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Evaluation of some Ethanobotanical Plant Extracts for Fungitoxicity against *Myrothecium roridum*

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Ethanolic extracts of twenty-one plant species were tested *in vitro* for their fungitoxic properties against leaf spot causing pathogen of mulberry *Myrothecium roridum* by poisoned food technique. Ethanolic extracts of twenty-plant spp. reduced mycelial growth of *M. roridum* significantly except *E. pulcherrima.* Highest inhibition of *M. roridum* colony growth observed in 10% extracts of *E. citriodora* (49.45%) followed by *D. metel* (39.45%), *Chromolaena odoratum* (25.56%) and *A. sativum* (25.00%). Among the concentration tested, 10% concentration was found significantly higher effective on reducing colony growth followed by 5 and 2.5%. Aqueous extract fresh leaves/ bulb of seven short-listed plant spp. (inhibition > 15% in ethanolic extracts) revealed that *D. metel* inhibited (23.43%) followed by *E. citriodora* (14.66%), *C. odoratum* (13.53%). On dry leaf extracts *D. metel* was found more effective than *E. citriodora.* The results indicated that *D. metel, E. citriodora, C. odoratum* and *A. sativum* having high fungitoxicity against *M. roridum* and ethanolic extract found more effective than aqueous extract.

Key words: Mulberry, plant extracts, Fungitoxicity, *Myrothecium roridum*

Introduction

Myrothecium roridum Tode ex Fr. is one of the major leaf spot causing pathogen of mulberry (*Morus* sp.). The pathogen causes disease on mulberry leaf during monsoon (Maji, 2002, 2003). As a result the mulberry leaf yield and quality drastically reduced. Feeding of diseased leaves affect silkworm larvae and cocoon quality. Chemical control of the disease has been developed. But it has limitation as its involved risk to silkworm due to residual toxicity and toxicity to non-targeted organisms. Besides, indiscriminate use of such chemicals resulted in evolution of resistant strains (Aditya Chowdhuri, 1991). The use of plant extracts and phytoproducts is gaining attention due to their proven nature specificity, biodegradibilty, low toxicity and minimum residual toxicity on the ecosystem (Aditya Chowdhuri, 1991; Pan and Deb. 1997). There are enough evidences from earlier works that several plant species possess antifungal/antibacterial properties (Grainge and Ahmed, 1988; Kurucheve *et al.*, 1997; Pan and Deb 1997; Sunderraj *et al.*, 1996; Raja and Kurucheve, 1998). In the present study, twenty-one ethanobotanical plants were screened for their fungitoxicity against *M. roridum.*

Materials and Methods

Preparation of extracts

Twenty-one locally available plant species presented in Table 1 were selected for the study. Fresh leaves/bulb were collected and washed with tap water and blotted to dry. For preparation of ethanolic extract 100 gm leaves/ bulbs were crushed with equal amount (w/v) of 25% ethanol and the resulting paste were kept 24 hrs in amber bottle at room temperature. The extracts were filtered through muslin cloth and centrifuged at 2500 rpm for 10 minutes to clear the supernatant, which form (100%) plant extract. For preparation of aqueous extract 100 g leaves/bulb crushed with equal amount of distilled water.

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Table 1. Plant species used in the study

Scientific name	Vernacular name	Family
Adhatoda zeylanica Medic.	Vasakh	Acanthaceae
Allium sativum L.	Garlic/Rasun	Amaryllidaceae
Andrographis paniculata Nees.	Kalmegh	Acanthaceae
Azadirachta indica A.Juss.	Neem	Meliaceae
Cassia tora L.	Chakunda	Caesalpiniaceae
Cassia sophera L.	Kalkasunde	Caesalpiniaceae
Caesalpinia pulcherrima Sw.	Gol mohar	Caesalpiniaceae
Catharanthus roseus (L.) Don.	Nayantara	Apocynaceae
Calotropis gigentia (L.) R.Br.	Akanda	Ascelepiadaceae
Chromolaena odoratum (L.) King & Robinson.	--	Compositae
Clerodendrom viscosum Vent.	Ghetu	Verbenace
Datura metel L.	Dhatura	Solanace
Eucalyptus citriodora L	Eucalyptus	Myrataceae
Euphorbia pulcherrima R. Grah.	Poinsettia	Euphorbiaceae
Holarrhena antidysenterica (L)Wall	Kurchi	Apocynaceae
Mirabilis jalapa L	Sandhyamalati	Nyctaginaceae
Moringa oleifera Lamk.	Sajina	Moringaceae
Ocimum sanctum L	Tulsi	Labiatae
Polyalthia longifolia (Sonn) Thw. Enum	Debdaru	Annonaceae
Tagetes patula L.	Marigold	Compositae
Thevetia peruviana (Pers) Schum .	Kalkephul	Apocynaceae

