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Study on Antifungal Activity of *Acorus calamus* L. and *Allium sativum* L. against Some Pathogenic Fungi

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Aqueous leaf extract of *Acorus calamus* L. (*A. calamus*) alone and its combination with bulb extract of *Allium sativum* L. (*A. sativum*) were evaluated for their antifungal activity against three phytopathogenic strains of fungi namely *Alternaria alternata* Fr. Keissl. (*A.alternata*), *Aspergillus flavus* Link. (*A.flavus*), and *Fusarium solani* F. sp. Pisi. (*F.solani*) using poisoned food technique at different concentrations (conc.) (5%, 10%, 15% and 20%). Aqueous leaf extract of *A. calamus* alone was found to be most effective in inhibition against *A. alternata* , followed by *F. solani* and *A. flavus*. Aqueous leaf extract of *A.calamus* in combination with bulb extract of *A. sativum*, was found to be most effective in inhibition against *F. solani* , followed by *A. alternata* , and against *A. flavus* . Aqueous leaf extract of *A. calamus* alone was found to be fungistatic against all three test phytopathogenic fungi. Combination of aqueous leaf extract of *A. calamus* and bulb extract of *A. sativum* also found to be fungistatic againt all three pathogenic fungi except *F. solani*, against which it was found to be fungicidal at 20% conc. Both aqueous leaf extract of *A. calamus* alone as well as its combination with bulb extract of *A. sativum* were found to have broad fungitoxic spectrum. Present study forms basis for its further evaluation under field conditions as a biofungicide.

Key words: Leaf extract, Fungicides, Antifungal, Fungitoxic spectrum.

Fungi are known to cause several diseases in cultivated crops. Every year agricultural production suffers huge losses due to various pre and post harvest fungal diseases. During storage also fungi affects food stuffs and grains very badly by producing mycotoxins. To overcome these losses chemical or synthetic fungicides have been used since long time[1]. These were undoubtedly found effective in controlling these plant diseases and are cheap and easily available in market. But their blind use has lead to drastic effects like environmental pollution, serious health problems because of toxic residues, development of resistance in target organisms[2].

In recent years, there has been continuous search for eco-friendly methods for prevention of fungal plant diseases. Exploitation of plant derived fungicides has been drawn considerable attention. These provide better alternative for synthetic fungicides which cause various environmental as well as health problems[3]. Many plants are known to possess antifungal properties, so these could be exploited commercially as a source of biofungicide[5]. Therefore, in present piece of research work aqueous leaf extract of *Acorus calamus* L. alone and its combination with bulb extract of *Allium sativum* L. were evaluated for their antifungal activity at different concentrations (5%, 10%, 15% and 20%) against three phytopathogenic fungi

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namely, *Alternaria alternata* Fr. Keissl. (*A.alternata*), *Aspergillus flavus* Link. (*A.flavus*), and *Fusarium solani* F. sp. Pisi. (*F.solani*) under *in vitro* conditions.

MATERIAL AND METHODS

Materials used in present study were leaves of *A. calamus* and bulbs of *A. sativum*. The fungal cultures of three pathogenic fungi namely *A. alternata, A. flavus* and *F. solani*.Fungal culture of pathogenic fungus *A. flavus* (Saktiman 3Nst) was obtained from Herbal Pesticide Laboratory, Banaras Hindu University, Varanasi. Fungal cultures of other two test fungi namely, *A. alternata* and *F. solani* were obtained from Plant Pathology Laboratory, Central Potato Research Institute, Shimla. Potato dextrose agar (PDA) media (Potato, 200 g; dextrose 20 g; agar, 15 g in 1 ml of distilled water) (HiMedia, Mumbai, India) was used throughout investigation.

Fresh leaves of *A. calamus* and bulbs of *A. sativum* were collected, washed under tap water, then in distilled water and then kept between folds of filter paper to remove excess of water from external surface. On drying 100 g of fresh leaves of *A. calamus* and 50 g of peeled bulbs of *A. sativum* were chopped well, then 100 ml and 50 ml of distilled water was added separately (1:1 w/v) respectively. These were ground to fine powder and paste respectively, soaked as such for 2 h. Soaked materials were then filtered through muslin cloth and leaf extract of *A. calamus* and bulb extract of *A. sativum* were then collected with the help of funnel in separate conical flasks [5]. Finally, filtrate then obtained were used as stock solutions. To study antifungal activity of plant extracts, the poisoned food technique was used[6]. In this technique, different concentration of leaf extract alone and in combination with bulb extract of *A. sativum* viz. 5%, 10%, 15% and 20% were prepared by taking 5 ml, 10 ml, 15 ml and 20 ml of plant extracts and 95 ml, 90 ml, 85 ml and 80 ml of PDA medium in four beakers respectively. Plant extracts and PDA medium in all four beakers were mixed thoroughly. This mixture was then poured into sterile petriplates. Medium of control sets contained distilled water instead of plant extracts[7]. Discs of each test fungi cut from periphery of 7 days old culture and were inoculated aseptically in each of treatment and control sets. The petriplates were incubated at 28 ± 2ÚC for 7 days in incubation chamber. Diameters of fungal colony of treatment and control sets were measured in mutually perpendicular directions on 7th day. The percent inhibition of radial growth of test fungus by leaf extract and its combination were calculated[5, 8]. Three replicates were taken for each set.

