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**Divya Bharathi AR**  
 Department of Plant Pathology,  
 College of Agriculture, University  
 of Agricultural Science Dharwad,  
 Karnataka, India

**Benagi VI**  
 Department of Plant Pathology,  
 College of Agriculture, University  
 of Agricultural Science Dharwad,  
 Karnataka, India

## ***In vitro* evaluation of botanicals and bio-agents against *Sclerotium rolfii* Sacc. incitant of wilt complex disease of betelvine (*Piper betle* L.)**

**Divya Bharathi AR and Benagi VI**

### **Abstract**

A laboratory experiment was conducted to study the antifungal activity of plant extracts and antagonistic activity of bio control agents against *Sclerotium rolfii* Sacc. inciting agent of betelvine wilt complex disease. Among the botanicals evaluated maximum per cent inhibition (85.00 %) of mycelial growth was recorded in the combination of garlic bulb extract, black tulsii leaf extract and rhizome extract of turmeric (1:1:1) at 15 per cent concentration. Among the fungal and bacterial bio control agents tested, *T. harzianum* (IOF isolate) and *T. harzianum* (Kakol isolate) were effective in inhibiting *S. rolfii* with 71.33 and 70.37 per cent respectively, which were statistically on par with each other.

**Keywords:** Betelvine, wilt complex, *Sclerotium rolfii*, botanicals and bio-agents

### **1. Introduction**

Betelvine (*Piper betle* L.) is a dioecious perennial creeper belongs to the family *Piperaceae*. It is one of the important commercial horticulture crops valued for its heart shaped green leaves which possess medicinal properties. Successful cultivation of betelvine suffers from root and aerial diseases among these wilt/ root rot caused by many fungal pathogens like *Phytophthora* spp., *Rhizoctonia solani*, *R. bataticola*, *Fusarium* spp., *Pythium* spp. and *Sclerotium rolfii* along with root knot nematode *Meloidogyne incognita* results in significant yield losses (Brahmankar *et al.*, 2011) <sup>[1]</sup>.

Wilt or foot rot caused by *Sclerotium rolfii* has become a major limiting factor for successful cultivation of betelvine. Chowdary (1945) <sup>[2]</sup> reported some diseases of betelvine in Sylhet, Assam with special emphasis on sclerotium wilt. *S. rolfii* is a polyphagous and most destructive soil borne fungus with wide host range and was first reported by Rolfs (1892) <sup>[3]</sup> as a cause of tomato blight in Florida.

### **2. Material and methods**

#### **2.1 *In vitro* evaluation of plant extracts**

Commonly available plant materials *viz.*, Black tulsii leaves, Neem leaves, Garlic bulbs 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 (1947) <sup>[4]</sup>.

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

Where

I = Per cent inhibition of mycelial growth

C = Growth of mycelium in control.

T = Growth of mycelium in treatment.

**Correspondence**  
**Divya Bharathi AR**  
 Department of Plant Pathology,  
 College of Agriculture, University  
 of Agricultural Science Dharwad,  
 Karnataka, India

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

The antagonistic micro-organisms like *Trichoderma harzianum* (Rifai), *Trichoderma viride* (Pers.), *Pseudomonas fluorescens* (Flugge) Migula and *Bacillus subtilis* (Ehrenberg) Cohn obtained from Department of Plant Pathology and Institute of Organic Farming, UAS, Dharwad along with isolates of *T. harzianum* and *P. fluorescens* from Kakol village were evaluated for their antagonistic effect under *in vitro* conditions against betelvine wilt pathogens by dual culture technique.

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.

