Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 4466 - 4471
Received 05 March 2021; Accepted 01 April 2021.

Effect of Some Plant Extracts: *In Vitro* Growth of Alternaria Sps

Naina Srivastava

Department of Botany D.A.V. (P.G.) College, Dehradun, Uttar Pradesh, India

Abstract:

Alternaria usually attacks aerial parts of its host and symptoms appear as small, circular, dark spots. Control of this pathogen has become an obligatory requirement. In the present study, specie of *Alternaria* (*Alternaria alternata*) were selected on the basis of the ability to cause economically important plant diseases.Bioassays revealed that among aqueous and methanol extracts; methanol extract caused maximum inhibition of both the *Alternaria* species at high concentration of 4% after 5 and 10 days of incubation period. *A. alternata* displayed maximum inhibition of 100% at higher concentration of 4% after 5 days and 94% after 10 days of incubation. *Alternaria* species, ubiquitous post harvest pathogens, contribute to the spoilage of 55% of the agricultural output. The study was to evaluate the antifungal activity of extracts some plant species against *Alternaria* spp.Methanolic extracts from different parts of *Polygonum perfoliatum Cymbopogon citratus ,Lantana camara* and *Mimosa pudica* were evaluated for potential antimicrobial activity against Alternaria strains. These plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address not fulfilled therapeutic needs.

Key words: *Alternaria alternata,* Methanolic extract, Agar well diffusion technique.

Introduction

Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extracts were determined medicinal plants contained substances inhibitory to conidial germination of *Alternaria* The present study aimed at evaluating the *in vitro* antimicrobial activity of methanolic extracts of medicinal plant against *Alternaria alternata, solani* using agar well diffusion technique. These extracts showed maximum activity, even at very low concentrations, and the same fungicide effects as chemical fungicide.This study concludes that aqueous and methanol extracts of aloe vera,*Polygonum perfoliatum Cymbopogon citratus ,Lantana camara* and *Mimosa pudica* possess the ability for substantial inhibition in growth of *A. alternata* and *A. tenuissima* and these extracts exhibit amazing fungicidal properties that support their traditional use as antiseptics formulations of plant extracts could have important roles in biologically based management strategies for control of diseases caused by Alternaria.

The plants used, as drugs are fairly innocuous and relatively free from toxic effects or were so toxic that lethal effects were well known. The nature has provided the storehouse of remedies to cure all ailments of mankind. There is no doubt that plants are a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis.

Alternaria genus is cosmopolitan in occurrence. The members of this species like *A.alternata, A. solani, A. porri, A. dauci, A. helianthi*, *A. carthami* and *A. macrospore*causing different diseases in their respective hosts (Rotem, 1998).

synthesis. There are several reports of antibiotic resistance of human pathogens to available antibiotics J., Mitsuyama et al, L .Gutmann et al, A. J Mathias., et al, R. Gangulyet al, Biomolecules of plant origin appear to be one of the alternatives for the control of theseantibiotic resistant human and plant pathogens besides small molecules from medicinal chemistry, natural

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 4466 - 4471
Received 05 March 2021; Accepted 01 April 2021.

products are still major sources.

Many researchers have tried to find safe and economical control of plant diseases by using extracts of different plant parts (Bajwa et al., 2004; Bajwa and Iftikhar, 2005; Shafique et al., 2011).

Among them, *A. solani* causing early blight of potato (*Solanum tuberosum)* and tomato (*Lycopersiconesculentum)* is the most destructive (Reni & Roeland**,** 2006) of field crops. It causes diseases on foliage (blight), basal stems of seedlings (collar rot and damping off), stems of adult plants (stem lesions), fruits (fruit rot) of tomato and may also infect egg plant and pepper. This disease can be very destructive if left uncontrolled, often resulting in complete defoliation of plants (Dater & Mayee, 1985). Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grow wild in different parts of our country Indian medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectiveness. In this study, we evaluated the antimicrobial activity of methanolic extracts of *Lantana camara* and *Mimosa pudica* against several pathogenic microorganisms.

Although, chemical control suggests being the best method to control *Alternaria* diseases however in recent years, use of chemicals has increased consumer concern and their use is becoming more restricted due to carcinogenic effects, residual toxicity problems, environmental pollution, occurrence of microbial resistance, high inputs etc. (El-Rokiek et al., 2006).

