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COMPRATIVE EVALUATION OF ANTIFUNGAL ACTIVITY OF *CUSCUTA REFLEXA* (MORNING GLORY) CONVOLVULACEAE

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ABSTRACT

Integrated disease management is a non-separable part of all the eco-friendly stable agricultural programs. Plant extracts have played significant role in the inhibition of pathogens and in the improvement of crop production. *Cuscuta reflexa* is a parasitic plant. Agriculturalists consider *Cuscuta* is only a destructive weed and attempt to eradicate it. The main purpose of this study was to assess its significance in developing plant based formulations for fungal disease management. Antifungal potential of C. *reflexa* extract was evaluated against two different pathogenic fungi namely, Candida albicans, Aspergillus fumigates Different concentrations of *C. reflexa* were prepared. Concentrations of *C. reflexa* were evaluated against fungal isolate by Mycelial dry weight method used to determine the MIC value of *cuscuta reflexa*. The fungi toxicity of extract in terms of inhibition zone diameter was calculated. Results indicated that fungal growth inhibition was directly proportional to the concentration of *C. reflexa* extract. *Cuscuta* extract exhibited significant antifungal activity against all test fungal isolates. However, extract was highly effective Candida albicans, Aspergillus fumigates. It was also found that concentration was significantly effective in reducing the Ketocanazoles growth of fungal isolates after 6 days of incubation. Further investigations however are required to analyze nature of antifungal compounds in *C. reflexa* and their stability.

KEYWORDS: *Cuscuta reflexa*, parasitic plants, antifungal extract, pathogenic fungi, parasitic weeds,MIC.

INTRODUCTION

Cuscuta reflexa is an angiospermic hustorial advance, obligate parasite belonging to family convolvulaceae. Members of this family are holoparasitic plants subsisting on other dicotyledonous plants. These plants can parasitize on very wide variety of plants including a number of agricultural and horticultural crop species such as alfalfa, lespedeza, flax, clover and potatoes. These can grow on common ornamental plants through the plans of India. Agriculturalists consider *Cuscuta* species a destructive weed and attempt to eradicate it. However on biological aspects, strong antifungal and antibacterial compounds have been extracted from *C. reflexa*. Its therapeutic properties such as anticancer, antidiabetic, antiviral, and anti-inflammatory are also well documented. Plant metabolites and plant-based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and in the improvement of crop production. Conventional breeding of disease-resistant cultivars and plant protection based on extensive use of agrochemicals represent still common strategies in the contest between human approaches to combat microbial food competitors and the evolutionary adaptability of bacterial and fungal phytopathogens. These are responsible for enormous crop losses worldwide and therefore threaten human nutrition. Integrated disease management is a non-separable part of all the eco-friendly stable agricultural programs. Biological control of plant diseases and plant pathogens is of great significance in forestry and agriculture. Therefore, new strategies to fight phytopathogens have to be explored. Keeping in view the strong phytochemical potential of *C. reflexa*, this study has been carried out to evaluate antifungal activity against important pathogenic fungal species with the ultimate aim of developing plant based formulations for plant disease management.

MATERIAL AND METHODS

Plant material

Fresh plant material of *C. reflexa* was collected from Six distinct different location of North India Muzaffarnagar ,Haridwar,Deharadun,Kumau,pantnagar,Nanital area.The plants were authenticated by Dr. Anju Pal, Dept.of Horticulture, G.B.Pant University of Agriculture. and Technology, Pantnagar, U.K

Test Microorganism

Candida albicans*.*(MTCC 183)

This organism occurs both in the form of oval yeast like bodies and as a thick septate psedo-hyphae. It occurs as a normal inhabitant of the mouth and intestinal tract. It is the cause of

thrush, a condition at one time common in children, in which white patches containing fungus are found in the mucous membrane of mouth.

Aspergillus fumigates (MTCC 870)

Aspergillus species are occasionally responsible for otomycosis, a superficial scaly infection of the skin of external auditory meatus. They also appear to be capable of invading the lungs; this, however is a very uncommon type of infection occurring as a rule only in patients with established pulmonary disease.

Preparation of aqueous extract

Hundred grams each of dried leaves of *C.reflexa* collected from different location of North India were macerated with 100 ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 120°C for 30 min. The extracts were preserved aseptically in brown bottles at 4°C until further use.

Preparation of plant Solvent extracts

Soxhlet extraction will be the method used for plant extraction. A portion of dried leaves (100 g) of *Cuscuta reflexa* was placed in a Soxhlet apparatus. Extraction was performed with 500 ml of an appropriate solvent (Ethanol, Methanol, Chloroform) with increased polarity for 24 h at 95^0C temperature not exceeding the boiling point of the solvent. The extract was filtered through a 45 µm filter paper and concentrated under vacuum. In this experiment three solvents were used: Ethanol, Chloroform and methanol. The resulting three solutions were concentrated in vacuoum to dryness to give Ethanol (4 g), Chloroform extract (10 g) and methanol extract MeOHE (12 g). The stock solutions were kept at 4°C until further use.

