Fungitoxic effect of *Adenocalymma alliaceum* Miers against *Fusarium oxysporum* f.sp. *gladioli*

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Fungitoxic effect of *Adenocalymma alliaceum* Miers against *Fusarium oxysporum* f.sp. *gladioli*

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Aqueous extract from the leaves of garlic creeper (*Adenocalymma alliaceum* Miers.) was investigated for their antifungal activity against 18 fungal organisms. The extract demonstrated wide spectrum fungitoxicty. Exposure of the spores of test fungi to the extract for 5 h inhibited the spore germination by 70-80 %, after exposure for 10 h, a 100% inhibition was observed. The inhibitory active principle of the extract was thermostable when kept for 1 h at 60°C and on boiling at 100°C for 4 min. However, the inhibitory activity of the extract decreased to 70 % when boiled for 6 min and was completely lost after boiling for 10 min.

Key words: *Adencoalymma alliaceum,* Antifungal activity, *Fusarium oxysporum* f.sp. *gladioli*

INTRODUCTION

Drawback of synthetic chemical methods have increased interest in developing further alternatives control measures particularly those that are environmentally social and biodegradable(Sharma and Tripathi, 2009). Thus replacement of synthetic fungicides by natural products particularly of plant origin, which are non-toxic and specific in their action, is gaining considerable attention. The rationale for exploiting plants for their antibiotic capabilities stems from the ability of plants to produce a wide array of secondary metabolites which presents a large and relatively untapped source of antifungal drugs (Amoo *et al.* 2009; Sharma and Tripathi, 2009).

Several herbal extracts have been reported to express antimicrobial activities. Inhibition of pathogens causing plant disease by the use of plant extracts and essential oils having antimicrobial properties have been observed from *Acalypha willensiana, Azadirachta indica, Datura metel, Eucalyptus comadulensis, E. citridora, Emblica offcinales, Allium sativum, Allium cepa, Lecus aspera, Ranunculus scleratus, Ocimum sanctum, Calotropis pocera* (Chohan *et al.* 2011). Wojdylo and Osgmianski (2007) suggested that there is a need to identify different types of medicinal plants with microbial activity. Obongoya *et al.* (2010) used *Azadirachta indica, Nicotiana tabacum* and *Vinca rosea* for the control of *Fusarium oxysporum* f. sp. *phaseoli.*

Medicinal creeper (*Adenocalymma alliaceum*) of family Bignoniaceae, is a wide spread climber shrub in North Brazil know as Ipod alho (garlic bush) because it has pungent garlic like smell. The leaves as well as the flower smell alliaceous and are used as substitute for garlic by natives, when the later is not accessible in the interior region. *Adenocalymma alliaceum* is one of the most important commercial cultivars because it contains a mixture of several compounds which are used as antisparmodics, diuretics, anesthesics, and narcotics. The compounds found in *Mansoa alliacea* and in some *Allium* species have biological proprieties that explain, at least in part, of the uses of “cipó-de-alho” in folk medicine. *M. alliacea* is commercialized as a medicinal plant, especially in Peru and in a smaller scale in the North of Brazil and it is the most studied species of the genus. There are very little chemical and biological studies on other *Mansoa*species. Other species of *Mansoa*

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have a garlic-like flavor and can be considered, together with *M. alliacea*, possible sources of diallyl sulfides (Zoghbi *et al.* 2009)

The present investigation was designed to determine the inhibitory effect of the leaf extract of *Adenocalymma alliaceum* against Eighteen fungal strains, out of which emphasis was given on one most devastating pathogen *Fusarium oxysporum* f. sp. *gladioli* that is the causal agent of diseases like *Fusarium* corn rot and yellows in gladiolus, which is one of the economically important crops of Asian countries. The damage to gladiolus plants due to these pathogens is as high as 40 % which gives the country a great economic loss. Hence, keeping in view the adverse effect of the fungicides on the agro-ecosystem, emphasis was given to find out the environmentally safe biocide from *Adenocalymma alliaceum* to manage the pathogen caused by *Fusarium oxysporum* f. sp. *gladioli*.

MATERIALS AND METHODS

Plant material

During the month of January and February the fresh leaves (100 g) of *Adenocalymma alliaceum* Miers were collected from the Botany Department, Lucknow University, Lucknow. The *in vitro* study was carried out in the Mycology and Plant Pathology research Laboratory, Lucknow.