Biological screening of plant extracts is carried out throughout the world for the determination of their antifungal activity. Synthetic chemicals used to control plant diseases not only pollute the environment, but are also harmful to human health. Because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly bio-compounds for the control of plant diseases using diffusates from different plants (Gerresten & Haagsma, 1951; Kumar *et al*., 1979; Naidu & John, 1981).

Many studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which showed antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antimicrobial potential (Mothana & Lindequist, 2005; Bajpai *et al*., 2005; Wojdylo *et al*., 2007).

The most common method for controlling these pathogens is the use of fungicides but the development of resistance in pathogenic fungi to common fungicides and increasing residual hazardous effects on human health and environmental pollution has given a thrust to search for new plant derivatives that can obstruct the fungal pathogencity. Use of natural products for the management of fungal diseases in the plants is considered as a good alternate to synthetic fungicides, due to their less negative impact on the environment. Many higher plants and their constituents have been successful in plant disease control and proved to be safe and nonphytotoxic; unlike chemical fungicides. Three weedy plants namely *Lantana camara* and *Capparis decidua* has been used for this purpose (Sharma & Kumar, 2009).

Plant diffusates are not only easy to prepare but are also non-polluting and low priced as compare to commercial fungicides. Keeping in view, some plant diffusates were evaluated for their antifungal activity against *Alternaria solani.*

Materials and Methods

This work was conducted in Department of Botany, D.G.(P.G.) COLLEGE KANPUR determine the antifungal activities of Aloe vera,*Polygonum perfoliatum Cymbopogon citratus ,Lantana camara* and *Mimosa pudica* selected fungal pathogen viz., *Alternaria alternata* in methanol by employing food poisoning technique (Naz *et al*., 2006).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 4466 - 4471
Received 05 March 2021; Accepted 01 April 2021.

Isolation of pathogens: pathogens viz., *Alternaria alternata* was selected for this experimental work. Pure culture of Diseased samples were surface sterilized with 5% Chlorox for one minute and washed three times with sterilized distilled water.

Preparation of pure culture: Pathogen were isolated with the help of sterilized forceps and plated on sterilized potato dextrose agar (PDA) medium (potato starch: 20 g, dextrose: 20 g, agar: 20 g and distilled water to make the volume 1 liter, which was sterilized in a gas operated autoclave at 15 pounds pressure per square inch (PSI) for 20 minutes. Plates were incubated at 25ºC and observed daily for emergence of colonies.Sub-culturing was done from single spore to obtain pure culture.

Collection and preservation of plants samples: Fresh leaves of *Aloe vera,Polygonum perfoliatum Cymbopogon citratus ,Lantana camara* and *Mimosa pudica* were collected from company garden kanpur. These were washed with tap water and air dried for one day to eliminate surface moisture. Then leaves were packed into envelop and kept in oven at 60°C temperature until dried. Dried leaves were grinded separately in an eclectic grinder to obtain powder which was than kept in plastic bags for further use.

Preparation of extracts: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method of Murray *et al.*, 1995 modified by Olurinola 1996 Hundred gram of the dried powdered plant were soaked separately in 500ml of 98% ethanol. These mixtures were refluxed followed by agitation at 200 rpm (revolution per minute) for 1 hour. The ethanolic extracts were squeezed and then filtered by muslin cloth. The extracts were placed into a wide tray to evaporate ethanol and added with water to make plant extracts.

Food poison technique: Diffusates were added in Potato dextrose agar (PDA) @ 10, 50, 100 and 200 g L-1 and poured into Petri dishes. PDA medium added only with ethanol and water served as control. Each Petri dish was inoculated with 5 mm plug of pure isolate taken from margins of actively growing culture of pathogen. Then Petri plates were incubated at 25° ± 2oC.

