RESEARCH ARTICLE

Antifungal activity of *Mirabilis jalapa*. L against selected fungi

Kumthekar Shrutika[1] and Pius Jessy[2],

[1]DDU, Kaushal Kendra, Greenhouse Management,Ramnarain Ruia College
[2]Department of Botany, Ramnarain Ruia College, Matunga, Mumbai-19.
shrutika2186@gmail.com

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ABSTRACT

The Four-o'clock plant, *Mirabilis jalapa* L.of family Nyctaginaceae is a popular ornamental plant grown worldwide for the beauty of its flowers and sweet fragrance. The plant is rich in many active compounds and has been used in traditional medicine due to the presence of these biomolecules of pharmacological importance. Evaluatation of antifungal activity of leaf, flower, root and seed of four cultivars (white, pink, yellow and multicolour flower) of *M. jalapa* on selected fungi –*Aspergillus niger* and *Fusarium oxysporum* was carried out by Minimum Inhibitory Concentration (MIC) and effect of extracts on spouraltion. The results of this study showed that extracts from all parts of four cultivars of *M. jalapa* were very active against selected fungi. MIC of the extracts was found to be in range of 11- 15 mg/ml. The extracts also significantly inhibited the sporulation in selected fungi. Among the cultivars Pink flower Cultivar and Multicolour flower cultivar and White flower cultivar showed potent antifungal activity against *F.oxysporum* compared to that of *A.niger*.

Key words: Antifungal activity, Minimum Inhibitory Concentration, *Mirabilis jalapa*,

INTRODUCTION

Plants are very important commercial source of chemical compounds. Secondary metabolities such as flavonoids, alkaloids and terpenoids produced by plants act as chemical defense against pests and diseases. Medicinal plants represent a rich source of antimicrobial agents. Uniyal *et al*. (2006) stated that a wide range of extracts of medicinal plant parts are used as raw drugs and possess varied medicinal properties. Some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use and many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, a vast majority have not been adequately evaluated (Balandrin *et al*., 1985).

Available literature on the phytochemical constituents of *M. jalapa* showed that the plant is rich in many active compounds including proteins, alkaloids, terpenes, flavonoids and steroids. It is a perennial herb and has been used in traditional medicine in many parts of the world for the treatment of various diseases (Nair *et a*l., 2004).

In the present study attempts were made to evaluate antifungal potential of leaf, flower, root and seed of four cultivars [white(WFC), pink(PFC), yellow(YFC) and multicolour flower(MFC)] of *M. jalapa* on two selected fungi.

MATERIALS AND METHODS

Fungal cultures:
Aspergillus niger (NFCCI 161) and *Fusarium oxysporum* (NFCCI 245) cultures were obtained from Agarkar Institute, Pune and was maintained on Potato Dextrose Agar (PDA) medium. Determination of Minimum Inhibitory Concentration (MIC) by broth dilution method (Astiti and Suprapta, 2012 - modified).

Spore Culture:
Spores were harvested in sterile d/w from the cultures of *A.niger* and *F. oxysporum* culutured on PD broth. This spore culture was used for carrying out MIC and effect of extracts on sporulation and fungal biomass. MIC was determined using dilution broth method (Astiti and Suprapta, 2012, modified). 1% - 1.5 % concentration of various plant extracts of *M.jalapa* were taken and diluted by the PD broth to make the total volume to 5 ml. 500 µl of spore culture was added in all test tubes. For each extract, positive and negative control with fungal spores and without fungal spores were also prepared. All the test tubes were incubated in dark at room temperature for 8 days. After 8 days, the concentration at which the fungal growth was inhibited was considered as the minimum inhibitory concentration (MIC) of the extract which was determined by visual observation and also by taking the fungal biomass.

Effect of extracts on Sporulation
Since the fungal growth was totally inhibited when 1.5% extract was used (from the earlier MIC experiment) effect of various extracts on sporulation was carried out only for 1, 1.1, 1.2, 1.3, 1.4 and 1.5% of extract prepared from different parts of four cultivars of *M.jalapa*. Total volume was made up to 5 ml with PD broth. 500 µl of spore culture was added in these test tubes. For each extract positive and negative control with fungal spores and without fungal spores were also prepared. All the test tubes were incubated in dark at room temperature for 8 days. After 8 days the effect of extracts on sporulation at various concentrations were carried out by counting the number of spores using haemocytometer under a light microscope.

