal properties. These play a vital role as medicine due to their antibiotic compounds. Secondary metabolites contain structures which having activity against pathogen due to presence of phenolic, saponin, alkaloids, terpenoids and flavonoids which play a vital role to inhibit growth of pathogenic fungi. This study focused on common weeds viz. Ageratum conyzoides and Withania somnifera which found in bare area their no requirement of crop production. These exhibit antifungal, antimicrobial, anti-inflammatory, analgesic as well as anti-diuretic properties. The phytopathogenic fungus, such as A. solani was taken to assess the anti-fungal potential of these plants. Antifungal activity of A. conyzoides leaf (3.1cm), flower (1.75cm) and W. somnifera (0.8 cm) reported as zone of inhibition (ZOI).

KEYWORDS: *Ethanomedicinal, Antifungal, Weed, Withania sonifera, Ageratum conyzoides Flavonoids.*

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INTRODUCTION

Fungi, which make up a substantial group of microbes and have over 2,50,00 species, are important opportunistic human diseases as well as important agricultural plant infections (Odds, 2000). About 60% of the population is employed in agriculture, which makes it a significant economic sector in India (GOI, 2011). Since a very long time, ethnomedicine plants have been regarded as a source of human sustenance and medications. Due to their lack of adverse effects or complete lack, herbal medications are used by about 80% of people worldwide, especially in underdeveloped nations (Aggarwal et al., 2019). The number of scientific study on these plants used in traditional medicine has substantially increased in recent years (Sharma et al., 2015). Weeds are a major concern for agricultural production because of their negative impact on output. Some of these are regarded as traditional plants used to treat human illnesses in Ayurveda, which was discovered by humans. Some of the plants used in ethnomedicine are regarded as traditional plants, while others are weeds impede the growth of the primary crop (Akbar et al., 2017). These are regarded as wild plants that often grow quickly and are not valued where they are growing. They can be found in abundance and in their native habitat in bare areas like *Ageratum, Hemp, Amaranthus, Chenopodium,* etc. Various plant families, including Amaranthaceae, Asteraceae, Umbelliferae, Solanaceae etc., are home to these weeds. The Amaranthaceae family contains 800 species and 64 genera, the majority of which are weeds and few shrubs (Pamila and Karpagam, 2017). The *Alternanthera* genus contains 80 different plant species. One of those 80 species, *Alternanthera philoxeroides*, is significant. *A.*

philoxeroides is a widespread alligator weed that can be found in tropical and subtropical regions of the world. Additionally, other *Alternanthera* species, including *Alternanthera sessilis* and *A. philoxeroides*, are used around the world to treat a variety of diseases (Kumari and Krishnan, 2016). Fungi have the greatest impact on illnesses and crop output losses. The fact that pathogenic fungi are developing a resistance to synthetic fungicides is quite worrying. The *Alternaria* genus is one of the most common and widespread fungi on the phyllosphere (Lopes and Martins, 2008). A genus of Deutromycetes fungi is called *Alternaria.* Necrotrophic fungi are the general term for the *Alternaria* species. They have the ability to form large, typically green, black, or grey colonies. Because it can cause plant blights, *Alternaria* is a common cause for concern. Currently, the total annual domestic output is thought to be roughly 2148.26 thousand tones over an area of 127.75 thousand hectares of land. Early potato blight is caused by *Alternaria solani*, which also causes significant harm to the crop (Anonymous, 2010). It is a serious foliar disease that affects potatoes and reduces output by 20–50%. On the plants, it causes tiny, darker lesions that enlarge into expanding black blotches of decomposing tissue. *A. solani* overwinters as mycelium or conidia in soil, diseased tubers, plant detritus, or on other hosts belonging to the same family of plants. Cultural practices include crop rotation, tillage, removal and burning of diseased plant waste, and eradication of weed hosts assist manage the disease by lowering the inoculum level for following plantings (Mal et al., 2023). Because *Alternaria* spreads so quickly, controlling it can be simple. The present study focusedon two ethnomedicinal plants *A. conyzoides and W. somnifera* to evaluate the antifungal activity against *A. solani.*

A. conyzoides, sometimes called Appa grass or Goat weed, is a member of the Asteraceae family. It is an annual herb that is polymorphic, aromatic, and endemic to tropical America. *Ageratum* is taken from the Greek word "a geras" which means "non-ageing" and refers to the longevity of the entire plant. *Conyzoid* is derived from "konyz" which is the Greek name of *Inulahelenium* through which the plant is similar to *A. conyzoides* (Kissman, et al. 1993). *W. somnifera* Dunal (Ashwagandha) is widely used in Ayurvedic medicine, the traditional medical system of India. It is a small woody shrub and 30-150 cm tall, upright, evergreen, tomentose shrub that can be found on bunds and in waste areas all over the drier sections of India. Simple leaves and thick, meaty, whitish-brown roots berries are small, globose, and yellow when ripe, enclosed in the persistent calyx; seeds are reniform and yellow (Chatterjee et al., 1995).