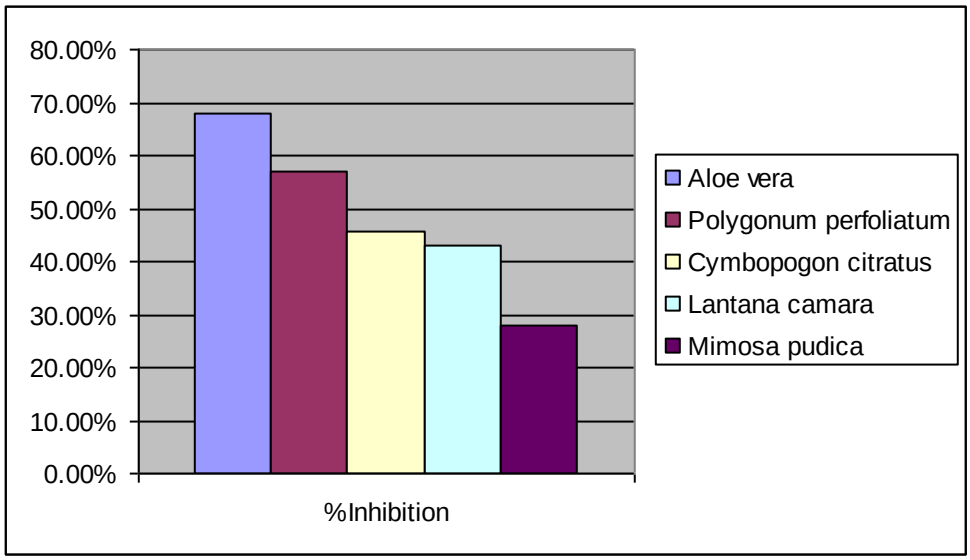
Mycelial growth was recorded when the growth of three selected pathogens were completed in the control treatment. Each treatment was repeated five times. Mean radial mycelial growth of each plant diffusates was recorded and data were subjected to statistical analysis. Radial mycelial growths on different diffusates were transformed into inhibition percentage by using the following formula (Naz *et al*., 2006):

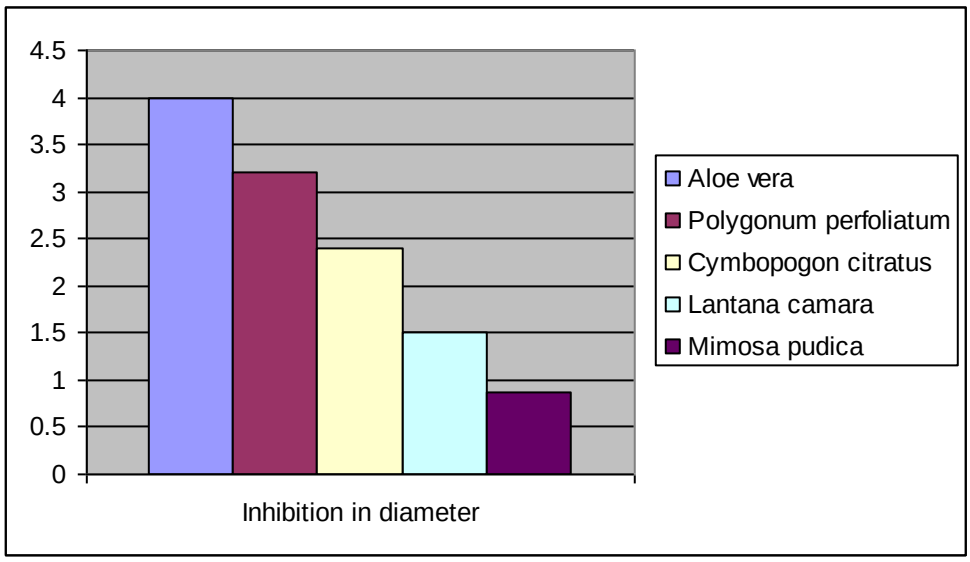
$$\text{Mycelial growth on diffusates Inhibition percentage} = 100 - \frac{\text{Mycelial growth}}{\text{control}} \times 100$$

The agar plate diffusion plate method (Nene & Thaplliyal, 1979) was used to test antifungal activity of plants against plant pathogen.

Name of plants	%Inhibition	Inhibition in diameter
Aloe vera	*(77.85%)*	4nm
Polygonum perfoliatum	(66.96%)	3.2nm
Cymbopogon citratus	(55.59%)	2.4nm
Lantana camara	(52.90%)	1.5nm
Mimosa pudica	(37.87%)	.88nm

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 4466 - 4471
Received 05 March 2021; Accepted 01 April 2021.





