IN VITRO EVALUATION OF LEAF EXTRACT OF SOME PLANTS AGAINST PATHOGENIC FUNGI OF *IMPATIENS BALSAMINA* L.

Gunjan Joshi, D.K. Jain and P.N. Singh

Department of Botany, Meerut College, Meerut (U.P.)

Abstract: Leaf extracts of eight plants (*Azadirachta indica, Aegle marmelos, Bougainvillea spectabilis, Catharanthus roseus, Datura stramonium, Lantana camara, Ocimum sanctum* and *Parthenium hysterophorus*) were evaluated for their fungitoxic activity against *Alternaria alternata* and *Colletotrichum capsici* isolated from the leaves of *Impatiens balsamina* L. by using surface sterilization method. Poisoned food technique was used to study the *in vitro* effect of leaf extracts (10% conc.). Maximum inhibition of radial mycelial growth of *Alternaria alternata* and *Colletotrichum capsici* were observed by the leaf extract of *Aegle marmelos* (60.59% and 54.59% respectively). Leaf extracts of *Azadirachta indica, Lantana camara* and *Parthenium hysterophorus* also showed considerable amount of inhibition.

Keywords:

INTRODUCTION

The plants of *Impatiens balsamina* L. (commonly named as Jewelweed, Jewel balsam weed, garden balsam and touch-me-not) are tender, succulent herb commonly grown as bedding and house plants. This plant has medicinal and ornamental values. *Alternaria alternata* (Fr) Keissler and *Colletotrichum capsici* (Syd.) E.J. Butler and Bisby are two main pathogens of *Impatiens balsamina*, causing leaf spot disease.

Many higher plants produce secondary metabolites which have a major role in plant defensive mechanism. These secondary metabolites may be exploited for the management of fungal diseases. Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999; Harborne, 1998; Gottlieb *et al*., 2002). Exploitation of naturally available chemicals from plants would be more realistic and ecologically sound method for plant protection. Leaf extracts are cost effective and are available cheaply and in plenty. Leaf extracts have been reported to reduce diseases caused by various pathogens. Hence the present study has been planned with the aim of evaluating the antifungal properties of leaf extract of eight plants viz. *Azadirachta indica*, *Aegle marmelos*, *Bougainvillea spectabilis*, *Catharanthus roseus*, *Datura stramonium*, *Lantana camara*, *Ocimum sanctum* and *Parthenium hysterophorus* against the fungal pathogens of *Impatiens balsamina*.

MATERIAL AND METHOD

Isolation of leaf pathogens: Leaves of *Impatiens balsamina* infected with *Alternaria alternata* and *Colletotrichum capsici* were collected separately. Leaf discs of 6 mm diameter containing lesions were cut by sterilized cork borer. They were surface sterilized with 0.01% mercuric chloride for 60 seconds, washed in three changes of sterilized distilled water, blotted dry and five such leaf disc were placed in each Petri dish containing molten, cool and sterilized PDA medium. The developing fungi were identified. Both fungi were tested for their pathogenicity to *Impatiens balsamina* using Koch's postulates. The pure culture of the pathogens were maintained on PDA.

Preparation of aqueous leaf extracts and their *in vitro* evaluation against pathogens by poisoned food technique: Fresh leaves of selected eight plants (*Azadirachta indica, Aegle marmelos, Bougainvillea spectabilis, Catharanthus roseus, Datura stramonium, Lantana camara, Ocimum sanctum* and *Parthenium hysterophorus*) were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and morter by adding 100 ml sterile distilled water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution. To study the antifungal mechanism of plant extracts the poisoned food technique was used (Nene and Thapliyal, 1982).Ten ml of stock solution were mixed with 90 ml. of sterilized molten PDA media to get 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile Petri plates, mycelium of five mm. size discs form periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed on the centre of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 25 ± 2^0 C for eight days and radial mycelial growth was measured.

The efficacy of leaf extracts or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the formula (Vincent, 1947).

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent inhibition
C = Radial mycelial growth in control
T = Radial mycelial growth in treatment

Table 1: Fungitoxic activity of plant extracts against *Alternaria alternata* and *Colletotrichum capsici*

Name of Plant	Per cent inhibition of mycelial growth of *Alternaria alternata* over control	Per cent inhibition of mycelial growth of *Colletotrichum capsici* over control
Azadirachta indica A. Juss	48.47 (44.13)*	31.86 (34.17)
Aegle marmelos (L.) Corr.Serr.	60.59 (51.12)	54.59 (47.64)
Bougainvillea spectabilis Willd.	32.56 (34.80)	20.17 (26.69)
Catharanthus roseus (L.) G.Don.	22.68 (28.44)	15.19 (22.94)
Datura stramonium L.	31.26 (34.00)	26.12 (30.74)
Lantana camara L.	43.58 (41.38)	45.77 (42.78)
Ocimum sanctum L.	12.78 (20.95)	15.15 (22.91)
Parthenium hysterophorus L.	42.26 (40.55)	41.60 (40.17)
SE(m) ±	0.89	0.47
CD (5%)	2.75	1.45
CV	4.23	2.62

