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In vitro evaluation of plant extracts and biocontrol agents against *Sclerotium rolfsii* Sacc. Causing wilt of betelvine

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Abstract
Betelvine (*Piper betle* Linn.) is most prone to infestation by several soil borne pathogens, among them wilt disease caused by *Sclerotium rolfsii* Sacc. is one of the most destructive one. The present study was carried out to evaluate aqueous extract of eight different plant species and four antagonistic biocontrol agents under *in vitro* condition for their inhibitory effect on mycelial growth of *S. rolfsii*. Among the plant extracts tested at three different concentrations, 15 per cent concentration of all plant extracts were significantly found superior to 5 and 10 per cent. At 15 per cent concentration of plant extracts, maximum 62.19 per cent inhibition of mycelial growth was recorded in tulsi leaf extract followed by marigold leaf extract (57.11%). Among the biocontrol agents tested *Trichoderma harzianum* I showed maximum antagonistic effect and found to be significantly superior in inhibiting the mycelial growth of *S. rolfsii* (62.64%).

Keywords: Betelvine, biocontrol agents, *in vitro*, mycelia, plant extracts, *Sclerotium rolfsii*

Introduction
Betelvine (*Piper betle* Linn.) is a perennial, shade loving ever green creeper belongs to the family Piperaceae. It is commercially cultivated in many parts of the world especially in the tropical and sub-tropical countries. Every part of the vine has high medicinal value, the presence of phenolic compound hydroxyl-chavicol, with anti-carcinogenic property has also been identified in betel leaves [1].

Betelvine wilt caused by *Sclerotium rolfsii* Sacc. is one of the most destructive disease of betelvine. The vines of all stages are susceptible to the disease. The infection usually starts at the collar region. Whitish cottony mycelium is seen on the stem and roots. The stem portion shows rotting of tissues at the point of attack and the plants show dropping of leaves and withering finally dry up. The extent of losses varies from 5-90 percent [2, 3]. The fungus can overwinter as mycelium in infected tissues or plant debris or as sclerotia near soil surface or buried in soil which serve as a major source of primary infection by germinating in response to alcohols and other volatile compounds released from decomposing plant material [4].

At present the disease is mainly managed by the use of agrochemicals such as fungicides. However, the indiscriminate use of chemicals not only hazardous to living being but also break the natural ecological balance by killing the beneficial and/or antagonist microorganisms. On the other hand, plant extracts, biocontrol agents and soil amended materials *etc.* suppress the pathogen and protect the plants that is environmentally safe, durable and cost effective alternative to chemicals and avoid the development of resistant strains of the pathogen [5]. Hence, the present study was made to screen out the effectiveness of plant extracts extracts and biocontrol agents under *in vitro* condition for developing suitable management strategy by integration of both chemicals and non-chemicals against the pathogen.

Material and Methods
To assess the growth inhibition and antagonistic properties of plant extracts and biocontrol agents respectively, *in vitro* experiment was carried out in the plant pathology laboratory of UAHS, Shivamogga.

***In vitro* evaluation of plant extracts**

Fresh samples of commonly available plant materials *viz.*, Tulsi leaves, Marigold leaves, Neem leaves, Noni leaves and Turmeric rhizomes, were collected and used for extraction. Hot water extraction of these plant materials was done by w/v (100g/100ml) basis and the concentrate was stored in refrigerator for further use. Antifungal activity of the plant extracts were tested by following poisoned food technique. The Desired quantity of the concentrate was mixed with sterilized and cooled Potato dextrose agar medium at the time of pouring to get 5, 10 and 15 per cent concentration. Twenty ml of the medium was poured into petriplate, mycelial disc of the fungus was placed at the centre of the petriplate and were replicated thrice. The per cent inhibition over control was worked out according to equation given by Vincent [6].

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

Where

I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

***In vitro* evaluation of bio-agents**

The efficacy of four antagonistic bio-agents *viz*., *Trichoderma harzianum* (Rifai) I (UAS Dharwad isolate), *Trichoderma harzianum* (Rifai) II (UAHS Shivamogga isolate), *Pseudomonas fluorescens* (Flugge) Migula and *Bacillus subtilis* (Ehrenberg) Cohn were obtained from Department of Plant Pathology, UAHS, Shimoga and were tested against *S. rolfsii* for radial growth inhibition on the PDA media using through dual culture technique under *in vitro* condition.

For this study both bio-agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus. Twenty ml of sterilized and cooled Potato dextrose agar was poured into sterilized petriplates. Fungal antagonists were evaluated by inoculating the pathogen at one side and the antagonist exactly opposite side to it in the same petriplate by leaving 3-4 cm gap. For this, actively growing culture was used. In case of bacterial antagonist evaluation, two mycelial discs of pathogen were inoculated and bacterial antagonist was streaked in the centre of the plate. The plates were replicated five times. After required period of incubation *i.e.* after control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was calculated as described above.

Results and Discussion

Efficacy of plant extracts extracts and bioagents was studied under *in vitro* condition by following dual culture method and the results obtained are presented under the following heads with relevant discussion.

