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In-vitro evaluation of some plant extracts for antimicrobial activity

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Aqueous extracts of *Tephrosia condida* (Roxb.) DC and *Boehmeria nivea* Gand. prepared from detached fresh leaves was evaluated for antimicrobial properties against five fungal pathogens viz. *Phytophthora parasitica, Fusarium udum, Macrophomina phaseolina, Acremonium kiliense* and *Alternaria brassicicola* and two bacterial species *Bacillus subtilis* and *Bacillus cereus.* High degree of inhibition of the fungal pathogen *Acremonium kiliense* as well as the bacterial species of *Bacillus cereus* and *B. subtilis* was observed with extract of *T. candida.* The extract could well inhibit the formation of sclerotia of *Macrophomina phaseolina* but not the fungal growth of *Phytophthora parasitica.* The extract, sterilized either through bacteriological G-5 filter of steam sterilized, was most effective against *Fusarium udum.* Extract of *Boehmeria nivea* inhibited growth of *Acremonium kiliense* and both the bacterial species but was not effective against *P. parasitica, F. udum and M. phaseolina.* the extracts also inhibited germination of fungal spores *in-vitro.* Extract of *T. candida* was thermostable and showed promising antimicrobial activity against all the test organisms except *P. parasitica.*

Key words : *Tephrosia candida, Boehmeria nivea,* plant extract, biological control

INTRODUCTION

Chemical therapy is a common practice for controlling plant disease. Continuous uses of synthetic pesticide to control various diseases of crop plants pose problem of pesticide resistance to pathogen and destruction of beneficial microbes. Growing concern about harmful effect of chemical pesticides on environment and human health and with the advent of progress in biotechnology, search for safer, ecofriendly biological control measures is more important in the niche of biodiversity. With the objective of finding alternatives of synthetic chemical fungicides, attempts have been made to control different microbial organisms with plant extracts by different workers (Rao and Thirumalachar, 1960 ; Dorozhkin *et al.*, 1973). Plant extracts from families Compositae, Cruciferae, Labiatae, Ranunculaceae and Solanaceae were found more effective against different fungal pathogens. In the present studies plant extracts of *Tephrosia candida* (Roxb.) DC and *Boehmeria nivea* Gand. have been tested against fungal pathogesns viz. *Phytophthora parasitica* Dast., *Fusarium udum* Butler, *Macrophomina phaseolina* (Tassi) Goid., *Acremonium kiliense* Gratz., *Alternaria brassicicola* (Schw) Wiltshire causing diseases in various crop plants. Two bacterial species *Bacillus subtilis* Cohn emend. Prazmowski and *Bacillus cereus* Frankland and Frankland were also included in the screening test.

MATERIALS AND METHODS

Disease free fresh leaves and young shoot tips of *Tephrasia candida* (Bilakhani) family Leguminosae and *Boehmeria nivea* (Ramie) family Urticaceae was collected from the C. R. I. J. A. F farm at Barrackpore, W. B. Aqueous extracts of the plants were prepared by macerating leaves and yound shoot tips in different ratios in gm/ml of 100, 50 and 25% conc. pH of the filtered aqueous extracts was recorded. The extract of *T. candida* was sterilized either through bacteriological sintered glass grade-5 filter, or steam sterilized in autoclave. Solvent extracts of *T. candida* and *B. nivea* was prepared by chloroform. For evaluation of inhibitory antimicrobial properties of the plant extracts against

the fungal pathogens and bacteria, paper disc diffusion assay technique (Gnanamanickam and Smith, 1980) was followed. Potato Dextrose Agar plates were inoculated with the test organisms and 10 mm dia paper discs, cut from Whatman no. 4 filter paper, sterilized and soaked aseptically in plant extracts, were placed in the centre of petri plates and incubated at 27°C temperature. Paper discs soaked in sterile distilled water served as control. After incubation for a desired time of 3-5 days the petri plates were surveyed for development of inhibition zone around the extract soaked paper discs. Triplicates were maintained for replication in each treatment.

Spore suspension of *Alternaria brassicicola* and sclerotial suspension of *Macrophomina phaseolina* was prepared mixed with plant extract adjusted to contain 2.5×10^5 conidia and sclerotia respectively per ml of 100%, and 1.25×10^5 per ml of 50 and 25% conc. of plant extract. Conidial suspension of *Fusarium udum* was prepared at 5×10^5 per ml of 100% and 2.5×10^5 per ml of 50 and 25% plant extracts. 0.1 ml each of the suspension mixture was placed on glass slides in four replications and incubated in moist chamber at 27°C temperature for 24 hours. Germination of spore and development of germ tube were studied under microscope.