Statistical analysis:
The data were subjected to Analysis of Variance (ANOVA). Statistical analysis was performed using IRRISTAT software (IRRI,2003). Treatment means were compared using Least Significance Difference (LSD) values at $p \leq 0.05$. Differences among treatments were tested by Ducan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Minium Inhibitory Concentration (MIC)
The results of determination of MIC of the extracts from various parts of *M. jalapa* against *A.niger* and *F. oxysporum* have been tabulated in table 1. It is evident that irrespective of cultivars, in general extracts from all parts were very active against selected fungi. There was no much difference in MIC. The extracts from various parts of *M.jalapa* exhibited MIC ranging from 11 to 15mg/ml against *A.niger* and *F. oxysporum*.

Among the cultivars, pink flower cultivar (PFC) and multicoloured flower cultivar(MFC) was found to be more effective against *A.niger* and White flower coloured cultivar(WFC) and MFC against *F. oxysporum*. Among the fungi the inhibitory effect was found to be moreagainst *F. oxysporum* compared to *A.niger* (Table 1).

Effect Of Extracts On Sporulation
Among the various concentrations of extracts, irrespective of cultivars and plant parts significant reduction in spore density was observed at 13 or 14mg/ml of the extract. Complete inhibition (100% inhibition) of sporulation in *A.niger* was observed when 14mg/ml of extract was used where as in *F.oxysporum* it was at 13 mg/ml. A decrease in spore density was observed with increasing concentrations of the extracts (Table 2).

Table 1: Minimum inhibitory concentration of extracts of flower, leaf, root, and seed of white, pink, yellow and multicolour flower cultivars of *M.jalapa.* against *A. niger* and *F. oxysporum.*

***M.jalapa* Plant part** / **Cultivars**	**MIC of Plant extracts (mg/ml)** ± SD	
	A.niger	***F. oxysporum.***
Flower		
WFC	14 ± 0.04	**11± 0**
PFC	15 ± 0.02	15 ± 0.02
YFC	15 ± 0	14 ± 0.04
MFC	15 ± 0	12 ± 0
Leaf		
WFC	14 ± 0.02	13 ± 0.04
PFC	**13 ± 0.04**	13 ± 0
YFC	14 ± 0.04	13 ± 0.02
MFC	15 ± 0	**11 ± 0**
Root		
WFC	15 ± 0	**11 ± 0**
PFC	**13 ± 0**	14 ± 0
YFC	14 ± 0	15 ± 0
MFC	14 ± 0.04	15 ± 0
Seed		
WFC	15 ± 0	14 ± 0.02
PFC	14 ± 0	14 ± 0
YFC	**13 ± 0**	15 ± 0
MFC	15± 0	**13 ± 0**

Table 2: Inhibitory activity of various concentrations of flower, leaf, root and seed extract of *M.jalapa* on sporulation of *A. niger* and *F. oxysporum*.

	Spore density ml^{-1} of *A.niger* (x 10^5 spores)				**Spore density ml^{-1} of *F.oxysporum* (x 10^5 spores)**			
	EXTRACTS							
Concentration (mg/ml)	**Flower**	**Leaf**	**Root**	**Seed**	**Flower**	**Leaf**	**Root**	**Seed**
0 (control)	65.1^a	65.1^a	65.1^a	65.1^a	143.1^a	143.1^a	143.1^a	143.1^a
10	34.7^b	23.2^b	17.2^b	37.8^b	15.2^b	13.2^b	16.0^b	16.0^b
11	27.1^c	17.3^c	11.0^c	26.7^c	7.5^c	9.5^c	5.8^c	8.3^c
12	17.8^d	13.0^d	6.6^d	24.6^c	4.7^d	6.6^d	2.3^d	5.3^d
13	9.7^e	9.5^e	1.8^e	8.1^d	3.0^e	**0^e**	**0^e**	1.5^e
14	0^f	0^f	0^f	0^e	0^f	0^e	0^e	0^f
15	0^f	0^g	0^g	0^f	0^g	0^e	0^e	0^g
LSD	1.0	1.3	1.5	2.2	1.4	1.5	1.4	1.6

Among the **cultivars** irrespective of various concentrations significant reduction in sporulation in *A. niger* and *F.oxysporum* was observed when extracts from **various parts of WFC** was used (Table 3). It was evident that irrespective of cultivars, and plant parts all extracts were significantly active against sporulation of selected fungi. Extracts were found to be more active against *F. oxysporum* than *A.niger.* In positive control, spore density was found to be 64 x 10^5 spores for *A.niger* and 145 x 10^5 spores for *F. oxysporum*.

Table 3: Inhibitory activity of flower, leaf, root and seed extract of four cultivars of *M.jalapa* on sporulation of *A.niger* and *F.oxysporum*.