* Figures in parentheses are arc sin transformed values

RESULT

In the present investigation eight aqueous leaf extracts were evaluated under *in vitro* condition to know the fungitoxic nature of these extracts against *Alternaria alternata* and *Colletotrichum capsici*. Leaf extracts were found effective in reducing the mycelial growth of both the pathogens. Maximum reduction of mycelial growth of *Alternaria alternata* was observed with *Aegle marmelos* (60.59%) which was significantly superior over all other treatments followed by *Azadirachta indica* (48.47%). Next effective leaf extracts were *Lantana camara* (43.58%) and *Parthenium hysterophorus* (42.26%) which were at par with each other. Whereas least growth inhibition was observed in case of *Ocimum sanctum* (12.78%) (Table 1,Fig. 1).

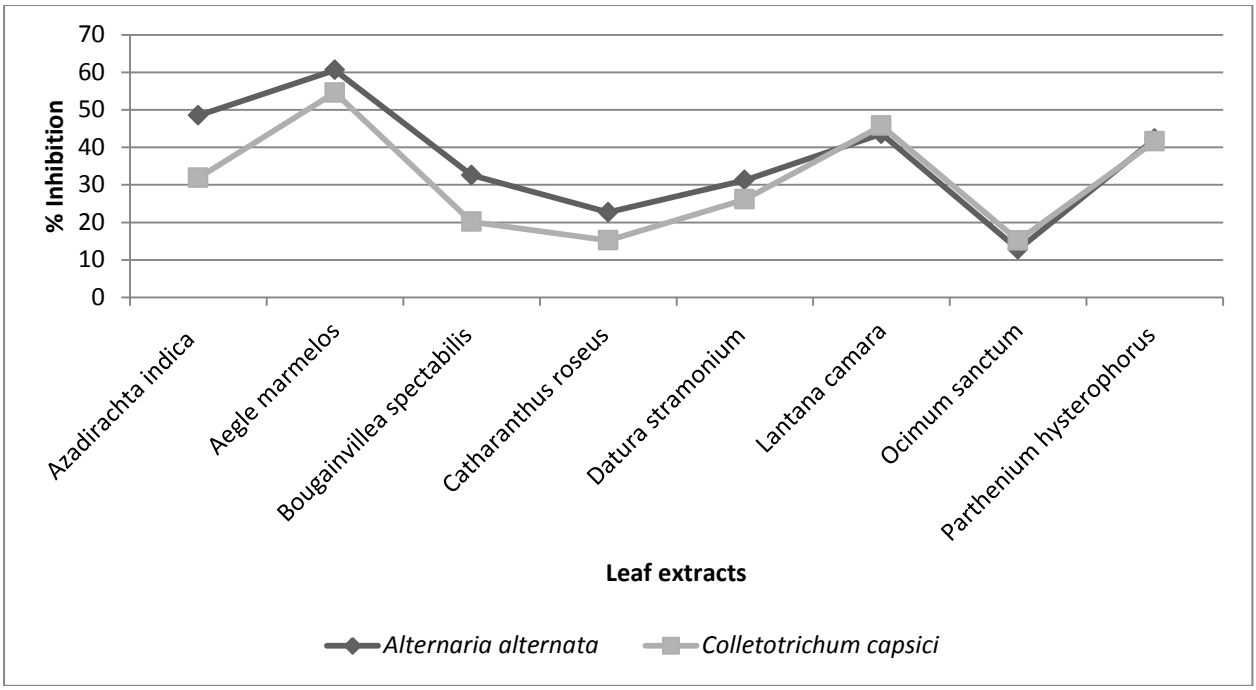

Figure1: Fungitoxic activity of plant extracts against mycelial growth of *Alternaria alternata* and *Colletotrichum capsici*.

Among the eight leaf extracts tested against *Colletotrichum capsici*, *Aegle marmelos* (54.59%) was significantly superior over all other leaf extracts followed by *Lantana camara* (45.77%), *Parthenium hysterophorus* (41.60%) and *Azadirachta indica* (31.86%). *Ocimum sanctum* (15.15%) and *Catharanthus roseus* (15.19%) were least effective in reducing the fungal growth (Table 1,Fig. 1).

DISCUSSION

Contrary to the problems associated with the use of synthetic chemicals, botanicals are environmentally non-pollutive, indigenously available, easily accessible, non phytotoxic, systemic ephemeral, readily biodegradable, relatively cost effective and hence constitute a suitable plant protection in the strategy of biological management of diseases. Hence, screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and to consider as one of the components in the integrated disease management.