$$\text{Percentage mycelial inhibition} = \frac{dc - dt}{dc} \times 100 ,$$

dc = average diameter of fungal colony in control sets, and *dt* = average diameter of fungal colony in treatment sets.

Nature of toxicity (fungistatic or fungicidal) was also assessed[9, 6]. For assessing nature of toxicity of aqueous leaf extract of *A. calamus* alone and its combination with bulb extract of *A. sativum*, on the 7th day inhibited discs were taken out from plates and washed with sterilized distilled water and reinoculated aseptically to plates containing 10 ml of fresh PDA medium. The revival of growth of fungal discs was observed after next 7 days and percent inhibition mycelial growth with respect to control set was calculated.

Fungi toxic spectrum of leaf extract of *A. calamus* alone and its combination with bulb extract of *A. sativum* was determined against six phytopathogenic fungi at 20% concentration by usual poisoned food technique using PDA medium. The percent inhibition was recorded and fungitoxic spectrum was studied.

RESULTS AND DISCUSSION

Crops are damaged every year due to various diseases, out of which fungal diseases are very common. Synthetic fungicides have been used since very long time to control these diseases. Although these have aided in agricultural improvement, but have numerous side effects. Therefore, these days plant derived fungicides are preferred over synthetic fungicides which are non phytotoxic, systematic & biodegradable in nature[10]. Plants are very good source of natural chemicals[11]. Many economically important organic compounds, pharmaceuticals & pesticides have been derived from higher plants[12]. Some recent researches on

antifungal activity of extracts of many higher plants have shown the possibility of their exploitation as biofungicides for control of plant diseases[13].

Considering these as a first step in present investigation, leaf extract of one such important Himalayan medicinal plant *A. calamus* alone as well as in combination with bulb extract of *A. sativum* was screened *in vitro* for its antifungal activity against three phytopathogenic fungi (*A. alternata, A. flavus & F. solani*) using poisoned food technique[5,6] . Rhizomes, roots and essential oils from various plant parts of this plant have been reported to possess antifungal properties[14]. Many of previous and recent studies have reported several important biological activities especially antimicrobial activity of roots, rhizome and essential oils of *A. calamus*[15, 16, 17]. Recent studies on antimicrobial activity of *A. calamus* rhizome and leaf extract have shown that rhizome predominantly possess bioactivities (antifungal and antiyeast)

Table 1. Fungitoxic screening of aqueous leaf extract *of Acorus calamus* against *A. alternata, A. aspergillus* and *F. solani*

Concentration	Percent inhibition of growth of test fungi		
(%)	*A. alternata*	*A. flavus*	*F. solani*
5%	66.6±0.64	40.3±0.33	54.3±0.72
10%	70.7±0.73	52.0±0.58	57.7±0.63
15%	81.4±0.37	66.1±0.69	70.3±0.36
20%	85.5±0.64	73.1±1.10	73.3±0.63

Each data point represents mean of three replicates ± S. E

Table 2. Fungitoxic screening of aqueous leaf extract of *Acorus calamus* + Bulb extract of *Allium sativum* against pathogenic strains of fungi *A. alternata, A. flavus* and *F. solani*

Concentration	Percent inhibition of growth of test fungi		
(%)	*A. alternata*	*A. flavus*	*F. solani*
5%	85.8±0.36	53.6±0.36	83.3±0.63
10%	91.1±0.00	66.2±0.36	89.6±0.40
15%	94.4±0.00	84.7±0.36	95.5±0.00
20%	95.5±0.00	91.4±0.36	100.0±0.00

Each data point represents mean of three replicates ± S. E

Table 3. Nature of toxicity of leaf extract of *Acorus calamus* against *A. alternata, A. flavus* and *F. solani*

