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BIO-EFFICACY OF PLANT EXTRACTS AGAINST *ALTERNARIA ALTERNATA* (FR.) KEISSLER *IN VITRO*

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Number of plants has been found to possess antifungal properties, which are able to control certain fungal diseases of crops. Effect of *Boswellia serrata* Roxb ex. Coleb., *Gnidia glauca* (Fresen.) Gilg., *Ocimum americanum* L., *Mundulea sericea* (Wild.) A. Chev., and *Woodfordia fruticosa* (L.) Kurz. extracts are tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of *Alternaria alternata* (Fr.) Keissler. Extracts of *B. serrata* leaves were found significantly superior in inhibiting the mycelial growth 21.97, 33.98 and 42.81% of *A. alternata* at 5, 10 and 15 percent, respectively. Extract of *W. fruticosa* and *O. americanum* were found second best after *B. serrata,* while extracts of *G. glauca* and *M. sericea* were least effective in growth inhibition as compared to other plant extracts at all the three concentrations tried.

Keywords: *Alternaria alternata;* Antifungal; Inhibition; Plant extracts.

Introduction

Fungi are an important group of microorganisms responsible for various diseases of plants and cause a considerable loss in yield. A number of chemical fungicides are available in market for the crop protection. Some of them are excellent in terms of efficacy and cost benefit. However, their indiscriminate use has created the problems of air, soil and water pollution, development of resistance in target organisms and serious health hazards due to the toxicity of their residues. Efforts are being done for finding alternatives to chemical fungicides to overcome these problems. Plants are the richest source of organic chemicals on the earth and produce a wide variety of secondary metabolites, which can be used as defensive weapons. Plant extracts can be the potential alternatives to chemical agents that are hazardous to human and animal health. Number of plants has been found to possess antifungal properties, which are able to control certain fungal diseases of crops instead of spraying chemical fungicides.

Alka Denej[1] reported that phenolic compounds are good inhibitors of fungal pathogens and useful in controlling fungal diseases. Garg[2] studied the antifungal activity of essential oil of *Boswellia serrata* against seventeen pathogenic fungi. From the above reports it was clear that plants containing natural phenols could be used as biofungicides against the *Alternaria alternata.*

Alternata alternata causes leaf blight in tomato, leaf spot on papaya, marigold, chilli and green gram. Tandon and Shivkumar[3] reported *A. alternata* to be pathogenic on fruits of tomato, apple, bean, lemon and Carandas (*Carissa carandas).* Singh and Suhag[4] reported that *A. alternata* isolated from radish were pathogenic on spinach, sarson, cabbage, cauliflower and tomato. Madan and Chand[5] reported *A. alternata* to be pathogenic on phalsa, peach, pomegranate, chickpea, linseed, papaya, bottlegourd and tomato.

Material and Methods

Leaves of *Boswellia serrata* and *Woodfordia fruticosa* were tested for their antifungal properties against the important pathogen *Alternaria alternata* as they contain natural phenols. In addition *Mundulea sericea, Gnidia glauca* and *Ocimum americanum* were also tested for their fungicidal properties. Healthy leaves of *B. serrata, O. americanum, M. sericea, W. fruticosa* and *G. glauca* collected from the Harishchandragad-Kalsubai Wild Life Sanctuary, were tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of selected pathogen. It was isolated from wilted roots of chilli. Surface sterilized pieces of chilli roots were placed on potato dextrose agar medium in petriplates. Petriplates were then incubated at 28°C to 30° C temperatures. Repeated sub culturing was practiced to obtain pure fungal culture. During all these operations perfect aseptic conditions were maintained.

Fifty grams of plant leaves of each plant were cut into small pieces and minced with the help of grinder