The study showed that leaf extracts of different plants vary in their effect on the mycelial growth of *Alternaria alternata* and *Colletotrichum capsici.* Kurucheve *et al*. (1997) observed that the variation in the inhibitory effect of plant extracts may be due to qualitative and quantitative differences in antifungal principles. From the result, it was evident that among the eight different leaf extracts maximum growth inhibition of both the pathogen was caused by *Aegle marmelos* leaf extract. The present findings are in agreement with the results obtained by earlier workers (Senthilnathan and Narasimhan, (1994); Balakumar *et al*.,(2011). Leaves of *Aegle marmelos* yield an essential oil, 4 alkaloids besides aegelenine and aegeline; also condensed tannins, phlobotannins, flavan-3-oil, leucoanthocyanins anthocyanins, flavonoid glycosides, skimmianine, β-sitosterol, rutin and marmesinin. These compounds stated either alone or in combination may be the reason for its antifungal activity.

It is obvious from the study that aqueous leaf extracts of *Azadirachta indica* recorded the antifungal activity against both the pathogen tested. There are other studies showing similar effects against *Alternaria alternata* and *Colletotrichum capsici* (Shivpuri *et al*.,1997; Singh and Majumdar, 2001), which further confirms the presence of antifungal compounds in the test species. The fungicidal spectrum of *Azadirachta indica* has been attributed to azadiractin which belongs to C_{25} terpenoids. In the present investigation inhibition of *Alternaria alternata* and *Colletotrichum capsici* by *Azadirachta indica* leaf extract may also be because of the same reasons. Leaf extract of *Lantana camara* was effective in the inhibition of both the pathogen which is supported by the study of following workers (Saksena and Tripathi, 1985; Saraf *et al*., 2011). The antimicrobial activity may be due to the presence of triterpene secondary metabolite in the extract. *Parthenium hysterophorus* leaf extract also showed antifungal activity against pathogens. Similar effect of *Parthenium hysterophorus* against *Drechslera hawaiiensis*, *Alternaria alternata* and *Fusarium moniliforme* was observed by Bajwa *et al*.,(2004).

CONCLUSION

In the present investigation, though complete inhibition of the pathogen was not observed in any of the plant extract tested but considerable amount of inhibition was noticed in some of them and holds promise with their use in management of leaf spot diseases of *Impatiens balsamina* caused by *Alternaria alternata* and *Colletotrichum capsici*.

REFERENCES

Bajwa, R., Shafique, S., Anjum, T. and Shafique, S. (2004). Antifungal activity of allelopathic plant extracts. IV. growth response of *Drechslera hawaiiensis*, *Alternaria alternata* and *Fusarium monilifrome* to aqueous extract of *Parthenium hysterophorus*. *Int. J. Agri. Biol*. **6**: 511-516.

Balakumar. S., Rajan, S., Thirunalasundari, T. and Jeeva, S. (2011). Antifungal activity of *Aegle marmelos* (L.) Correa (Rutaceae) leaf extract on dermatophytes. *Asian Pac. J. Trop. Biomed.* **1**: 309-312.

Gottlieb, O.R., Borin, M.R. and Brito, N.R. (2002). Integration of ethnobotany and phytochemistry: dream or reality? *Phytochemistry* **60**: 145–152.

Harborne, J.B. (1998). Phytochemical methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed., Chapman & Hall Pub., London, UK.

Kurucheve, V., Gerard, E.J. and Jayaraj, J. (1997). Screening of higher plants for fungitoxicity against *Rhizoctonia solani in vitro*. *Indian Phytopathology* **50**: 235-241.

Nene, Y.L. and Thapliyal, P. N. (1982). *Fungicide in Plant Disease Control*, III Edition: Oxford and IBH publishing Co. Pvt. Ltd., New Delhi.

Saksena, N. and Tripathi, H.H.S. (1985). Plant volatiles in relation to fungistati. *Fitotherapis*, **56**: 243-244.

Saraf, A., Quereshi, S., Sharma, K. and Khan, N.A. (2011). Antimicrobial activity of *Lantana camara* L. *J. Exp. Sci.* **2**: 50-54

Senthilnathan, V. and Narasimhan, V. (1994). Effect of plant extracts on mycelial growth and spore germination of *Alternaria tenuissima* inciting the blight of onion and the nature of the antifungal components, In: (Eds. Sivaprakasam, K. and Seetharaman, K.) *Crop Diseases Innovative Techniques and Management* pp.307-313, Kalyani Publisher, New Delhi.

Shivapuri, A., Sharma, O.P. and Jhamaria, S.L. (1997). Fungitoxic properties of plant extracts against pathogenic fungi. *J. Mycol. Pl. Path*. **27**: 29-31.

Singh, J. and Majumdar, V.L. (2001). Efficacy of plant extracts against *Alternaria alternata*, the incitant of fruit rot of pomegranate (*Punica granatum* L.). *Indian J. Mycol. and Pl. Path*. **31**: 346-349.

Varma, J. and Dubey, N.K. (1999). Prospectives of botanical and microbial products as pesticides of tomorrow. *Curr. Sci.* **76**: 172–179.

Vincent, J. M. (1947). Distortion of fungal hyphae in presence of certain inhibitors. *Nature* **159**: 850.