Fungi	Concentration	Percent inhibition		Nature of
	(%)	Treated	Re inoculated	toxicity
A. alternata	15	81.4±0.37	73.3±0.63	Static
	20	85.5±0.64	75.5±0.63	Static
A. flavus	15	66.1±0.69	70.3±0.39	Static
	20	73.1±1.10	74.0±0.97	Static
F. solani	15	70.3±0.36	62.9±4.28	Static
	20	73.3±0.63	71.1±0.63	Static

Each data point represents the mean of three replicates ± S.E

than any other plant part such as leaf that has less bioactive effects[18]. Present study focused particularly on antifungal activity of leaf extract of *A. calamus* alone and its combination with bulb extract of *A. sativum* , its nature of toxicity and fungitoxic spectrum. Results obtained are depicted in tables and shown in plates ahead.

Fungitoxic screening of aqueous leaf extract *of A. calamus* against *A. alternata, A. flavus* and *F. solani*

As it is evident from table-1 and plate-1 that aqueous leaf extract of *A. calamus* has shown the % inhibition of radial growth of all of three test fungi at different conc. and % inhibition increased

Table 4. Nature of toxicity of leaf extract of *Acorus calamus* + Bulb extract of Allium sativum against *A. alternata, A. flavus* and *F. solani*

Fungi	Concentration (%)	Percent inhibition		Nature of toxicity
		Treated	Re inoculated	
A. alternata	15	94.4±0.00	71.8±0.97	Static
	20	95.5±0.00	71.4±0.37	Static
A. flavus	15	84.7±0.36	64.4±5.70	Static
	20	91.4±0.36	71.1±1.13	Static
F. solani	15	95.5±0.00	59.6±5.23	Static
	20	100.0±0.00	0.0±0.0	Cidal

Each data point represents the mean of three replicates ± S.E

Table 5. Fungitoxic spectrum of aqueous leaf extract of *Acorus calamus* against 6 tested fungi at 20% concentration

Tested fungi	Percent inhibition of growth of test fungi
Aspergillus flavus	73.1±1.10
Alternaria alternata	85.5±0.63
Fusarium solani	73.3±0.63
Helminthosporium oryzae Breda de Hann.	100.0±0.00
Penicillium italicum Wehmer.	100.0±0.00
Pythium debaryanum Hesse.	100.0±0.00

Each data point represents the mean of three replicates ±S. E

Table 6. Fungitoxic spectrum of aqueous leaf extract of *Acorus calamus* + Bulb extract of *Allium sativum* against 6 tested fungi at 20% concentration

Tested fungi	Percent inhibition of growth of test fungi
Aspergillus flavus	91.4±0.36
Alternaria alternata	95.5±0.00
Fusarium solani	100.0±0.00
Helminthosporium oryzae Breda de Hann.	100.0±0.00
Penicillium italicum Wehmer.	100.0±0.00
Pythium debaryanum Hesse.	100.0±0.00

Each data point represents the mean of three replicates ±S. E

with increasing conc.

Results depicted in table-1, indicate that at 20 % conc. leaf extract of *A. calamus* provided most effective inhibition against *A. alternata* (85.5 %), followed by *F. solani* (73.3 %) then *A. flavus* (73.1 %).

Fungitoxic screening of leaf extract of *A. calamus* + bulb extract of *A. sativum:*

As it is evident from the Table-2 and plate-2, that fungitoxic activity of aqueous leaf extract of *Acorus calamus* has been enhanced in combination with bulb extract of *Allium sativum.*

Results depicted table-2, have indicated that at 20 % conc., combination of leaf extract of *A. calamus* + bulb extract of *A. sativum* provided most effective inhibition against *F. solani* (100 %), followed by *A. alternata* (95.5 %) and then A. flavus (91.4 %). Both showed good synergistic effect in combination.

Nature of toxicity of aqueous leaf extract of *A. calamus* against toxigenic strains of fungi *A. alternata, A. flavus* and *F. solani*

