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RESEARCH ARTICLE

INVESTIGATION OF ANTIFUNGAL ACTIVITY OF *CICER ARIETINUM* L. LEAVES AGAINST IMPORTANT SEED BORNE FUNGI

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ABSTRACT:
In vitro evaluation of aqueous extract of leaves of *Cicer arietinum* against five fungi namely *F. oxysporum* , *Aspergillus flavus*, *Curvularia lunata*, *Cladosporium cladosporioides* and *Penicillium* sp. Among the five fungi tested, *A.flavus* recorded maximum antifungal activity of 93.0% inhibition at 50%concentration tested. In 40% of aqueous extract it was recorded 78.3% inhibition and moderate activity was observed in 20 and 30% concentration and recorded 63.3% and 49.3% respectively. *A.flavus* was followed by *Penicillium* species and was recorded 91.3% inhibition at 50% concentration. In 30 and 40% concentration it was recorded 671% and 79.3% inhibition respectively. Least activity was observed in 10% concentration. In *F.oxysporum*, at 50% concentration, 88.0% inhibition was recorded followed by 67.2%, 51.1%, 35.2% and 22.0% inhibition in 40, 30, 20 and 10% concentration tested. *C.lunata* recorded significant activity of 80.3% inhibition in 50% concentration and least inhibition was observed in 10% concentration(15.0%). In *C.cladosporioides* it was recorded 74.5% inhibition in 50% concentration and at 10% concentration , it was recorded 25.3% inhibition. Moderate activity was observed in 20, 30 and 40% concentration. Compared to synthetic fungicide Dithane M 45 and Bavistin at 2.0% recommended concentration, 100% inhibition was observed in all the test fungi.
Key words: Aqueous extract, Fungi, *Cicer arietinum*, Dithane M 45, Bavisin

INTRODUCTION:

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases [1]. The plant extracts have been developed and proposed for use as antimicrobial substances [2]. Herbal medicines represent one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs [3]. Over the past 25 years, there has been an increased interest in the investigation of plant materials as a source of antimicrobial agents. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action. In last few decades, the continuous escalation of resistant fungi against a wide range of antifungal drugs necessitates discovering novel unconventional sources of antifungal treatment. As a result of the indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antimicrobes [4]. Medicinal plants are an integral component of research development in microbiology and pharmaceutical industry. Natural products from plants traditionally have provided the pharmaceutical industry with one of its most important sources of lead compounds and up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version [5]. Many synthetic chemicals are widely used for the management of seed borne fungi which are both efficient and effective. Many of these synthetic fungicides are known for their non-biodegradable nature and residual toxicity [6]. Pesticide pollution of soil and water bodies is well documented in the literature [7]. This has driven plant pathologists to search for alternative eco-friendly methods for the management of plant diseases. Plant based remedies would be of immense value in developing ecofriendly remedies due to their easy biodegradability, less phytotoxic and more systemic nature[8]. Considering all the ill effects of synthetic fungicides, Reports available on *C.arietinum* [14,15] tested for antibacterial activity. Not much reports are available for antifungal activity. Hence in the present study, leaf of *Cicer arietinum* L. belongs to family Fabaceae were investigated for antifungal activity *in vitro* condition.

MATERIALS AND METHOD:

Test fungi: Five species of fungi viz., *F. oxysporum* , *Aspergillus flavus, Curvularia lunata, Cladosporium cladosporioides* and *Penicillium* sp. isolated from maize seeds were used as test fungi for antifungal activity assay.

Test Plant: Shade dried, healthy leaves of *Cicer arietinum* L. were collected. The leaves were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter, and used for preparation of extracts [9].

Extraction:

Aqueous extraction: One hundred grams of the thoroughly washed and air dried healthy leaves of *C. arietinum* were macerated with 100ml sterile distilled water in a waring blender (Waring international, new hart-ford, CT, USA) for 5 min. The macerate was filtered through double layered muslin cloth, and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120^0 C for 10 minutes, which served as 100% aqueous mother extract. The extract was preserved aseptically in a sterile brown bottle at 50 C until further use [10].

Antifungal activity assay by poisoned food technique

Aqueous extract: Different concentrations of the CDA medium with the aqueous extracts of leaf extract of *C. arietinum* viz., 10, 20, 30, 40, and 50% were prepared and poured into sterile petriplates allowed to cool and solidify. Five mm mycelium disc of seven day old cultures of species of fungi were placed at the centre of the petriplates and incubated at 25 $\pm 1^o$ C. The CDA medium without the aqueous extract but with the same concentration of sterile distilled water served as control. The colony diameter was measured in mm. For each treatment three replicates were maintained. The percent inhibition of mycelial growth if any was determined by the formulae PI = C-T/CX100 Where C= Diameter of control colony, T=Diameter of treated colony. Minimal inhibitory concentration (MIC) for each of the test fungi was determined [11, 12] .The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