Dry leaf extracts were prepared by drying 100 g of fresh leaves at 80°C temperature and powdered in grinder. The resultant powder soaked in 100 ml hot water (80°C) for 10 min. and filtered through muslin cloth and centrifuged at 2500 rpm for 10 min. This form standard plant extracts 100%.

Bioassay of plant extracts against *M. roridum*

The fungitoxicity of leaf extracts was determined following poison food technique. Potato Dextrose Agar (PDA) medium was amended with 5% solvent extracts and PDA with out extract served as control. The sterilised medium was poured in to 90 mm sterilised Petri plate at 15 ml/ plate. Three replications were maintained for each treatment. Agar block (6 mm diameter) containing 7 days old mycelia of *M. roridum* placed centrally on PDA in Petri plate and incubated at 28°C for 10 days in incubator. Colony diameter of the test fungi was measured 10 days after incubation. Percentage of mycelial growth inhibition on different treatment was calculated by using Shahi (1999) formula.

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

Where, I = Percent growth inhibition, T = Colony diameter on treated and C = Colony diameter in control.

Data analysis

The data were subjected to analysis of variance (ANOVA) to determine significant differences among the various treatments using F- ratio test. To compare the treatment means, critical differences were calculated by Fishers least significant differences test at $\alpha = 5\%$.

Results

Ethanolic extracts

Ethanolic extracts twenty plant reduced mycelial growth significantly except *E. pulcherrima* (Table 2). Highest inhibition of colony growth was recorded in 10% *E. citriodora* (49.45%), followed by *D. metel* (39.45%), *C. odoratum* (25.56%), *A. sativum* (25%), *C. sophera* (19.45 %), *C. viscosum* (16.66%) and *C. tora* (16.11%). Five plant species viz., *C. gigentia, M. jalapa, O. sativum, T. patula* and *T. peruviana* inhibited colony growth (15%), which was statistically at par. Other plant species were less effective on inhibition of colony growth. Among the test concentrations, 10% concentration was significantly higher effective than 5% and 2.5%.

Aqueous extracts

Seven plants short listed plant extracts (on the basis of inhibition i.e. >15% at 10% ethanolic extracts) were tested at 2.5, 5 and 10% concentration. Among the seven extracts tested, six plants extract *D. metel, E. citriodora, A. sativum, C. odoratum C. tora,* and *C. viscosum* significantly reduced colony growth *M. roridum* (Table 3).

Table 2. Effect of ethnolic extracts of different plant species on radial growth of *M. roridum*