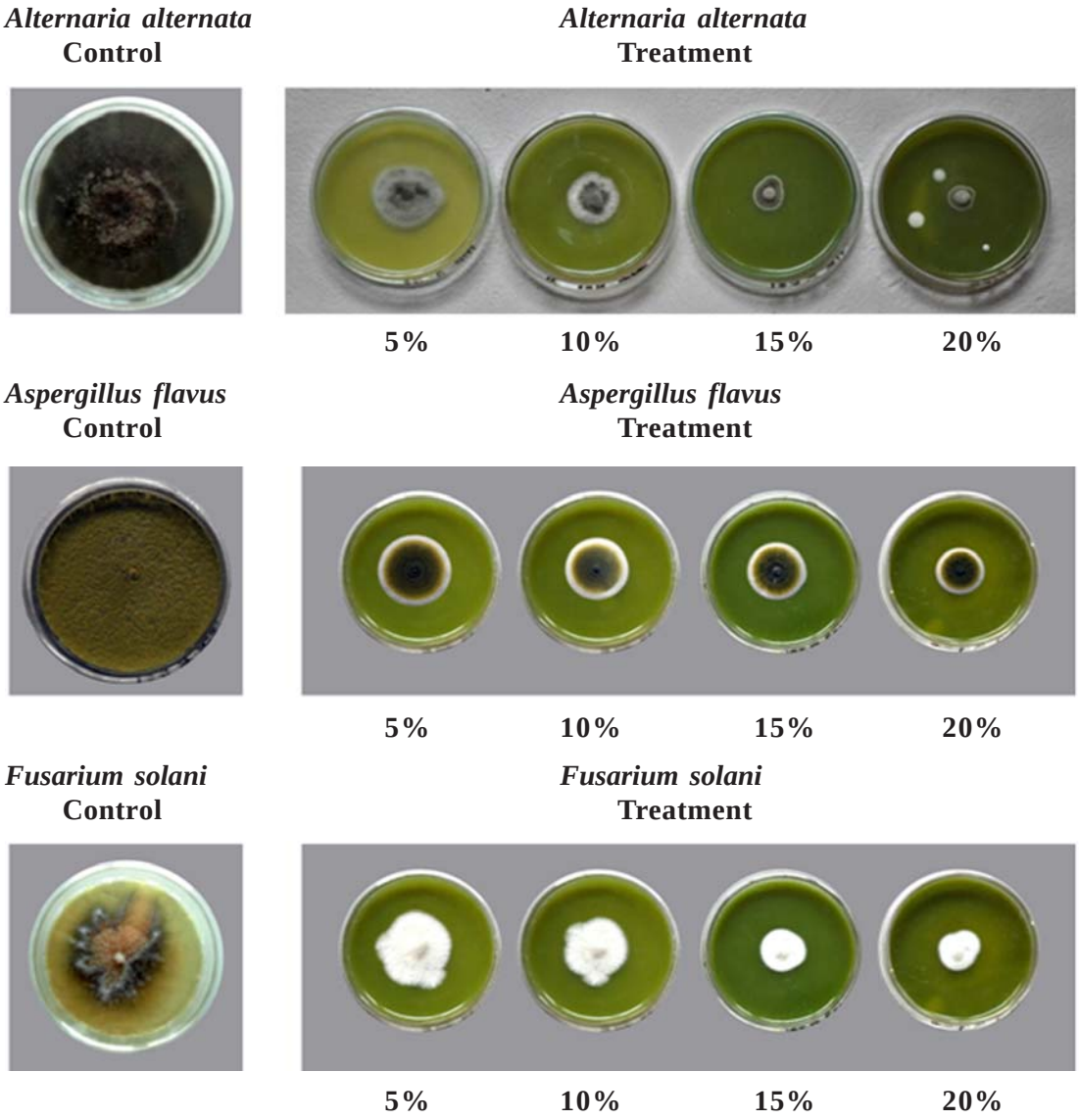

Plate 1. B. Photograph of fungitoxic screening of *A. calamus* against *Alternaria alternata*, *Aspergillus flavus* and *Fusarium solani*

Results depicted in table-3 have indicated nature of toxicity of leaf extract of *A. calamus*. It was found to be fungistatic against all three test fungi at 15 and 20 % conc. as the growth revives.

Nature of toxicity of aqueous leaf extract of *A. calamus* + bulb extract of *A. sativum* against toxigenic strains of fungi *A. alternata, A. flavus* and *F. solani.*

Results depicted in table-4 have indicated nature of toxicity of leaf extract of *A. calamus* + bulb extract of *A. sativum*. It was found to be fungistatic at 15 % and 20 % conc against all three test fungi except for *F. solani* where it was found to be fungicidal against *F. solani* at 20 % conc.

Fungitoxic spectrum of aqueous leaf extract of *A. calamus* against 5 tested fungi

Results depicted in table-5 have shown fungitoxic spectrum of leaf extract of *A. calamus.* It had been found to show a broad fungtoxic spectrum as found effective in inhibiting all six test fungi at 20 % conc.