SYNTHETIC FUNGICIDE:

Two synthetic chemicals viz., Dithane M-45 and Bavistin at 2 grams per liter of recommended dose were used for comparison against plant extract. CDA medium with 2 percent concentration of Dithane M-45 and Bavistin were prepared. Eighteen ml of CDA media with synthetic fungicide was poured into petri plates. Five mm mycelial discs from the margins of seven day old cultures of fungal species were placed in the center of the CDA medium. The inoculated plates were incubated at 25 ± 1^0 C for seven days and ten replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined by the formulae PI = C-T/CX100 Where C= Diameter of control colony, T=Diameter of treated colony [12]. The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

RESULT:

Among the five species of fungi tested, *A.flavus* recorded 93.0% inhibition at 50%, 78.3% inhibition at 40%, 63.3% inhibition at 30% concentration, 49.3% inhibition in 20% concentration and 31.5% inhibition at 10% concentration respectively. *A.flavus* was followed by *Penicillium* species and recorded 91.3% inhibition at 50% concentration. *F.oxysporum* recorded 88.0% inhibition at 50% concentration and 22.0% inhibition at 10% concentration. *C.lunata* recorded 80.3% inhibition at 50% concentration and 15.0% inhibition at 10% concentration. Moderate activity was observed in *C.cladosporioides* and recorded 74.5% inhibition and 25.3% inhibition at 10% concentration. Compared to synthetic fungicide Dithane M 45 and Bavistin, in all the species of fungi 100% inhibition was observed in both Dithane M 45 and Bavistin tested at 2.0% concentration (Table 1).

Table 1: Antifungal activity of aqueous extract of leaves of *Cicer arietinum* L. against seed borne fungi

Fungi	Mycelial Growth Inhibition (%)						
	Concentration of Aqueous Extract					Dithane M-45	Bavistin
	10%	20%	30%	40%	50%	2%	2%
F. oxysporum	$22.0^a\pm0.0$	$35.2^b\pm0.0$	$51.1^c\pm0.1$	$67.2^d\pm0.2$	$88.0^e\pm0.2$	$100.0^a\pm0.0$	$100.0^a\pm0.0$
A. flavus	$31.5^a\pm0.1$	$49.3^b\pm0.0$	$63.3^c\pm0.0$	$78.3^d\pm0.1$	$93.0^e\pm0.1$	$100.0^a\pm0.0$	$100.0^a\pm0.0$
C. lunata	$15.0^a\pm0.0$	$35.6^b\pm0.1$	$51.6^c\pm0.2$	$67.4^d\pm0.2$	$80.3^e\pm0.2$	$100.0^a\pm0.1$	$100.0^a\pm0.1$
C.cladosporioides	$25.3^a\pm0.2$	$42.3^b\pm0.0$	$54.7^c\pm0.1$	$65.1^d\pm0.2$	$74.5^e\pm0.0$	$100.0^a\pm0.0$	$100.0^a\pm0.2$
Penicillium sp.	$27.2^a\pm0.0$	$49.6^b\pm0.2$	$67.1^c\pm0.0$	$79.3^d\pm0.0$	$91.3^e\pm0.0$	$100.0^a\pm0.2$	$100.0^a\pm0.1$

- ***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:

Seed-borne diseases have been found to affect the growth and productivity of crop plants. A seed borne pathogen present externally or internally or associated with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection [13]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. They increase prevalence of resistant strains of fungi and the recent appearance of strains. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. In developing countries, microorganisms are frequently a cause of prevailing diseases, presenting a serious public health issue

in a significant segment of the population as uncovered by either private or official health care systems [3]. Many synthetic chemicals are widely used for the management of seed borne fungi which are both efficient and effective. Thiram and bavistin are some of the most commonly used synthetic chemicals for the seed treatment of maize. Many of these synthetic fungicides are known for their non-biodegradable nature and residual toxicity [6]. Pesticide pollution of soil and water bodies is well documented in the literature [7]. This has driven plant pathologists to search for alternative eco-friendly methods for the management of plant diseases. One approach that has been used for the discovery of antimicrobial agents from plants is based on the evaluation of traditional medicinal plant extracts [14]. In the present study, aqueous extract of leaf of *Cicer arietinum* L. belongs to family Fabaceae were investigated for antifungal activity *in vitro* condition.

CONCLUSION:

From the above observation it can be concluded that, leaves of *C. arietinum* showed a promising result against *F. oxysporum, Aspergillus flavus, Curvularia lunata, Cladosporium cladosporioides* and *Penicillium* sp. A further antifungal assay is needed to test different fungi in different solvent extracts. A further isolation of bioactive principle is needed from different solvent extract and its purification and characterization is needed. Further the bioactive compound can be tested for antibacterial of both human and plant pathogens which is playing an important role in agriculture and human health.

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CONFLICT OF INTEREST: The authors confirm there is no conflict of interest.

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