RESULTS

Disc diffusion assay

The result of paper disc diffusion assay envisaged that, aqueious extract of *Tephrosia candida* at 100% and 50% concentrations was most effective and high degree of inhibition of the fungal pathogen *Acremonium kiliense* as well as the bacterial species of *Bacillus cereus* and *Bacillus subtilis* was obserfed. The extract could well inhibit the reproductive unit sclerotia of *Macrophomina phaseolina* but not the fungal growth of *Phytophthora parasitica* appreciably. Extract of *T. candida,* sterilized both through bacteriological G-5 filter and steam sterilized in autoclave at 100% and 50% concentrations was most effective against growth of *Fusarium udum* Extract of *Boehmeria nivea* at same (100% and 50%) concentrations inhibited the fungal growth of *Acremomium kiliense* and both the bacterial species forming a noticeable inhibition zone on the culture plates. The extract however was not effective against the fungal pathogens *Macrophomina phaseolina, Fusarium udum* and *Phytophthora parasitica* (Tables 1 and 2).

Table 1 : Bioassay of antimicrobial activity of *Tephrosia candida* extract by disc diffusion method

Plant Extract	Nature of Extract	Conc. (%)	Pathogen	Diameter of Paper disc (mm)	Diameter of Inhibition zone (mm)
Tephrosia candida Leaf and Young shoot tip	Aqueous Extract	100	*Acremonium kiliense*	10	25.50
			Macrophomina phaseolina		10.00
			Fusarium udum		12.70
			Phytophthora parasitica		0
			Bacillus cereus		13.33
			Bacillus subtilis		12.00
		50	*Acremonium kiliense*	10	17.86
			Macrophomina phaseolina		4.50
			Fusarium udum		5.80
			Phytophthora parasitica		0
			Bacillus cereus		1.00
			Bacillus subtilis		5.40
	Mixture Solvent Extract Ethanol + Benzene	1:20	*Acremonium kiliense*	10	3.10
			Macrophomina phaseolina		1.50
			Fusarium udum		1.00
			Phytophthora parasitica		0
			Bacillus cereus		0.10
			Bacillus subtilis		0.10
	Petroleum Ether Extract	1:20	*Acremonium kiliense*	10	0
			Macrophomina phaseolina		0
			Fusarium udum		0
			Phytophthora parasitica		0
			Bacillus cereus		0
			Bacillus subtilis		0
Control (D.W.)	—		*Acremonium kiliense*	10	0
			Macrophomina phaseolina		0
			Fusarium udum		0
			Phytophthora parasitica		0
			Bacillus cereus		0
			Bacillus subtilis		0

Spore germination

Germination of *Alternaria brassicicola* spores was highly inhibited up to 80.64% with 100% aqueous extract of *Tephrosia candida* while *Boehmeria nivea* extract was less effective inhibiting spore germination up to 49.08%. *At* 100% conc. it showed inhibition of germ tube growth of *A. brassicicola* up to 88.50% which was at per with that of *T. candida* (85.88%). Steam sterilized extract of *T. candida* at 100, 50 and 35% conc. showed high degree of inhibition in spore germination of *Fusarium udum* up to

79.95, 63.93 and 58.29% respectively. Bacteriological G-5 filter sterilized extract inhibited the spore germination by 85.92, 49.31 and 40.68% respectively. Both the extract inhibited germ tube growth up to 96.90%. 100, 50 and 25% steam sterilized *T. candida* extract inhibited the germination of sclerotia in *Macrophomina phaseolina* up to 86.20, 13.17, and 18.83% respectively. Steam sterilized extract of *T. candida* at 100% conc. inhibited germ tube growth appreciably up to 71.52% as compared to control (Tables 3, 4 and 5 and Fig. 1-3).

Table 2 : Bioassy of antimicrobial activity of *Boehmeria nivea* extract by disc diffusion method

Plant Extract	Nature of Extract	Conc. (%)	Pathogen	Diameter of Paper disc (mm)	Diameter of Inhibition zone (mm)
Boehmeria nivea Leaf and Young shoot tip	Aqueous Extract	100	*Acremonium kiliense*	10	8.75
			Macrophomina phaseolina		0
			Fusarium udum		0
			Phytophthora parasitica		0
			Bacillus cereus		9.33
			Bacillus subtilis		9.37
	Aqueous Extract	50	*Acremonium kiliense*	10	6.0
			Macrophomina phaseolina		0
			Fusarium udum		0
			Phytophthora parasitica		0
			Bacillus cereus		1.90
			Bacillus subtilis		2.01
	Chloroform 1:20		*Acremonium kiliense*	10	1.8
			Macrophomina phaseolina		0
			Fusarium udum		0
			Phytophthora parasitica		0
			Bacillus cereus		0.9
			Bacillus subtilis		1.1
Control (D.W.)	—		*Acremonium kiliense*	10	0
			Macrophomina phaseolina		0
			Fusarium udum		0
			Phytophthora parasitica		0
			Bacillus cereus		0
			Bacillus subtilis		0