**Table 1:** *In vitro* evaluation of botanicals against *Sclerotium rolfsii* causing wilt complex disease.

Name of the botanical	Parts used	Per cent inhibition			
		5 %	10%	15%	Mean
Black tulsii	Leaves	2.04 (8.07) *	9.63 (18.07)	12.22 (20.45)	7.96 (15.53)
Garlic	Bulbs	22.96 (28.63)	28.33 (32.16)	36.85 (37.38)	29.38 (32.72)
Neem	Leaves	30.37 (33.44)	42.96 (40.95)	53.52 (47.02)	42.28 (40.47)
Turmeric	Rhizomes	8.71 (17.09)	9.63 (18.06)	38.85 (38.55)	19.06 (24.57)
Black tulsii + neem	Leaves+leaves (1:1)	37.04 (37.49)	45.00 (42.13)	60.00 (50.77)	47.34 (43.46)
Garlic + turmeric	Bulb+rhizome (1:1)	36.48 (37.16)	37.59 (37.81)	40.37 (39.45)	38.15 (38.14)
Garlic + black tulsii+ turmeric	Bulb+leaves+rhizome (1:1:1)	44.44 (41.81)	52.22 (46.27)	85.00 (67.22)	60.55 (51.77)
Mean		26.00 (29.10)	32.19 (33.64)	46.69 (42.98)	34.96 (35.24)
		S. Em.±		C.D. at 1%	
	Botanical (B)	0.32		1.23	
	Concentration (C)	0.21		0.81	
	Interaction (BxC)	0.56		2.14	

\*Arcsine values

The antifungal property of black tulsii 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 (Dipasqua *et al.*, 2007) [7]. In turmeric polyphenolic compound curcumin has antimicrobial activities against different bacteria, viruses, fungi (Moghadamtousi *et al.*, 2014) [8]. The antimicrobial properties of garlic were attributed to the presence of sulphur as an active principle (Mangamma and Sreeramulu, 1991) [9].

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

The results presented in table 2 revealed that the, efficacy of biocontrol agents against *S. rolfsii* was significant. Among fungal bio-agents namely *T. harzianum* (IOF isolate) and *T. harzianum* (Kakol isolate) are more effective in inhibiting *S. rolfsii* with 71.33 and 70.37 per cent respectively, which were statistically on par with each other. Among bacterial bio-agents *Pseudomonas fluorescens* showed maximum inhibition (62.10 %). Similar findings were reported by Nagamma and Nagaraja (2015) [10] Maximum inhibition of mycelial growth (71.67%) was noticed in *T. harzianum* (Bacteriology lab isolate) which was followed by *T. viride* (Microbiology lab) (63.33%). Least inhibition was observed in *T. harzianum*

## 3. Results and Discussion

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads.

### 3.1 *In vitro* evaluation of plant extracts

The results revealed that the efficacy of botanicals against *S. rolfsii* was statistically significant. Among the seven botanicals tested, garlic bulb extract + black tulsii leaf extract and rhizome extract of turmeric (1:1:1) at 15 per cent concentration gave higher level of inhibition (85.00 %) of mycelial growth, black tulsii leaf extract + neem leaf extract (1:1) at the concentration of 15 per cent were next best (60.00 %). Least mycelial inhibition (12.22 %) was recorded in black tulsii leaf extract at the concentration of 15 per cent (table 1). The results are in agreement the findings of Muthukumar (2015) [5] reported the efficacy of 16 essential oils against mycelia growth of *S. rolfsii*, among these citronella, lemongrass, tulsii and turmeric oils found to be effective at 1.0 per cent concentration. Begum *et al.* (2014) [6] found that, among botanicals tested at 5 and 10 per cent concentrations, significantly highest average inhibition was recorded with neem (74.81%), followed by tulsii (67.10%) and nirgudi (65.81%).

GKVK isolate (31.67%). Parmar *et al.* (2015) [11] screened the six *Trichoderma* strains among them *T. viride* (NBIIITv 23) inhibited 61 per cent growth of *S. rolfsii* followed by *T. harzianum* (NBII Th 1) 55 per cent, respectively.

**Table 2:** *In vitro* evaluation of bio-agents against *Sclerotium rolfsii*

Bio-agents	Per cent inhibition
<i>Trichoderma harzianum</i> (*IOF isolate)	71.33# (57.63)
<i>Trichoderma harzianum</i> (Kakol isolate)	70.37 (57.02)
<i>Trichoderma viride</i>	66.17 (54.43)
<i>Pseudomonas fluorescens</i> (IOF isolate)	62.10 (52.00)
<i>Pseudomonas fluorescens</i> (Kakol isolate)	57.55 (49.34)
<i>Bacillus subtilis</i> (IOF isolate)	51.44 (45.82)
S. Em. ±	0.40
C.D. @ 1%	1.60

\*Arcsine values, # = mean of five replications

\*IOF isolate – Institute of Organic Farming, UAS, Dharwad

## 4. Conclusion

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

the plant extracts garlic bulb extract + black tulsii +rhizome extract of turmeric (1:1:1) and antagonists *Trichoderma* spp. can be used against *S. rolfsii* under field condition.

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