Results and Discussion:

Inhibition in radial mycelial growth (rmg) of *Alternaria solani* induced by five medicinal plants:

Highest Inhibition in radial mycelial growth % induced by *Alternaria solani* was exhibited by *Aloe vera (67.85%) Polygonum perfoliatum*(56.96%) followed by *Cymbopogon citratus* (45.59%),,*Lantana camara* (42.90%) and *Mimosa pudica* (27.87%) difference found in inhibition of radial mycelial growth between *Cymbopogon citratus* (45.59%) and ,*Lantana camara* (42.90%), whereas significant difference in inhibition of radial mycelial growth was found between *Polygonum perfoliatum* (56.96%) and *Cymbopogon citratus* (45.59%). Lemongrass extract decreased *Fusarium verticillioides* growth in PDA by 90 and 100% at 500 and 1000 ppm, respectively, being in accordance with the present study (Mishra and Dubey, 1994). In the same way, Baratta et al. (1998) reported 91% inhibition of the growth of *A. niger* in liquid culture media when treated with 1000 ppm lemongrass extract.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 4466 - 4471
Received 05 March 2021; Accepted 01 April 2021.

Results were found cognizant with previous findings of Hassanein *et al*., (2008) that the extract obtained from *Lantana camara* completely suppressed the growth of *Alternaria solani* on PDA *In vitro*.Statistically, significant increase in radial mycelial growth of *Alternaria solani* was observed with an increase in concentration of the diffusates (Fig. 2). Among 5 concentrations, the highest inhibition in radial mycelial growth was 55.72% at 200g/l followed by 48.60% at 100g/l, 44.51 at 50g/l and lowest inhibition was recorded 24.49 at 10g/l as compared to control. Similar results were recorded from preliminary investigations by Hassanein *et al*., (2008) reporting antifungal activity of *Azadirachta indica* leaf extract against *Alternaria solani*.

There are many examples in literature which support these findings. Similarly, Bajwa et al. (2007) carried out the study on antifungal activity of aqueous and n-hexane shoot extracts of *Aloe vera* against *A. alternata*, *Alternaria citri* and *A. tenuissima*. They reported that inhibitory effect was variable with applied concentrations and caused a significant inhibition in biomass production of the three test fungi. *Alternaria alternata* had strong antifungal properties towards *Aloe vera* gel at same concentration . The result inclose conformity with the finding of Yolanta & Galon (1995) who tested antifungal activity of natural *Aloe vera* gel on plant pathogenic fungi *Alternaria alternata*.

The current results demonstrate that selected medicinal plant diffusates effectively suppressed the radial mycelial growth of Alternaria. The highest inhibition (77.85%) was recorded at 200g/l concentration in radial mycelial growth of pathogen, followed by (66.96%) followed by *Cymbopogon citratus* (45.59%),,*Lantana camara* (52.90%) and *Mimosa pudica* (37.87%) 100g/l (80.20%), 50g/l (62.11%), whereas the least inhibition of radial mycelial growth (42.90%) was observed at 10g/l. Statistically, the rate of increase in radial mycelial growth inhibition was regressed against five different concentrations of plant diffusates.

Biocontrol is the safest and economical method of controlling plant pathogens by using extracts of different plant parts . Presently, the study was conducted to assess the *in vitro* efficacy of aqueous and methanol extracts of *C. citratus* against two pathogenic test species of *Alternaria.*

Reference:

1. Rotem, J. 1998. *The genus Alternaria: Biology, Epidemiology and Pathogenicity*. *Am.*
2. *Phytopathol*., Society Press, St. Paul, Minnesota.
3. Reni, C. and V.E. Roeland. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *J. Phytopathol.*, 72(13): 335-347.
4. Dater, V.V. and C.D. Mayee. 1985. Chemical management of early blight of tomato. *J. Maha Agri.Univ*., 10(3): 278-280.
5. Gerretsen, F.C. and N. Haagsma. 1951 Occurrence of antifungal substances in *Brassica repa,Brassica olleracea* and *Beta vulgaris*. *Nature* (London), 168-659.
6. Kumar, B.P., M.A.S. Charya and S.M. Reddy. 1979. Screening of plants extracts for antifungal properties. *New Botanist*, 6: 41-43.
7. Naidu, V.D. and V.T. John. 1982. *In vitro* inhibition of rice fungal pathogens by extracts from higher plants. *Int. Rice Res. Newsletter*, 6: 12.
8. Mothana, R.A.A. and U. Lindequist. 2005. Antimicrobial activity of some medicinal plants of the island Soqotra. *J. Ethnopharmacol*., 96: 177-181.
9. Bajpai, M., A. Pande, S.K. Tewari and D. Prakash. 2005. Phenolic contents and antioxidant activity of some food and medicinal plants. *Int. J. F. Sci. Nutrition*, 96(4): 287-291.
10. Wojdylo, A.J., Oszmianski and R. Czemerys. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *F. Chem*., 105: 940-949.
11. Sharma, B. and P. Kamar. 2009. *In vitro* antifungal potency of some plant extracts against
12. *Fusarium oxysporum. Int. J. G. Pharmacy*, 3(1): 63-65.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 4466 - 4471
Received 05 March 2021; Accepted 01 April 2021.

13. Sharma, V.N. and C.L. Jandaik. 1994. Effect of some plant materials in controlling different
14. moulds in *Agaricus bisporus* (Lang) Imb. *J. Ind. Mycol. and Pl. Pathol*., 24(30): 183-185.
15. J., Mitsuyama , R Hiruma., A .Yamaguchi. and T .Sawai.. *Antimicrob. Agents Chemothe.*, **1987**, 31,379–384.
16. L .Gutmann., D .Billot-Klein., R .Williamson., F. W Goldstein., J Mounier, F Acar and E Collatz. *Antimicrob. Agents Chemothe.*, **1988**, 32, 195–201.
    A. J Mathias., R. K Somashekar., S Sumithra. and S Subramanya..*Indian J. Microbio.*, **2000**,40, 183–190.
17. R. Ganguly, P Mishra. and A .Sharma,. *Indian J. Microbio.*, **2001**, 41, 211– 213.
18. Bajwa R, Iftikhar S (2005). Antifungal activity of allelopathic plant extracts V1: *In vitro* control of fungal pathogens by aqueous leaf extracts of eucalyptus. Mycopath, 3(1 and 2): 7-12
19. Shafique S, Bajwa R, Shafique S, Akhtar N, Hanif S (2011). Fungitoxic Activity of Aqueous and Organic Solvent Extracts of *Tagetes erectus* on Phytopathogenic Fungus *Ascochyta Rabiei.* Pak. J. Bot., 43(1): 59-64.
20. El-Rokiek KG, El-SAhahawy TA, Sharara FA (2006). New approach to use rice straw waste for weed control. II: The effect of rice straw extract and fusillade on some weeds infesting soybean. Int. J. Agric. Biol., 8(2): 269-275.
21. Naz, F., C.A. Rauf, I.U. Haque and I. Ahmad. 2006. Management of *Rhizoctonia solani* with plant diffusates and chemicals. *Pak. J. Phytopathol*., 18(1): 36-43.
22. P. R Murray, E. J Baron, M. A Pfaller, F. C Tenover, H. R Yolken, *Manual of Clinical Microbiology*, 6th Edition. ASM Press, Washington. DC,
23. P. F Olurinola, *A laboratory manual of pharmaceutical microbiology*. Idu, Abuja, Nigeria, **1996**, 69-105.
24. Bajwa R, Shafique S, Shafique S (2007). Appraisal of antifungal activity of *Aloe* **1995**, 15-18. *vera.* Mycopath, 5: 5-9.
25. Mishra AK, Dubey NK (1994). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. App. Env. Microbiol., 60: 1101-1105.
26. Baratta MT, Dorman HJD, Deans SG, Figueieredo AC, Barroso JG, Ruberto G (1998). Antimicrobial and antioxidant properties of some commercial essential oils. Flav. Frag. J., 13: 235-244.
27. Yoltana, S. and R.B. Golan. 1995. *Aloe vera* gel activity against plant pathogenic fungi.