MATERIAL AND METHOD

A. conyzoide and *W. somnifera* plant species were collected in March 2023 in their natural habitat from village Mullana. The collected plants were identified at the Department of Bio-Sciences and Technology, MMDU Mullana, Ambala, Haryana, India. Whole plant collected in an open bag and carried in laboratory then washed under tap water then distilled water. Fresh 10grams leaf and flower were taken in 100 ml of distilled water separately and grind with the help of mortar pestle and filter by muslin cloth and centrifuged at 10,000 rpm for 15 minutes, then supernatant taken in conical flask and autoclave at 121^0 C at 15 lbs. Pour plating method applied to check antifungal activity against phytopathogenic fungi. *Alternaria solani* fungus isolated from potato leaf (Pandey & Tripathi, 2014)

Antifungal Activity assay

Anti-fungal activity Assay

Alternaria solani fungal strains were plated independently in triplicate using 1 ml, of fresh plant extracts in PDA agar medium respectively. Using the food poisoning method, which counts colony forming units (CFU), antifungal assay was determined. Although control plates were utilized without extract, fresh water extract of both plants was applied to the media. Six mm-diameter agar discs containing fungal mycelia were cut from the edges of the pure cultures that were still growing after a day using a sterilized cork-borer. The center of the petri dish was then aseptically inoculated with these discs (Deepika et al., 2011; Salhi et al., 2017). The 1 ml of fresh extract was used in PDA for *A. solani*, and the petri plates were incubated for 144 hours at 28°C.

RESULTS

Antifungal assay against *A. solani*

Antifungal activity of *A. conyzoides* leaf, flower and *W. somnifera* whole plant (leaf and flower) extract having tendency to inhibit growth of *A. solani* fungi. The leaf and Flower extract extracts of *A. conyzoides* in distilled water show best results. Leaf part show high antifungal activity (5.25cm) as compared to control (7cm) followed by flower part (3.9 cm) zone of inhibition as compare to control. *W. somnifera* also having antifungal activity against *A.solani*1ml extract of *W. somnifera* apply on fungi then observed 3rd, 5th and 7th day zone of inhibition 4.2cm as comparared control 5 cm (table 1-3 and fig 1-3). Table 4 reveled the Zone of Inhibitions (ZOI) of fresh distilled water extract of *A. conyzoides* and *W. somnifera* against *A. solani* in cm. *A. conyzoides* leaf and flower part found higher antifungal as compared as *W. somnifera.*

Table 1: Antifungal activity of *Ageratum conyzoides* leaf fresh Distilled water extract (1 ml) against *Alternaria solani,* (Colony diameter in cm)

Samples	3rd day	Control	5th day	Control	7th day	Control
R1	2.5	2.8	3	4.8	3.9	7
R2	2.5	2.8	3	4.8	3.9	7
R3	2.5	2.8	3	4.8	3.9	7

Table 2: Antifungal activity of *Ageratum conyzoides* flower fresh Distilled water extract (1 ml) against *Alternaria solani* (Colony diameter in cm)

Samples	3rd day	Control	5th day	Control	7th day	Control
R1	1.6	2.5	3.35	4.95	5.25	7
R2	1.6	2.5	3.35	4.95	5.25	7
R3	1.6	2.5	3.35	4.95	5.25	7

Table 3: Antifungal activity of fresh Distilled water extract of *Withania somnifera* (1 ml) against *Alternaria solani* (Colony diameter in cm)

Samples	3rd day	Control	5th day	Control	7th day	Control
R1	1.1	1.35	2.25	2.55	4.2	5
R2	1.1	1.35	2.25	2.55	4.2	5
R3	1.1	1.35	2.25	2.55	4.2	5

Table 4: Antifungal activity of fresh Distilled water extract of *Ageratum conyzoides* and *Withania somnifera* against *Alternaria solani.* ZOI in cm

Plant extract	3rd Day(cm)	5th Day(cm)	7th Day(cm)
Ageratum conyzoides (Leaf)	0.3	1.8	3.1
Ageratum conyzoides (Flower)	0.65	1.6	1.75
Withania somnifera	0.25	0.3	0.8