Plant species	Colony diameter (mm)						
	10 days after inoculation				Inhibition %		
	2.5%	5%	10%	Mean	2.5%	5%	10%
Adhatoda zeylanica	59.66	59.33	54.66	57.89	0.56	1.11	8.86
Allium sativum	53.00	45.33	45.00	47.77	11.66	24.45	25.00
Andrographis paniculata	60.00	59.66	57.00	58.88	0.00	0.56	5.00
Azadirachta indica.	59.66	59.66	57.33	58.88	0.56	0.56	4.45
Cassia tora	54.66	49.33	50.33	51.44	8.88	17.78	16.11
Cassia sophera	58.00	54.66	48.33	53.66	3.33	8.90	19.45
Caesalpinia pulcherrima	55.66	55.33	56.00	55.33	8.90	7.78	6.66
Catharanthus roseus	55.00	55.00	55.00	55.00	8.33	8.39	8.33
Calotropis gigentia	56.00	54.66	51.00	53.88	6.66	8.88	15.00
Clerodendrom viscosum.	55.00	50.00	50.00	51.66	8.33	16.66	16.66
Chromolaena odoratum	55.00	48.00	44.66	47.55	16.16	20.00	25.56
Datura metel	44.33	39.33	36.33	40.00	26.12	34.45	39.45
Eucalyptus citriodora	56.00	51.00	30.33	45.77	6.66	15.00	49.45
Euphorbia pulcherrima	59.66	59.33	60.00	59.66	0.56	1.11	0.00
Holarrhena antidysenterica	55.66	54.33	54.33	54.88	7.23	8.90	9.45
Mirabilis jalapa	54.33	54.00	51.00	53.11	9.45	10.00	15.00
Moringa oleifera	59.00	54.33	54.33	55.88	1.69	9.45	9.45
Ocimum sanctum	54.00	55.33	51.33	53.55	10.00	7.78	14.45
Polyalthia longifolia	55.33	54.33	54.33	54.66	7.78	9.45	9.45
Tagetes patula	60.33	54.66	51.00	55.33	0	8.90	15.00
Thevetia peruviana	52.33	53.33	51.33	52.33	12.78	11.11	14.45
Control	60.00	60.00	60.00	60.00			
Mean	55.76	53.69	51.03				
CD at 5%							
Plant		0.76					
Concentration		0.28					
Plant × Conc.		1.32					

Highest inhibition of colony growth was recoded in *D. metel* (23.43%), followed by *E. citriodora* (14.66%), *A. sativum* (11.04%). Among the test concentrations, 5% and 10% concentration was at par significantly higher effective than 2.5%.

Dry leaf Powder extract

Dry leaf powder extracts of six plant extracts tested, among them five plant extracts viz., *E. citriodora, D. metel, C. tora, C. odoratum* and C. *viscosum* significantly reduced *M. roridum* colony growth (Table 4). Highest inhibition of colony growth was recoded in 10% concentration *D. metel* (25.43%), followed by *E. citriodora* (10.29%), *C. viscosum* (8.57%). Among the test concentrations, 2.5% and 10% concentration was at par and 5% was significantly higher than 10% concentration.

Discussion

The pesticidal properties of many plants have been known for long time. During recent years plant products and their derivatives are under intensive investigation in search of eco-friendly management of plant disease. The inhibitory effect of plant extracts might be attributed to the presence of antifungal properties in them. Several workers established fungitoxicity of higher plant extracts of *A. vasica, A. sativum, A. indica, C. gigantia, D. metel, Moringa oleifera, O. sanctum, P. longifolia, C. roseus, C. pulcherrima, E. citriodora* and *T. peruviana* (Achimu and Scholesser, 1992; Grainge and Ahmed, 1988; Kurucheve, *et al.*, 1997; Kurucheve and Padmavathi, 1998; Mishra and Tiwari, 1992; Sarvamangala *et al.*, 1993; Sundarraj *et al.*, 1996; Narain and Sathpathi, 1978). Several workers reported that antifungal properties of bulb of *A. sativum* both *in*

Table 3. Effect of short listed fresh aqueous plant extract radial growth of *M. roridum*

Plant species	Colony diameter (mm) 10 days after inoculation			Mean
	2.5%	5%	10%	
Allium sativum	55.00 (5.71)	52.33 (10.29)	48.33 (17.14)	51.89 (11.04)
Cassia tora	56.00 (4.00)	46.67 (20.00)	59.67 (0.00)	54.11 (7.27)
Cassia sophera	58.00 (0.57)	58.67 (0.00)	61.00 (0.00)	59.22 (0.00)
Chromoleana odorata	52.67 (9.71)	51.67 (11.43)	47.00 (19.43)	50.44 (13.53)
Clerodendrum viscosum.	55.33 (5.14)	56.00 (4.00)	56.33 (3.43)	55.89 (4.18)
Datura fastuosa	44.67 (23.43)	44.67 (23.43)	44.67 (23.43)	44.67 (23.43)
Eucalyptus citriodora	53.00 (9.14)	50.33 (13.17)	46.00 (21.14)	49.78 (14.66)
Control	58.33	58.33	58.33	58.33
Mean	54.13	52.33	52.67	
CD at 5%				
Plant		0.91		
Concentration		0.56		
Plant × Concentration		1.58		