Preparation of aqueous extract of leaves of garlic creeper

Collected leaves were properly washed with 70% ethanol and water separately. Final wash with distilled/sterilized water removes the trace of ethanol. An aqueous extract was prepared by blending fresh leaves with distilled water (10 ml/g fresh weight) i.e.10% in a blender for 2 minutes at 25 $\pm 1^0$C. The homogenate was filtered through a layer of muslin cloth. The filtrate was centrifuged at 12000 rpm for 20 minutes. The supernatant was sterilized through a Millipore filter (0.2 µm) and served as the stock solution. All dilution were made with sterile distil water when necessary.

Screening of antifungal activities

The antifungal activities of the plant extract was screened against *Alternaria alternate, Alternaria brassicae, Alternaria brassicicola, Alternaria carthami, Alternaria mali, Aspergillus niger, Botrydiplodia theobromae, Cladosporium cladosporioides, Colletotrichum capsici, Curvularia lunata, Fusarium roseum, Fusarium oxysporum* f. sp. *ciceri, Fusarium oxysporum* f. sp.*gladioli, Fusarium udum, Penicillium aurantio–griseum, Penicillium digitatum, Penicillium expansum* and *Penicillium itaclicum* were isolated from infected plant parts of their respective host and maintained on Potato Dextrose Agar (PDA) media at $27^0 \pm 2^0$C and store at 4 ^{0}C.

Effect of the leaf extract on spore germination of some fungi

Five concentrations of extract i.e. 1:0,1:1, 1:2,1:3,1:4,and 1:5 (extract : sterile distilled water) and one control (sterile distilled water alone) were tested for spore germination. Aliquots of 0.1 ml from each were placed on separate microscopic glass slide in three replicates and mixed with fungal spores obtained from 10 day old cultures from the different fungi: Slides containing the spores were placed in the moist chamber and incubated at 25 $\pm 2^0$C for 24 h. Each slide was then fixed in lactophenol- cotton blue and examined under high power (× 40) microscope for recording number of spore/conidia germinated using haemocytometer which about 100 spores on each slide were screened randomly for germination.

Further, studies were carried out on the spore germination of *Fusarium oxysporum* f. sp. *gladioli* only by leaf extract to observe the antifungal activity.

Effect of the duration of exposure of the leaf extract to the spore of *Fusarium oxysporum* f. sp. *gladioli*

Spores were mixed with the leaf extract and kept for 1, 5, 10, 15, and 24 h at 25 $\pm 2^0$C .After the specified periods of time, the cotton blue stained spore suspension was filtered through Whatman filter paper No 1. The spores retained on the filter paper were washed, 4-5 times with distilled water to remove traces of the leaf extract. Such spores were mixed in a drop of sterile distilled water on glass slides and incubated for 24 h at 25 $\pm 2^0$C. Control sets were prepared in the same way in sterile distilled water only.

Thermostabilty of the extract

The leaf extract was kept for 1 h at 30, 40, 50 and

60 ^{0}C and tested for its activity against fungal spores. The leaf extract was also boiled (100 ^{0}C) in separate culture tubes by direct heating for 1, 3, 5, 10, and 15 min. The precipitate obtained on boiling was allowed to settle, and the supernatant cooled to room temperature and tested again for its effect on spore germination.

Shelf life of the extract

The leaf extract was stored at 20, 10, 22, and 37 ^{0}C for 42 days. After every seven days, aliquots were drawn and tested for their efficacy on spore germination.

Pot trials of with botanical (in vivo)

To observe the effect of Aqueous extract of the garlic creeper leaves at their recommended dose during in vivo experiments, pot trials was conducted to control the Fusarium wilt of *Gladiolus* caused by *Fusarium oxysporum* f. sp. *gladioli*. The pathogens inoculum was mixed in the pot soil to achieve the pathogens population density of 2×10^4 CFU g^{-1}. The experiments inculcated 3 treatments

(1) Plant treated with aqueous extract of garlic creeper leaves and pathogen
(2) Plant treated with pathogen
(3) Plant not treated (control)

The seedling vigour (SV) one month after sowing was calculated.
Seedling vigour= (shoot length + root length) × percentage germination (%)
Mortality in plants was calculated according to the following formula

$$\text{Mortality (\%)} = \frac{\text{Total no of plants died in pot}}{\text{Total no of plant in pot}} \times 100$$

The per cent seedling mortality due to *Fusarium* wilt was calculated in each treatment and the dated was recorded one month after sowing.

