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In vitro studies of some plant extracts against *Rhizoctonia solani* Kuhn infecting FCV tobacco in Karnataka Light Soil, Karnataka, India.

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The antifungal effect of 10 plant extracts viz., *Thevetia peruviana*, *Ocimum basilicum, Piper betel*, *Murraya koenigii*, *Chrysanthemum coronarium*, *Polyalthia longifolia*, *Catharanthus roseus, Pelargonium graveolens, Moringa officinalis and Lawsonia inermis* were evaluated by poisoned food technique against *Rhizoctonia solani* Kuhn, the causal organism of sore shin disease of tobacco. Among all the plants screened only four plants namely, *Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens* have recorded significant antifungal activity against *Rhizoctonia solani*. The effective concentration inhibiting the growth of mycelium and sclerotia formation of the pathogen by the plant extract of *Piper betel* was at 50% concentration, *Lawsonia inermis* inhibited the growth at 75%, and *Polyalthia longifolia* and *Pelargonium graveolens* suppressed the growth at 100% concentration. Organic solvents *viz.,* n-hexane, ethyl acetate and methanolic extracts of four plants *viz*., *Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens* were investigated for their antifungal activity against this phytopathogenic fungus. Ethyl acetate extracts showed 100% inhibition of mycelial growth and sclerotia formation of *Rhizoctonia solani* at 1000 ppm. Methanol extracts moderately inhibited the growth at 1000 ppm and n-hexane extracts were not effective against the test organism. Aqueous extract of *Piper betel* can be recommended to the farmers for the control of *Rhizoctonia solani.*

Key words: *Rhizoctonia solani, Piper betel, Lawsonia inermis* and antifungal activity.

Introduction

Tobacco is an important commercial crop affected by various fungal, bacterial and viral diseases. Sore shin caused by *Rhizoctonia solani* is prevalent in almost all tobacco growing areas of the world (Lucas, 1975; Gopalachari,

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1984). It was recorded for the first time in Karnataka Light Soil(KLS) nurseries during the nursery survey conducted in 2005 (Anonymous, 2006).The pathogen is gaining importance with severe damage to seedlings in isolated pockets as well as sporadically in tray nurseries. Management of this disease is difficult due to viability of sclerotia in the soil for several years. Different methods have been used to control *R. solani* such as cultural practices, solarization and chemical control (Baker and Cook, 1979; Dubey, 2001). The conventional synthetic chemicals have raised many problems like pollution, development of resistant strains of the pathogens, slow biodegradation and some of them are even carcinogenic (Brent and Hollomon, 1998; Barnard *et al*.,1997). Botanicals are now emerging as safer and more compatible approach to control phytopathogens (Kumbhar *et al*.,2000). Higher plants are known to posses fungitoxicity against spore germination and mycelial growth of phytopathogenic fungi (Varma and Dubey, 1999) . The plant world is a rich store house of natural chemicals that could be exploited for use as pesticides (Satish *et al.,* 2008). The total number of plant chemicals may exceed 400,000 and of these 10000 are secondary metabolites whose major role in the plant is reportedly defensive (Grayer and Harborne, 1994). *In vitro* studies were carried out to explore the fungicidal efficacy of some plant leaf extracts and solvent extracts against *R. solani,* the causal organism of sore shin of tobacco. Numerous studies conducted revealed antifungal activity of many plant extracts against *R. solani* (Castellanos *et al*.,2000; Lean *et al*.,1999; Srivastava and Singh, 2001).

Hence, in the present study 10 plant extracts and 4 solvent extracts were tested *in vitro* against *Rhizoctonia solani,* which can be exploited in the plant disease management.

Materials and methods

Test fungi

Infected seedlings were collected during the disease survey in 2006-2008.The infected root bits of 1-2 cm were surface sterilized using 70% ethyl alcohol. These surface sterilized roots were plated on Czapek-Dox-Agar (CDA) and incubated at 25±2 °C for seven days.

Plant materials

Fresh disease free leaves of 10 plant species were collected from Mysore, Karnataka, India. (Table 1).

Table 1. List of plant species tested for antifungal activity.

Name of the plant	Family
Catharanthus roseus L	Apocyanaceae
Chrysanthemum coronarium L.	Asteraceae
Lawsonia inermis L.	Lythraceae
Morinda officinalis L.	Moringaceae
Murraya koenigii L.	Rutaceae
Ocimum basilicum L.	Lamiaceae
Piper betel L.	Piperaceae
Pelargonium graveolens L.	Geraniaceae
Polyalthia longifolia Thw.	Anonaceae
Thevetia peruviana Pers.	Apocyanaceae

Preparation of extracts

Aqueous extract

The selected leaf samples (100 gm) of all plants were thoroughly washed, blot dried and macerated with 100 ml sterile distilled water in a blender (Preethi mixer grinder, India) for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatmann No.1 filter paper and sterilized at 121°C for 20 min, which served as the mother extract.

Solvent extract

Thoroughly washed mature leaves of all the test plants were shade dried and then powdered with the help of a blender. Thirty grams of the powder was filled in the thimble and extracted successively with n-hexane, ethyl acetate and methanol using a soxhlet extractor for 48 h. All extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight brown bottle until further use. All extracts were subjected to antifungal activity against the test fungi.