Figures with in parenthesis is percentage of inhibition of colony growth

Table 4. Effect of short listed dry leaf extract on radial growth of *M. roridum*

Plant species	Colony diameter (mm) 10 days after inoculation			Mean
	2.5%	5%	10%	
Cassia tora	59.66 (1.96)	65.33 (0.00)	60.00 (0.00)	61.66 (0.00)
Cassia sophera	59.00 (3.28)	57.00 (6.55)	58.00 (4.92)	58.00 (4.91)
Chromoleana odorata	59.33 (2.79)	59.33 (2.77)	56.67 (8.19)	58.44 (4.19)
Clerodendrum viscosum.	55.33 (9.29)	56.66 (7.11)	55.33 (9.29)	55.77 (8.57)
Datura metel	46.66 (23.51)	46.00 (24.59)	43.33 (28.97)	45.33 (25.69)
Eucalyptus citriodora	54.33 (10.93)	55.00 (9.83)	55.00 (9.83)	54.77 (10.29)
Control	61.00	61.00	61.00	61.00
Mean	56.47	57.19	55.62	
CD at 5%				
Plant		1.46		
Concentration		0.96		
Plant × Concentration		2.54		

Figures with in parenthesis is percentage of inhibition of colony growth

vivo and *in vitro* (Adetumbi and Lau, 1983; Agarwal, 1978; Ammer *et al.*, 1980, Appleton and Tansey, 1975; Arya *et al.*, 1995, Bisht and Khulber, 1995, More and Atkins, 1977, Srinivas *et al.*, 1997; Tansey and Appleton, 1985). The antimicrobial properties of *A. sativum* ascribed mainly due to allicin (Cao *et al.*, 2001). Fresh aqueous and ethanolic as well as dry extracts of *D. metel* inhibited mycelial growth of *M. roridum*. Fungitoxic principal of *D. metel* may be due to presence of poisons alkaloids *viz.* hyosciamine, atropine and scopolamine in the leaves. Several workers reported that fungitoxic property of Eucalyptus leaves remains in the oil part (Chanegriha, *et al.*, 1998; Dellacassa, *et al.*, 1989; Gundidza, *et al.*, 1993; Hajji, *et al.*, 1993; Hmamouchi, *et al.*, 1990; Shahi, *et al.*, 1999 Yadav and Dubey, 1994; Pattnaik *et al.*, 1996). Comparative less efficacies of dry *E. citriodora* leaf extracts was may be due to volatile nature of Eucalyptus oil. Ethanolic extracts of *C. tora* and *C. sophera* found comparatively higher fungitoxicity than aqueous extract which indicates that the fungitoxic principle is more soluble in solvent. Extracts of *Cassia tora* leaf and seed is used for treatment of fungal borne skin disease like ringworm and psorosis (Bhattacharya, 1980; Malhotra *et al.*, 2003; Oudhia, 2002). Acharya and Chatterjee (1975) reported that the chrysphonic acid-9-anthrone is the antifungal principle of *C. tora*. Garg and Siddique (1992) reported that *Ocimum* sp. has strong inhibitory effect on growth of many fungi and the antifungal property is due to presences of eugenol. The leaves contain an active principle vasicine (Agarwal and Ghosh, 1985; Bakhru,2001). The present study clearly indicated that plant extracts of *D. metel, E. citriodora, A. sativum* were found highly fungitoxic against *M. roridum*. Extracts of these plants can be exploited as safer botanical pesticides for managing *Myrothecium* leaf spot disease of mulberry.

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