Table 3 : Effect of extract of *Tephrosia candida* plant at different concentration on spore germination and germ tube development of *Fusarium udum*

Treatment	Conc. (%)	Spore germination (%)	Sporulation Inhibition (%)	Germ tube length (u)	Germ tube Inhibition (%)
Steam sterilized	100	19.23	79.95	3.83	96.05
,,	50	34.60	63.93	39.58	68.05
,,	25	40.00	58.29	106.37	14.15
G-5 filter steri-	100	13.51	85.92	3.84	96.90
,, lised	50	48.62	49.31	114.24	17.79
..	25	56.90	40.68	106.69	13.89
Control (D.W.)	0	95.92	0	123.90	0

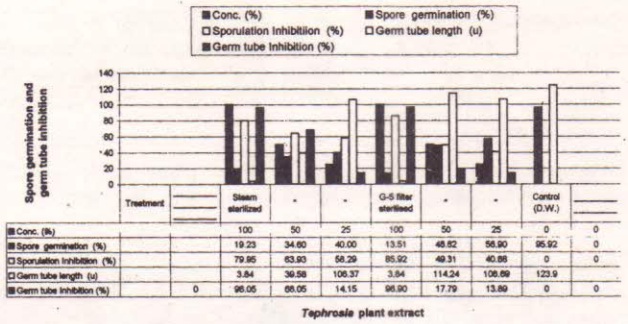


Fig. 1 : Effect of *Tephrosia candida* extract on sporulation and spore germination of *Fusarium udum*

Table 4 : Effect of extract of *Tephrosia candida* plant at different concentration on sclerotia germination and germ tube development of *Macrophomina phaseolina*

Treatment	Conc. (%)	Germination of sclerotia (%)	Germination Inhibition (%)	Germ tube length (u)	Germ tube Inhibition (%)
Steam sterilized	100	10.00	86.20	50.00	71.52
,,	50	62.71	13.17	151.28	13.72
,,	25	58.62	18.83	108.28	38.32
	100	40.00	44.61	149.60	14.80
,,	50	67.56	6.45	159.05	9.40
..	25	71.05	1.62	205.63	0
Control (D.W.)	0	72.22	0	175.56	0

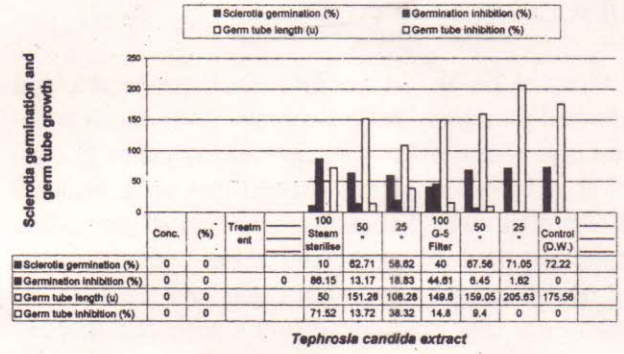
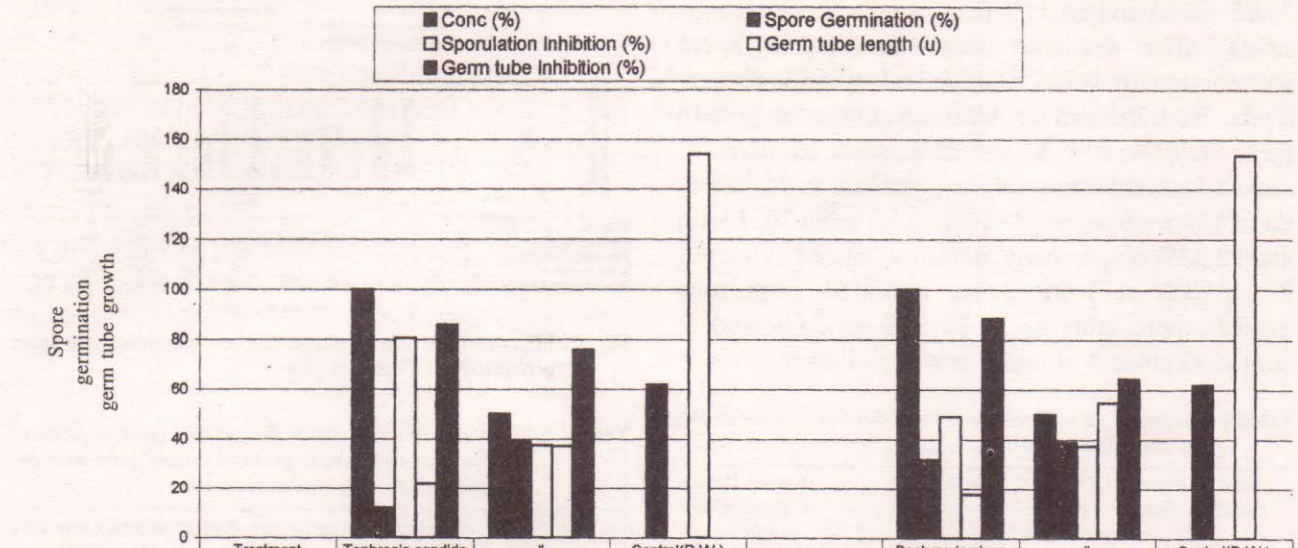