Antifungal activity assay

Mycelial dry weight method was carried out to determine the antifungal activity of the methanol extracts at concentrations ranging from 1 to 10 mg/ml according to the method of Rasooli and Abyaneh . The dermatophytes grown on SDA medium for a week were flooded with 0.85% saline. After settling of the larger particles, conidia were counted with a haemocytometer and diluted in saborauds dextrose broth to a final spore concentration of 1×10^6 spores/ml. For antidermatophytic assay in broth, 5 ml of sterile saborauds dextrose broth medium taken in screw capped tubes were inoculated with 20 µl of fungal suspension

and 1-10 mg/ml concentration of the extract. The tubes were incubated for a week at 30°C. The visible mycelial growth in the tubes expressed the degree of activity of the extract. Fungal mycelia from the above tubes were separated by passing through Whatman No. 1 filter paper. The filter paper was allowed to dry at 60°C to reach a constant weight. Fungal growth inhibition was calculated by considering the control and sample mycelial dry weights. Ketaconozole was used as a standard antifungal agent. The percentage of growth inhibition was calculated according to the formula:

Per cent growth inhibition = [(A control – A test) / A control] × 100

Statistical analysis

The experimental results are expressed as mean ± standard deviation (SD) of triplicate measurements. The data was subjected to One Way Analysis of Variance (ANOVA) and the significance of differences between the sample means was calculated by Turkeys test. Data was considered statistically significant at P value ≤ 0.05. Statistical analysis was performed using Graph Pad statistical software.

RESULTS AND DISCUSSION

In this research Mycelial dry weight method was used to determine the MIC values of *Cuscuta reflexa* Collected from distinct different location of North india (Muzaffarnagar, Haridwar, Deharadun, Kumau, Pantnagar, Nainital) leaf methanol extracts against two *Candida albicans and ,Aspergillus fumigates*,. Table 1 represents the MIC values and the per cent inhibition of methanol leaf extracts of *Cuscuta reflexa* Collected from distinct different location of North india (Muzaffarnagar, Haridwar, Deharadun,Kumau, Pantnagar, Nainital) against the Fungi as compared to the antifungal drug, ketaconozole. Among the two Fungus, *Aspergillus fumigates* was found to be more susceptible to the methanol leaf extract of all the Six plants. *C.reflexa Muzaffarnagar sample* extract had the MIC values of 1.75 mg/ml (74.6% growth inhibition) and 3.0 mg/ml (70.9% growth inhibition) against *C.reflexa Haridwar sample*, *C.reflexa Deharadun sample*, *C.reflexa Kumau sample*, *C.reflexa Pantnagar sample* and *C.reflexa Nanital sample* respectively which was lower when compared to other plant extracts. The MIC value of *C.reflexa Haridwar sample* extract exceeded more than the used concentration range of 10 mg/ml against *C.reflexa Dehardun sample*. The MIC values obtained against *Candida albicans and, Aspergillus fumigates* with all the three plant extracts were higher than the standard antifungal drug ketoconazole.

Table 1: Minimum Inhibitory Concteration (MIC) of *cuscuta reflexa* collected from distinct different location of north India.

	Candida albicans		*Aspergillus fumigates*	
	MIC (mg/ ml)	**% growth inhibition**	**MIC (mg/ ml)**	**% growth Inhibition**
Ketaconazole	0.4	90.3	0.25	93.0
C.reflexa Muzaffarnagar sample	3.0	86.9	1.75	84.6
C.reflexa Haridwar sample	9.5	84.5	8.0	83.3
C.reflexa Deharadun sample	6.5	84..3	4.0	85.0
C.reflexa Kumau sample	4.5	86.5	6.5	84.4
C.reflexa Pantnagar sample	6.2	84.6	5.5	85.8
C.reflexa Nainital sample	5.9	85.9	6.2	83.9

Values are means of three independent replicates Mean values with different superscripts are significantly different from each other as indicated by Turkey's HSD ($P \leq 0.05$)

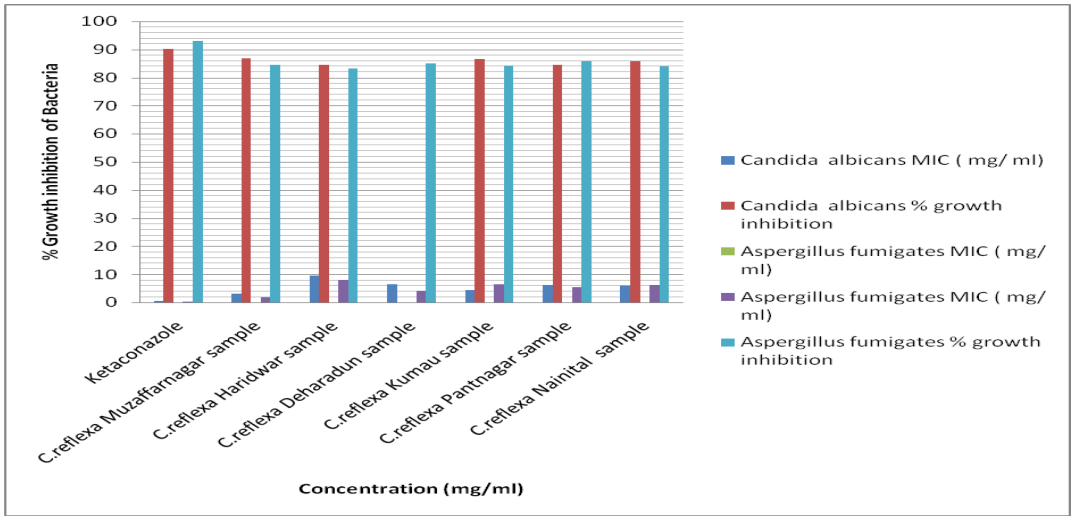

Fig 4.4.1: Graphical representation of Minimum Inhibitory Concentration (MIC) of *Cuscuta reflexa* Collected from distinct different location of North india (Muzaffarnagar, Haridwar, Deharadun, Kumau, Pantnagar, Nainital) leaf ethanol extracts against Fungus using mycelial dry weight method

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