Cultivars	EXTRACTS							
	Spore density ml^{-1} of *A.niger* (x 10^5 spores)				Spore density ml^{-1} of *F.oxysporum* (x 10^5 spores)			
	Flower	Leaf	Root	Seed	Flower	Leaf	Root	Seed
WFC	**17.7^{d}**	**10.5^{c}**	**12.4^{c}**	**15.0^{d}**	**22.0^{c}**	**21.5^{c}**	**20.7^{c}**	**20.8^{c}**
PFC	22.2^{b}	17.8^{b}	15.6^{a}	18.2^{c}	29.4^{a}	29.2^{a}	26.2^{a}	25.5^{b}
YFC	19.8^{c}	27.3^{a}	14.1^{b}	29.0^{b}	23.7^{b}	23.7^{b}	26.2^{a}	27.7^{a}
MFC	28.5^{a}	18.4^{b}	16.0^{a}	30.9^{a}	24.3^{b}	24.0^{b}	22.3^{b}	25.6^{b}
LSD	0.7	1.0	1.2	1.6	1.1	1.1	1.1	1.2

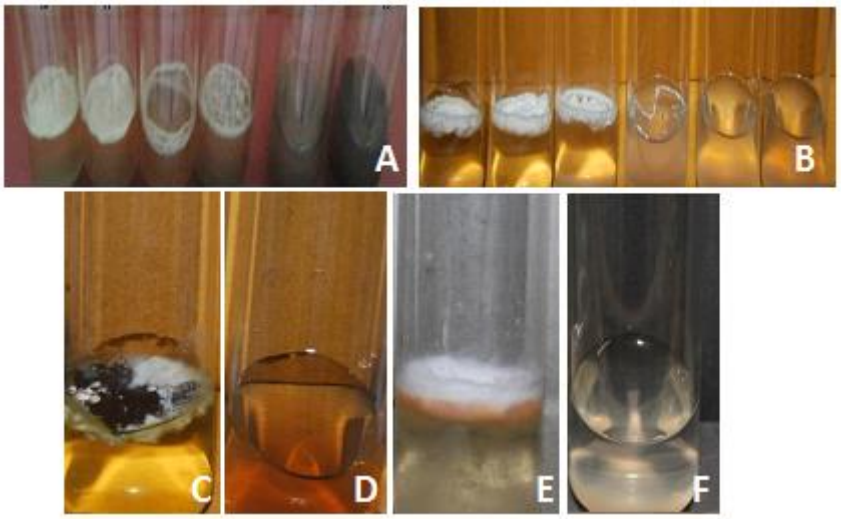

Fig.1 Effect of flower extracts of white flower cultivar of *M.jalapa* against sporulation in
A: *A.niger* **B:** *F.oxysporum* **C & D:** Positive and negative control -*A.niger*
E & F : Positive and negative control -*F.oxysporum*

In this study it was observed that extracts from all parts of four cultivars of *M. jalapa* were very active against selected fungi -*A.niger* and *F.oxysporum*. In general, significant reduction in sporulation and biomass in *A.niger* and *F. oxysporum* was observed when treated with flower and root extracts. Antifungal potential of the extracts were found to be significantly high against *F. oxysporum* compared to *A.niger*.

Though there are many reports on antibacterial activity of *M. jalapa* (De Bolle *et al.,* 1996; Kusamba *et al.,* 1991; Dimayuga,1998; Oladunmoye *et al*., 2007; Oskay and Sari, 2007; Sharma *et al*., 2010 and Ullah *et al.,*2011; Zachariah *et al.,* 2012), only few reports are available on its antifungal activity (Hajji *et al.,* 2010; Kumar *et al.,* 2010 and Muthumani *et al.,* 2008). To our knowledge there are no reports, where a comparative study of antifungal activity was carried out with various parts of four cultivars of *M.jalapa.* Aqueous tuber extract (Hajji *et al.,* 2010), leaf extract (Muthumani *et al.,* 2008) and Biocidal protein – Mj – AMP (Ikeda *et al.,* 1987) from *M.jalapa* was reported to show a wide range of antifungal activity. *Mucuna pruriens* seeds showed antifungal activity against *A. carneus, A.flavus* and *Candida albicans* (Marimuthu *et al.,*2013).

Inhibitory activities exhibited by *M.jalapa* may be due to the presence of tannins, alkaloids, flavonoids, terpenoids or essential oils (Selvakumar *et al.,*2012; Akintobi *et al.,* 2011; Erasto *et al*.,2004). Similar observations were also made in the present study, where the highest antimicrobial activity exhibited by flower and root extracts also showed high amount of phenolics, flavonoids, tannins and alkaloids.

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