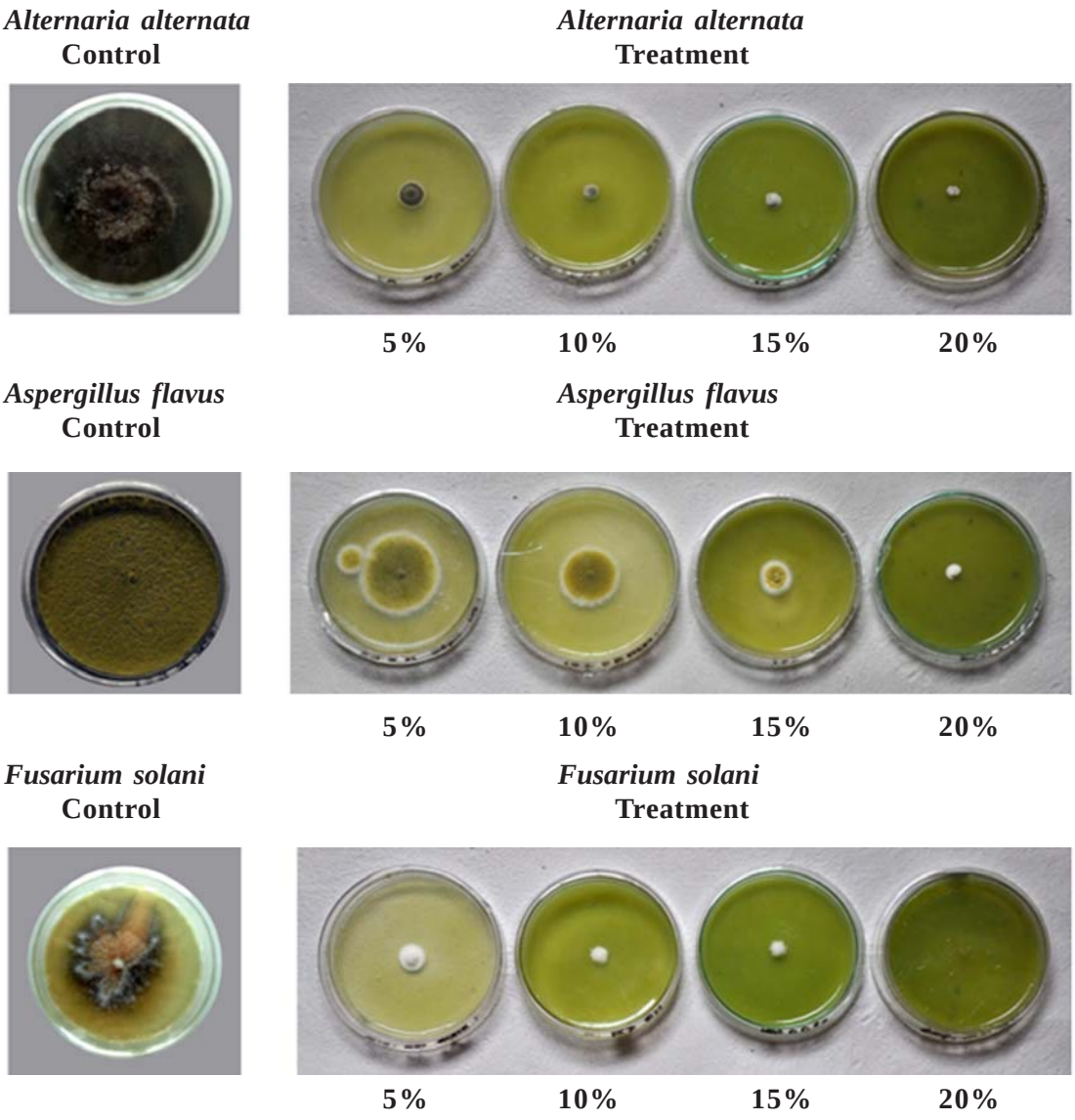

Plate 2. C. Photograph of fungitoxic screening of leaf extract of *A. calamus* + Bulb extra of *A. sativum* against *Alternaria alternata, Aspergillus flavus* and *Fusarium solani*

Fungitoxic spectrum of aqueous leaf extract of *A. calamus* + bulb extract of *A. sativum* against 5 tested fungi

It is evident from Table-6 that combination of aqueous leaf extract of *A. calamus* + bulb extract of *A. sativum* also had a broad Fungitoxic spectrum.

Overall analysis of results had shown that aqueous leaf extract of *A. calamus* alone as well as in combination with bulb extract of *A. sativum* were found to show antifungal activity against all three test fungi namely, *A. alternata, F. solani* and *A. flavus* under *in vitro* conditions. This piece of research work forms basis for its further evaluation against these phytopathogenic fungi in field conditions or under *in vivo* conditions and its assessments as new biofungicide.

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