Statistical analysis

Data for inhibition of spore germination were analysed using analysis of variance (ANOVA) or student's test.

RESULTS AND DISCUSSION

The effect of an aqueous extract from the leaves of garlic creeper (*Adenocalymnma alliaceum* Miers) at different dilutions on the spore germination of 18 fungal strains is shown in Table 1. There was a significant inhibition of fungal spore germination by the leaf extract at different dilutions.

The pure extract showed a 100% inhibition of test fungi tested except *B. theobromae*. At 1:3 dilution, *C.capsici* and *F. oxysporum* f. sp. *gladioli* exhibited 100% inhibition, while *A. brassicae* and *P. itaclicum* were least affected (49 %.).The degree of susceptibility of the different fungi varied at different dilutions. The extract in 1:4 dilutions was also inhibitory against several fungi tested: spore germination of *F. oxysporum* f. sp. *gladioli* was inhib-

Table 1 : Antifungal activity of garlic creeper (*Adencoalymma alliaceum* Miers) extract against different fungi

	% inhibition at different dilution					
Pathogens	1:0	1:1	1:2	1:3	1:4	1:5
Alternaria brassicae	100	100	100	49	28	21
A. brassicicola	100	100	100	56	29	22
A.carthami	100	100	98	65	46	30
A. mali	100	94	94	91	66	75
A. alternata	100	100	98	55	28	20
Aspergillus niger	100	98	91	70	67	25
Botrydiplodia theobromae	96	78	49	20	18	11
Cladosporium cladosporioides	100	100	87	51	49	29
Colletotrichum capsici	100	100	100	100	49	38
Curvularia lunata	100	100	98	93	39	21
Fusarium roseum	100	100	95	91	70	62
F. oxysporum f. sp. *ciceri*	100	100	100	78	52	39
F. oxysporum f. sp. *gladioli*	100	100	100	100	83	30
F. udum	100	100	100	59	62	41
Penicillium aurantio - griseum	100	100	100	70	56	19
P. digitatum	100	95	90	56	49	30
P. expansum	100	100	85	60	56	25
P. itaclicum	100	100	67	49	39	14

ited by 83%: while *A.alternata and A. brassicae* were least affected (28 %.).Dilution of extract at 1:5 was also inhibitory against several fungi tested: spore germination of *F. roseum* was inhibited by 62%: that of *P.itaclicum* was least affected (14 %.).

Table 2 : Effect of the duration of Exposure of the leaf extract of garlic creeper (*Adenocalymnma alliaceum* Miers) on spore germination of *F. oxysporum* f. sp. *gladioli*

Treatment	Inhibition of spore germination
1 h	50
5 h	75
10 h	100
15 h	100
24 h	100
Control	0.0

The species –specific inhibition of the spore germination was clearly observed with different species of the same genus indicating differences in susceptibility to the garlic creeper leaf extract.

Table 3 : Effect of leaf extract of garlic creeper (*Adenocalymnma alliaceum* Miers) kept at different temperatures on spore germination of *F. oxysporum* f. sp. *gladioli*

Treatment		Inhibition of spore germination
Extract kept for 1 h at 60^{0}C		100
Extract boiled for		
	1 min	100
	3 min	100
	5 min	100
	10 min	100
	15 min	100
Control		0.0

The exposure of the spore to the extract for different periods of time was studied. An exposure of 1 h inhibited spore germination by 50%, where as 100% inhibition was observed after 10 h exposure (Table 2). The low degree of inhibition of the spore germination after 1 h of exposure may be due to less absorption of the extract through the fungal spore wall (Chohan *et al* 2 011).

Table 4 : Effect of storage period of leaf extract of garlic creeper (*Adenocalymma alliaceum* Miers) at different temperatures on spore germination of *F. oxysporum* f. sp. *gladioli*

Storage period (days)	Inhibition of spore germination			
	-20 ºC	10 ºC	22 ºC	37 ºC
0	100	100	100	100
7	100	100	100	100
14	100	100	100	100
21	100	100	100	100
28	100	100	79	55
35	100	100	47	27
42	100	100	21	9
Control	0.0	0.0	0.0	0.0

The inhibitory effect of the leaf extract was not lost even at higher temperatures. The leaf extract kept for 1 h at temperatures up to 60 ºC exhibited 100% inhibition of the spore germination, as was also observed after boiling at 100 ºC for 4 min is shown in Table 3. This indicates that the extract was quite thermostable.