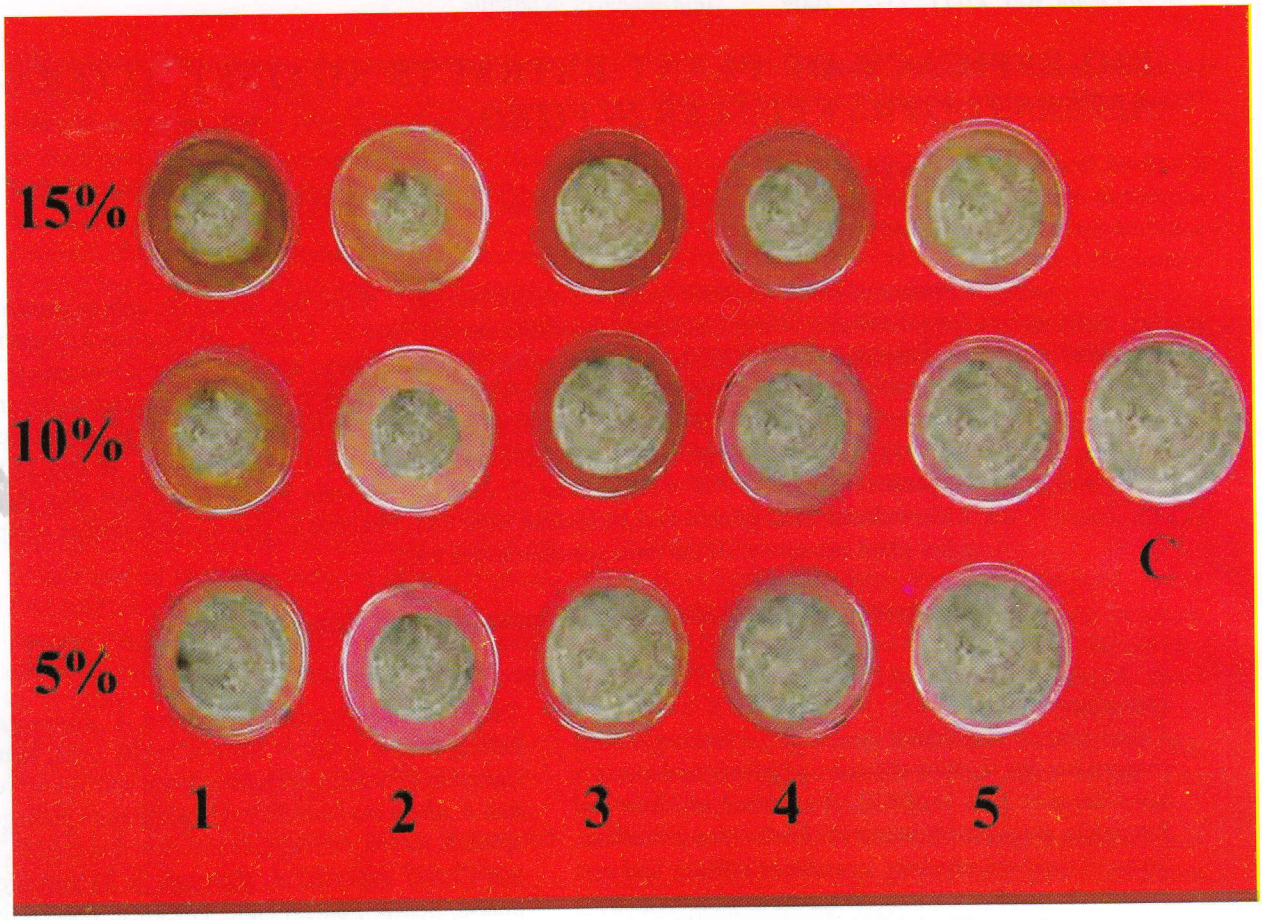

Fig.1. Effect of various plant extracts on the growth of *Alternaria alternata in vitro* at different concentrations.
(1. *Woodfordia fruticosa;* 2. *Boswellia serrata;* 3. *Ocimum americanum;* 4. *Gnidia glauca;* 5. *Mundulea sericea)*

Table 1. Effect of various plant extracts on the growth of *Alternaria alternata in vitro* at different concentrations.

Name of plant extracts used	Concentrations					
	5%		10%		15%	
	Av. colony diameter (mm)	Growth inhibition (%)	Av. colony diameter (mm)	Growth inhibition (%)	Av. colony diameter (mm)	Growth inhibition (%)
Woodfordia fruticosa	75.75	14.64	66.75	25.00	62.00	30.14
Boswellia serrata	69.25	21.97	58.75	33.98	50.75	42.81
Ocimum americanum	73.00	17.74	68.25	23.31	64.25	27.60
Gnidia glauca	78.25	11.83	72.00	19.10	68.25	23.09
Mundulea sericea	82.75	6.76	73.00	17.97	69.75	21.40
Control	88.75	-	89.00	-	88.75	-
S. Em.	0.54		0.62		0.49	
CD at 5%	1.63		1.86		1.47	
CV	1.41.		1.76		1.48	

by adding 50 ml sterilized distilled water. These phyto-extracts were filtered through double-layered muslin cloth in 150 ml conical flasks and plugged with non-absorbent cotton. These filtered extracts were autoclaved at 1.2 kg cm^{-2} pressure for 20 minutes. Autoclaved extract was individually added into previously sterilized PDA @ 5 percent (*i.e.* 1 ml extract +19 ml PDA), 10 percent (*i.e.* 2 ml extract +18 ml PDA) and 15 percent (*i.e.* 3 ml extract +17 ml PDA) and mixed thoroughly at the time of pouring in the previously sterlized petriplates. Petriplates were then inoculated aseptically, after solidification, by placing 5 mm disc at the center, cut aseptically with cork borer from 10 days old culture of test pathogen *i.e. Alternaria alternata,* separately. Four repetitions of each treatment for the test pathogen were maintained. The plates without phyto-extracts served as conrol. Petriplates were then incubated at room temperature[6]. Observations on colony diameter were recorded and statistically analyzed and percent growth inhibition was also worked out by using the following formula suggested by Vincent[7].

$$\text{Per cent growth inhibition} = \frac{C-T}{C} \times 100$$

Where, C= Growth of pathogen in control after incubation, T=Growth of pathogen in treatment after incubation

Results and Discussion

The extracts of *Boswellia serrata, Gnidia glauca, Mundulea sericea, Ocimum americanum* and *Woodfordia fruticosa* with three concentrations viz., 5, 10 and 15 percent were evaluated *in vitro* by poisoned food technique for their efficacy against *Alternaria alternata.* The results, presented in Table 1 and depicted in Fig. 1, indicated that the different plant extracts have varied efficacy against *A. alternata* at all the three concentrations tried.

The extracts of *B. serrata* leaves were inhibitory to the mycelial growth of *A. alternata,* even at 5 percent concentration tried, as compared to the control. It proved significantly superior in checking the fungal growth 21.97, 33.98 and 42.81% over the rest plant extracts at 5, 10 and 15 percent concentrations, respectively.

Extract of *O. americanum* (Rantulsi) leaves was second best at 5 percent concentration in cheking the fungal growth (17.74%). Next best in order of merit after *Boswellia* at 10 percent and 15 percent, was extract of *W. fruticosa* (25% and 30.14%). Extract of *G. glauca* and *M. sericea* exhibited slight inhibitory effect at all the three concentrations tried with respect to the other extracts.

Results indicated that the extracts of *B. serrata* leaves gave maximum inhibition followed by extract of *W. fruticosa* and *O. sanctum,* while extract of *G. glauca* and *M. sericeae* were ineffective at 5 and 10 percent concentrations.

Thus, the present study helps to avoid the hazardous effects of synthetic fungicides.

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