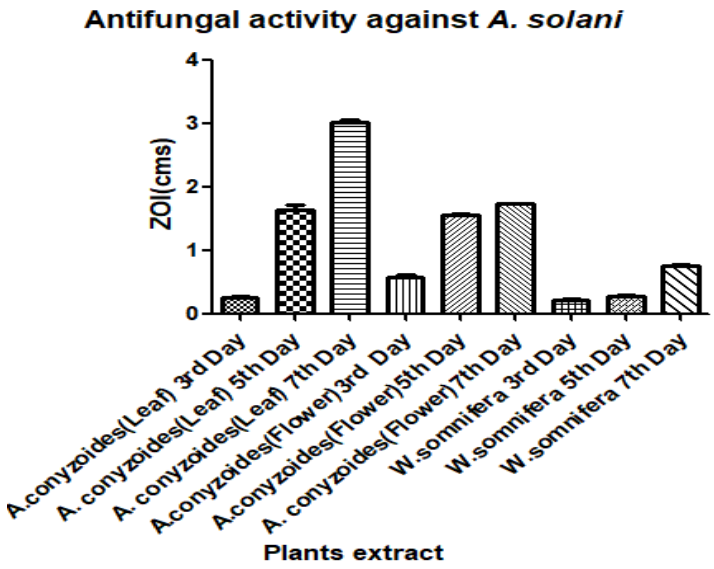

Figure 1: Antifungal activity of fresh extract of *Ageratum conyzoides* and *Withania somnifera* against *Altenaria solani.*

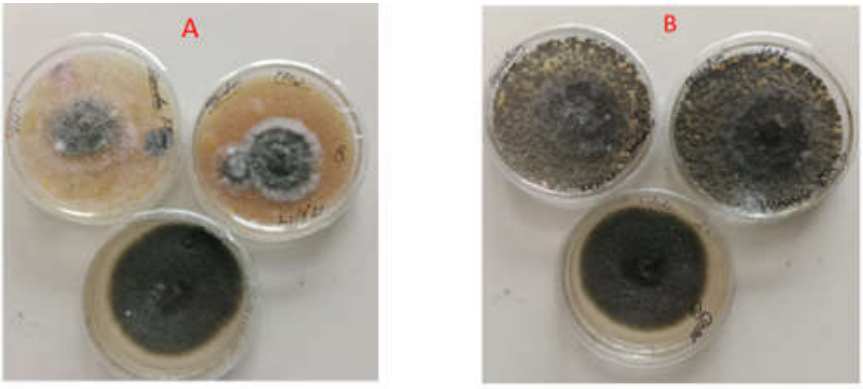

Figure 2: Antifungal activity of *Ageratum conyzoides* Flower (A) and Leaf (B) against *Altenaria solani.*

Figure 3: Antifungal activity of *Withania somnifera* against *Altenaria solani.*

DISCUSSION

This study revealed that, fresh distilled water extract of *W. somnifera* and *A. conyzoides* weeds resist the growth of *A. solani*. Similar antifungal activities of *A. conyzoides* extracts and essential oils have been demonstrated in previous studies against a variety of fungal species, including *Aspergillus niger* (Omole et al., 2019), *Penicillium notatum, Fusarium oxysporum* (Lian et al., 2019), *Puccinia arachidis* (Yusnawan and Inayati, 2018), and *Rhizopus stolon. Ageratum conyzoides* ethanol extract significantly inhibited the growth of *Phytophthora megakarya* (Ndacnou *et al.*, 2020), *F. lateritium, F. solani*, and *Cochliobolus lunatus* (Ilondu, 2013). Sub-fractions derived from *A. conyzoides* methanolic stem extracts generally inhibited the pathogen's growth in varying ways at varying concentrations. Other studies have also reported on the variations in antifungal activities of different sub-fractions of methanolic extracts of other plant species, namely *Chenopodium album, C. quinoa, C. murale, Coronopus didymus, Senna occidentalis,* and *Sisymbrium irio* (Javaid et al., 2017a, b; Naqvi et al., 2019; Khan and Javaid, 2020, Banaras et al., 2021). According to these researches a part of discussion that a fresh extract means crude extract having better antifungal activity as comparable as solvents, because herbal extract are eco-friendly low cost in lieu of chemicals against *A. solani*.

CONCLUSION

The antifungal activity of *W. somnifera* and *A. conyzoides* leaf and flower extracts in distilled water thoroughly investigated. The crude extract of both plants found antifungal to *A. solani* and the growth of fungus. The fungal infections caused a serious threat to crop plants and economy loss. The present study able to provide eco-friendly management of fungal disease with the use of ethnomedicinal weeds.

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