Antifungal activity assay

Aqueous extract

Antifungal activity of the plant extract was carried out by poison food technique (Nene and Thaplyal, 1979). Czapek Dox Agar medium (CDA) with 10, 25, 50, 75, 100% concentration of aqueous extracts of test plants namely, *Thevetia peruviana*, *Ocimum basilicum, Piper betel*, *Murraya koenigii*,

Chrysanthemum coronarium, *Polyalthia longifolia*, *Catharanthus roseus, Pelargonium graveolens , Moringa officinalis and Lawsonia inermis* were prepared. About 15 ml of the medium was poured into each petriplate and allowed to solidify. 5 mm disc of seven-day-old culture of *R. solani* were placed at the center of the petriplate and incubated at 25±2°C for seven days. After incubation period, radial colony growth (mm) were measured and recorded in each treatment. For each treatment three replicates were maintained. CDA medium without the aqueous extract served as control. The fungal toxicity of the extracts in terms of % inhibition of mycelial growth was calculated using the following formula:

% inhibition = dc – dt /dc x 100, where dc = average increase in mycelial growth in control, dt = average increase in mycelial growth in treatment.

Solvent extract

One gram of each of the dried evaporated solvent extract of *Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens* was separately dissolved in 10 ml of respective solvents *i*.e., n-hexane, ethyl acetate and methanol. Antifungal activity of the solvent extract was carried out by poisoned food technique (Nene and Thaplyal, 1987). The Czapek Dox Agar (CDA) containing 100,200,300,400,500 and 1000 ppm concentration of each solvent extract was prepared. The CDA medium amended with 100 µl of the solvent without any extract served as control. The solvent extract amended medium was poured into sterile 90 mm diameter petriplates (15 ml per plate).The mycelia disc (5 mm) obtained from the margin of seven-day-old culture was inoculated at the centre of the petriplate to both control and solvent extract amended CDA medium. The petriplates were incubated at 25 ± 2°C for seven days. The experiment was replicated three times. The diameter of the fungal colonies and growth characteristics in each petridish were recorded. The antifungal activity was expressed as percentage of mycelial growth inhibition with respect to control was computed using Srivatsava and Singh (2001) method.

Results

Antifungal activity assay

Aqueous extract

Among the 10 plants screened, aqueous extracts of *Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens* have recorded varied percentage of inhibition against *R. solani* at 100% concentration. *Piper betel* recorded significant antifungal activity at 50% concentration (Fig.1). *Lawsonia inermis* showed 100% inhibition at 100% concentration (Fig. 2). *Polyalthia longifolia* and *Pelargonium graveolens* showed moderate growth at 100% concentration , But did not completely inhibit the growth even at 100% concentration (Table.2).

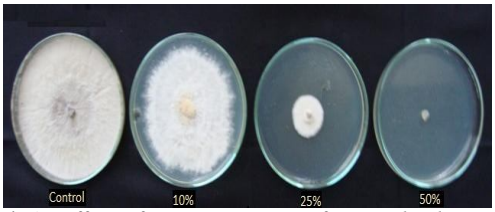

Fig.1: Effect of aqueous extract of *Piper betel* on *Rhizoctonia solani*

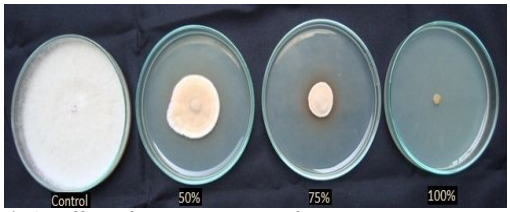

Fig.2: Effect of aqueous extract of *Lawsonia inermis* on *Rhizoctonia solani*

The aqueous extracts of *Piper betel* and *Polyalthia longifolia* completely suppressed the formation of sclerotia at 10% concentration. However, *Lawsonia inermis* suppressed the sclerotia formation at 25% . *Pelargonium graveolens* did not inhibit the sclerotia formation even at 100% concentration.

Table 2. Percentage inhibition (PI) of growth of *Rhizoctonia solani* by some aqueous plant extracts.

Plant extracts	Percentage of mycelial inhibition		
	Control	Concentration (%)	
		50%	100%
Lawsonia inermis	00^{a}	32^{b}	100^{c}
Pelargonium graveolens	00^{a}	00^{a}	22^{b}
Polyalthia longifolia	00^{a}	00^{a}	26^{b}
Piper betel	00^{a}	100^{c}	100^{c}

Figures having the same letters are not significantly different according to Ducan's multiple range test (P<0.05)

Solvent extract

The concentration of 1000ppm of ethyl acetate extracts of all the four plants was effective in the inhibition of mycelial growth of *R. solani.* Among the different solvent extracts tested against *Rhizoctonia solani,* 1000ppm of ethyl acetate extracts of *Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens* recorded significant antifungal activity (Table.3). Methanol extracts of *Lawsonia inermis* , *Pelargonium graveolens* ,*Polyalthia longifolia* and *Piper betel* showed 44%, 29%, 76% and 56% inhibition respectively. Total absence of inhibitory activities by n-hexane extracts on *R. solani* was observed.