Fig. 2 : Effect of *Tephrosia candida* extract on germination of scleriotia and germ tube growth of *Macrophomina phaseolina*

Table 5 : Effect different plant extracts on spore germination and germ tube length of *Alternaria brassicicola*

Treatment	Conc. (%)	Spore germination (%)	Sporulation Inhibition (%)	Germ tube length (u)	Germ tube Inhibition (%)
Tephrosia candida	100	12.00	80.64	21.77	85.88
,,	50	38.72	37.55	37.13	75.92
Control (D.W.)	0	62.00	0	154.21	0
Boehmeria nivea	100	31.57	49.08	17.66	88.50
..	50	38.88	37.29	54.72	64.38
Control (D.W.)	0	62.00	0	153.60	



Treatment	Tephrosia candida	"	Control(D.W.)		Boehmeria nivea	"	Control(D.W.)
Conc (%)	0	100	50	0		100	50	0
Spore Germination (%)		12.00	38.72	62.00		31.57	38.88	62.00
Sporulation Inhibition (%)	0	80.64	37.55	0		49.08	37.29	0
Germ tube length (u)		21.77	37.13	154.21		17.66	54.72	153.60
Germ tube Inhibition (%)	0	85.88	75.92	0		88.50	64.38	

Plant extract

Fig. 3 : Effect of extract on spore germination and germ tube growth of *Alternaria brassicicola*

DISCUSSION

Extract of *Tephrosia candida* was thermostable and showed promising antimicrobial activity against the test organizms. pH of the aqueous extract of *T. candida* at 100% conc. was 8.0 and that of *B. nivea* at 100% conc. was 5.0. Solvent extract of the plants could not produce appreciable result with clear zone of inhibition against the test organisms whereas the inhibition zone was conspicuous with aqueous extracts. Perhaps the specific chemical constituents responsible for such inhibition could not be dissolved in the particular solvents. Further investigation on the isolation and characterization of active principles of the extract of the *T. candida* are in progress.

Biocides of plant origin alone and in combination with fungal and bacterial biocontrol agents (BCA) have promising prospects in controlling plant pathogens are in evidence (Gupta *et al.*, 2002 ; Jisha *et al.*, 2002). Screening and selection of the beneficial agents for tolerance and compatibility with effective plant extract is therefore important, that can be used as eco-friendly biopesticide to replace the synthetic chemicals. Leaves and stems of *Tephrosia candida* has been reported to contain Amorphone (6-hydroxy 6a, 12a-dehydro rotenone) which induce insecticidal property against larvae of *Spodoptera litura* (Kole *et al.*, 1992). Leaf powder also has a repellent action against banana weevil (*Cosmopolites sordidus*) (Walangululu *et. al.*, 1993). One new rotenoid (12 a-hydroxy-beta-toxicarol) isolated from roots have activity against certain insects (Andrei *et. al.*, 1997). Moreover, *Tephrosia condida* has been reported to have weed controlling capacity (WCC) to about 77% against the Kans grass (*Saccharam spontaneum*) and other weeds (Saha and Sarkar, 1997). *T. candida* is also believed to control nematodes in tea plantation (Sharma, 2002). It is tolerant to a wide range of soil type and temperature, preferring acid soils. It has been suggested that *T. candida* increases N availability and organic carbon in the soil. It has potential for improving the productivity of acid soils under traditional system (Gichuru, 1991). The plant can be used for reclamation of problem soil. It being a legume plant has petential for the management of degraded land. Keeping the land fallow and covered with *T. candida* improved physical and chemical properties of

soil, increasing maize yield significantly perhaps by enhancing nutrient recycling in addition to N input through fixation (Gichuru, 1994). Thus the efficacy of this botanical may provide a base for developing an IPM system.

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