However, on boiling the extract for 6 min, the inhibition decreased to 70-80% and after boiling the extract for 10 min the inhibitory activity was totally lost. In contrast, boiling for over 10 min stimulated the spore germination with the formation of the 1-3 branched germ tube per spore as compared to the control where only one simple germ tube per spore was observed. Also, the length and thickness of the germ tube were increased. Thus, indicatins the loss of bioactivity of leaf extract.

The efficacy of the leaf extract was not lost up to 42 days at storage 20 ^{0}C and 10 ^{0}C and up to 21 days at other temperature tested. The inhibitory effect decreased after 28 day on storage at 22^{0}C and 37 ^{0}C and was lost after 42 days of storage at 37 ^{0}C (Table 4)**.**

The pH value of the extract of *Adenocalymma alliaceum* is 6.0 at the time of treatment, which is moderately acidic and this result indicated that the inhibition produced were certainly due to active principle present in this extract, not due to pH of the plant extract. It was experimentally established that all test fungi grow well in acidic pH. The result of this food poisons technique indicated the synergistic effect of the active principle present in the plant extract.

In pot trail conducted on *Gladiolus* to study the effect of Aqueous extract of the garlic creeper leaves was able to promote plant growth (Table 5). Botanical extract was found to be efficient in increasing all the parameters of growth as plant height (82.49 cm.) leaf no (14) fresh weight (356.00 g) dry weight (10.00 g) phenol (87.35) as compared to control. The per cent mortality of plant due to wilt disease was reduced in all treatment. The maximum per cent reduction in mortality of plants over control was found 10% botanical treatment.

Table 5: Plant growth promotion with botanical extract on *Gladiolus (in vivo)*

Parameter	Treatments (*Gladiolus)*			
	T1	T2	Control	LSD_P= (0.05)
Germination (%)	86.66(3.333)	45.33(6.666)	65.33(3.333)	24.890
Shoot length (cm)	80.14(0.011)	49.30(0.110)	60.25(0.051)	1.015
Root length (cm)	2.35(0.048)	1.74(0.003)	1.75(0.013)	0.502
Total plant height (cm)	82.49(0.029)	51.04(0.108)	62.00(0.043)	0.519
Leaf(no)	14(0.043)	6(0.030)	11(0.040)	0.418
Fresh weight (gm)	356.00(0.046)	150.00(0.003)	258.00(0.072)	0.330
Dry weight (gm)	10.00(0.015)	02.00(0.050)	06.00(0.105)	0.365
Total phenol content (Mg Gae/100 g)	87.35(2.361)	44.36(2.157)	75.23(2.165)	0.254
Index vigour	7148.58(36.090)	23136.64(42.969)	4050.46(65.106)	214.530
Mortality (%)	10.00(0.142)	60.00(0.035)	40.00(0.325)	0.236

Each value is mean of three replicates and Standard Error (±SE) is given along the mean values and LSD_P= (0.05) Least significant Difference. Control: not treated. (T1: Plant treated with aqueous extract of garlic creeper leaves and pathogen, T2: Plant treated with pathogen)

In the present investigation, leaf extracts of *Adenocalymma alliaceum* showed a wide spectrum of fungicidal activity, which is lost after boiling at 100 ^{0}C for 10 min, indicating that the active principle could possibly be an ester. Results signify the potentiality of *Adenocalymma alliaceum* as a source of antifungal therapies and hence further work is necessary to evaluate its active principle potentiality in *in vivo* studies on other pathogens as this biofungicidal botanics is environmentally safe and could replace the toxic and hazardous synthetic compounds. Simultaneously investigations are also needed to characterize, formulate and marketwise the active principles of the extract which may provide leads for the discovery of a novel antifungal compounds from *Adenocalymma alliaceum.* (Zoghbi *et al.* 2009)

The control of pathogens by using the fungicides is the most common and instant mean. Besides the development of resistance in pathogens against fungicides, environmental pollution and residual hazardous effects of fungicides on non target hosts are the main drawbacks in their use. Different plants having the fungicidal, insecticidal and nematicidal properties are present in the universe. There is a need to identify their principle ingredients and active ingredients and use them for the benefits of mankind.

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