Ethyl acetate extracts of *Polyalthia longifolia, Lawsonia inermis* , *Piper betel* and *Pelargonium graveolens* completely suppressed the formation of sclerotia at 100 ppm, 200 ppm, 300 ppm and 1000 ppm respectively. Methanol extracts of *Piper betel* and *Lawsonia inermis* showed complete inhibition at 500 ppm, *Polyalthia longifolia* at 400 ppm and *Pelargonium graveolens* at 300 ppm. n-hexane extracts of *Polyalthia longifolia* showed sclerotial inhibition at 1000 ppm. The other three plant extracts did not suppress the sclerotia formation even at 1000 ppm.

Table 3. Percentage inhibition (PI) of growth of *Rhizoctonia solani* by some solvent plant extracts.

Plant extracts	Percentage of mycelia inhibition					
	n-hexane		Ethyl acetate		Methanol	
	500ppm	1000ppm	500ppm	1000ppm	500ppm	1000ppm
Lawsonia inermis	00^{a}	00^{a}	100^{e}	100^{e}	22^{b}	44^{c}
Pelargonium graveolens	00^{a}	00^{a}	17^{b}	100^{e}	00^{a}	29^{b}
Polyalthia longifolia	00^{a}	00^{a}	84^{d}	100^{e}	63^{d}	76^{d}
Piper betel	00^{a}	00^{a}	44^{c}	100^{e}	39^{c}	56^{d}

Figures having the same letters are not significantly different according to Ducan's multiple range test ($P<0.05$)

Discussion

Biological control have attained importance in modern agriculture to curb the hazards of intensive use of chemicals for pest and disease control (Baker and Cook, 1979). *In vitro* studies were carried out to explore the fungicidal efficacy of some plant leaf extracts and solvent extracts against *R. solani,* the causal organism of sore shin of tobacco.

Among the 10 aqueous plant extracts studied, 4 plant extracts showed fungitoxic potentiality against *R. solani,* out of which maximum effect was seen in *Piper betel* and *Lawsonia inermis* in suppressing the growth and sclerotia formation. Previous research literature on the antifungal properties of various

plant extracts have shown that they have varying degrees of growth inhibitory effect on *R. solani* due to their different chemical composition. Several workers have identified the chemical compounds of these plants and showed that those fractions are very efficient in suppressing the growth of fungi. The fungicidal property of *Piper betel* might be due to the presence of hydrochavicol in the extracts as reported by Ali *et al.,* (2010) ; Nalina and Rahim (2007) and the presence of Lawsone *i.e*., 2 – hydroxyl – 1, 4 – Naphthaquinone in *Lawsonia inermis* (Ali *et al.,* 1995 ; Themnozhi *et al*., 2009 ; Natarajan and Lalithakumari, 1987). *Polyalthia longifolia* gave moderate effect against the pathogen and its fungitoxic activity may be due to the presence of Clerodanes and Diterpenoids (Faizi *et al.,* 2008). Somani (2009) also reported that *Polyalthia longifolia* extract possess inhibitory effect against *R. solani* causing Black scurf of potato. *Pelargonium graveolens* have also shown moderate fungicidal response against *R. solani*. Citronellal and geraniol present in *Pelargonium graveolens* is known to have antifungal property (Aggarwal, *et. al*., 2000). Previous reports are not available regarding the effect of *Piper betel* against the sore shin pathogen and the present work has revealed the effective concentration required to suppress the growth of *R. solani* causing sore shin disease. Wanchaitanawong *et al*., (2005) has reported that *Piper betel* is very effective against *Aspergillus niger, A. oryzae* and *Penicillium* spp.

Ethyl acetate extract of all the four plants showed potential *in vitro* antifungal effect against the test pathogen, *R. solani*. There are scanty reports on the ethyl acetate extracts of plants against *R. solani*. Methanol extracts inhibited the pathogen moderately. Total absence of inhibitory activities by n-hexane extracts on *R. solani* was observed. This is in accordance with Rhouma *et al.*, (2009) who has reported that the antimicrobial compounds of the Plant extracts are not soluble in non polar solvents like n-hexane.

The present work has revealed the effective concentration required to suppress the sclerotia formation also. This concentration is important because the survival structure in *R. solani* is sclerotia (Gopalachari, 1984; Lucas, 1975; Agrios, 2005).Hence the concentration which suppresses the sclerotia formation should be considered in formulating the effective dosage for the control of *R. solani*. In the present study, aqueous extracts of *Piper betel* and *Lawsonia inermis* and ethyl acetate extracts of all the 4 plants have shown promising results against *R. solani*. The results confirmed that these aqueous plant extracts and solvent extracts have antifungal properties on both mycelial growth and sclerotia formation. Further research work is warranted to identify the bioactive compounds having antifungal activity in these plant extracts. The results also clearly indicate that whenever plants are screened for the antimicrobial activity, testing should be done using both aqueous and solvent extracts. If aqueous

extracts are found effective, it will help in the formulation of ecofriendly control measure, which is cheap and can be recommended to the farmers.

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