In vitro* evaluation of plant extracts extracts against *S. rolfsii

The effect of plant extracts on the per cent inhibition of mycelial growth of *S. rolfsii* at three concentrations differed significantly. Among the eight plant extracts tested, maximum of per cent inhibition of mycelial growth (40.06%) was recorded in tulsi leaf extract which was significantly superior to all other tested plant extracts, followed by marigold leaf extract (37.81%), neem leaf extract (27.43%), turmeric rhizome extract (27.26%), noni leaf extract (25.80%), mehandi leaf extract (24.49%) and garlic bulb extract (24.14%). Least inhibition was recorded in eucalyptus leaf extract (21.37%). Among the tested three concentrations, 15 per cent concentration of all plant extracts was significantly found superior to 5 and 10 per cent. At 15 per cent concentration of plant extracts, maximum of 62.19 per cent inhibition of mycelial growth was recorded in tulsi leaf extract followed by marigold leaf extract (57.11%) and turmeric rhizome extract (53.44%). Further neem leaf extract, noni leaf extract, mehandi leaf extract, garlic bulb extract and eucalyptus leaf extract showed 50.59, 49.78, 47.89, 43.33 and 36.48 per cent inhibition respectively (table 1).

The findings are in line with the findings of Muthukumar [7] reported the efficacy of 16 essential oils against mycelia growth of *S. rolfsii*, among these citronella, lemongrass, tulsi and turmeric oils found to be effective at 1.0 per cent concentration and caused complete growth inhibition of pathogen. Begum *et al.* [8] found that, among botanicals tested at 5 and 10 per cent concentrations, significantly highest average inhibition was recorded with neem (74.81%), followed by tulsi (67.10%) and nirgudi (65.81%). The antifungal property of tulsi is due to phenolic compound such as eugenol, it is related to its lipophilic character in that they increase the fluidity and permeability of the cell membrane of microorganisms [9].

Table 1: Effect of different plant extracts on mycelial growth of *S. rolfsii*

Plant extractss	Common name	Plant part used	Per cent inhibition of mycelia growth			
			Concentrations (%)			Mean
			5	10	15	
Allium sativum L.	Garlic	Bulb	0.00 (0.00)*	29.07 (32.65)	43.33 (41.19)	24.14
Curcuma longa L.	Turmeric	Rhizome	0.00 (0.00)	28.33 (32.18)	53.44 (47.00)	27.26
Morinda citrifolia L.	Noni	Leaves	0.00 (0.00)	27.63 (31.73)	49.78 (44.90)	25.80
Eucalyptus tereticornis L.	Nilgiri	Leaves	0.00 (0.00)	27.63 (31.73)	36.48 (37.18)	21.37
Lawsonia inermis L.	Mehandi	Leaves	2.59 (9.27)	23.00 (28.67)	47.89 (43.81)	24.49
Ocimum sanctum L.	Tulsi	Leaves	6.44 (14.71)	51.56 (45.91)	62.19 (52.08)	40.06
Azadirachta indica Juss.	Neem	Leaves	3.52 (10.82)	28.19 (32.08)	50.59 (45.36)	27.43

Tagetes erecta L.	Marigold	Leaves	9.89 (18.34)	46.44 (42.98)	57.11 (49.11)	37.81
Control			0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	S.Em±			**CD at 1%**		
Plant extracts (B)	0.38			1.031		
Concentration (C)	0.24			0.634		
BxC	0.67			1.796		

* Figures in parenthesis are arcsine transformed values

In vitro evaluation of biocontrol agents against *S. rolfsii*

There were significant differences among all the tested bio-agents as presented in table 2. *Trichoderma harzianum* I was found to be significantly superior in inhibiting the mycelial growth of *S. rolfsii* (62.64%) followed by *Trichoderma harzianum* II (57.08%). There was no inhibition of mycelial growth of fungus in *Pseudomonas fluorescens* and *Bacillus subtilis* (0.00%).

Table 2: *In vitro* evaluation of bioagents against *S. rolfsii*

Bio agent	Per cent inhibition of mycelial growth
Trichoderma harzianum I	62.64# (52.35)*
Trichoderma harzianum II	57.08 (49.10)
Bacillus subtilis	0.00 (0.00)
Pseudomonas fluorescens	0.00 (0.00)
S.Em±	0.10
CD at 1%	0.43

* Figures in parenthesis are arcsine transformed values
\# mean of five replications

Similar types of observations were made by Basamma [10] and Kulkarni [11] who noticed 59.81 and 53.33 per cent inhibition of mycelial growth of *S. rolfsii* by *T. harzianum* these results indicated that *Trichoderma* isolates have competition, mycoparasitic and lysis effect on the pathogen. It may be due to production of antibiotic substance such as gliotoxin, viridin and some cell wall degrading enzymes which might have diffused air filled pores, which are detrimental to the growth of *S. rolfsii* as reported by Brain [12] and also certain biologically active heat stable metabolites such as ethyl acetate [13]. These results are also in agreement with results of Karthikeyan [14] and Mukharjee *et al*. [15]. Showed the inhibition of mycelial growth of *S. rolfsii* by *T. harzianum* is due to the penetration of the antagonist hyphae into hyphae of pathogen at the place of contact. The bioagents used in the present study are easily producible, biodegradable, less expensive and cause no environmental hazards to human health. These are ecologically safe and culturally more acceptable among the farmers.

The use of botanicals and bio-agents provide an alternative to the use of synthetic pesticides with the advantage of minimizing the cost of cultivation and also avoid the health hazards. From the *in vitro* findings, it can be suggested that there are indeed alternatives to replace the synthetic fungicides for management of this notorious soil borne fungi: *S. rolfsii*, which causes loss of multimillion dollars. It is possible that by combining these approaches (use of plant extracts and antagonistic microorganisms) an economically viable alternative for crop production system can be developed.

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