



## *In vitro* evaluation of different fungicides against *Sclerotium rolfii* causing wilt of betelvine

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### Abstract

A laboratory experiment was conducted to evaluate various chemical compounds against *Sclerotium rolfii* Sacc causing betelvine wilt complex disease. Among the fungicides evaluated *in vitro*, systemic fungicides propiconazole, difenconazole, thiophanate methyl, triadimefon and combi products viz., tricyclazole 18% + mancozeb 62%, carbendazim 12% + mancozeb 63%, thiram 37.5 % + carboxin% and tebuconazole 50% + trifloxystrobin 25% were found to be most effective showing cent per cent inhibition of mycelial growth at all the concentration tested. Whereas, non-systemic fungicide mancozeb was found to be effective only at higher concentration (0.3%) and the least per cent inhibition of the fungus was recorded by copper oxychloride (4.35%).

**Keywords:** combi product, inhibition, *in vitro*, non-systemic, systemic fungicide

### 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.

Betelvine wilt caused by *Sclerotium rolfii* Sacc. Is one of the most destructive disease of betel Vine. 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<sup>[1,2]</sup>.

Use of fungicides for the control of plant diseases is a common practice. As *Sclerotium* is a soil borne pathogen with a wide host range crop rotation may not be of much help. Hence, the present study was made to screen the fungicides to manage wilt disease within the reasonable limit to avoid fungicidal residue effect.

### Material and Methods

The experiment was conducted at Department of Plant Pathology, College of Agriculture, UAHS, Shivamogga. Efficacy of different non-systemic, systemic and combi-product fungicides at different concentrations were evaluated on radial growth of the test fungi by poisoned food technique<sup>[3]</sup>.

### Isolation of fungus

For the isolation of pathogen Potato dextrose agar media was used. Approximately 20 ml autoclaved PDA was poured in each sterilized Petri dish and allowed to solidify. The diseased plant specimens were cleaned properly. The diseased portion was cut into small bits along with the healthy portion with sterilized blade and transferred into sterilized Petri plates containing 0.01% sodium hypochlorite solution for surface sterilization. After a

minute the bits were transferred to sterilized water and washed by three sequential changes in sterilized distilled water to remove the traces of sodium hypochloride. Bits were blot dried by keeping them on sterilized filter paper to absorb the excess water. Three bits were aseptically transferred to solidified PDA medium in sterilized Petri dish at equidistance and incubated at room temperature (27 °C + 1 °C). All the operations were carried out aseptically and growth of organism was observed regularly.

### Evaluation of fungicides

The efficacy of systemic fungicides, non-systemic fungicides and combi products were assessed against *Sclerotium rolfii* for radial growth inhibition. These fungicides were evaluated in the laboratory by following Poison food technique. The systemic fungicides were tested at 0.05, 0.1 and 0.15 per cent concentrations, whereas the non-systemic and combi products were tried at 0.1, 0.2 and 0.3 per cent concentrations.

Required quantity of individual fungicide was added separately into sterilized molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. Mycelium discs of 5 mm size from seven days old culture was cut by a sterile Cork borer and one such disc was placed at the centre of each agar plate. The plate without any fungicide served as control. Three replications were maintained for each concentration. The plates were incubated at room temperature and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth over control, calculated by using the formula given by Vincent<sup>[4]</sup>.

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

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment

**Results and Discussion**

The efficacy of six systemic fungicides, four non-systemic fungicides and four combi products were assessed against *Sclerotium rolfsii* for radial growth inhibition under *in vitro* condition by following poison food technique and the results obtained are presented under the following heads with relevant discussion.

The mycelial growth inhibition of *S. rolfsii* at three concentrations by four non-systemic fungicides were recorded and presented in Table 1. Among four non-systemic fungicides used cent per cent inhibition of growth of *S. rolfsii* was recorded by captan and chlorothalonil followed by mancozeb (61.75%). Least per cent inhibition of the fungus was recorded in copper oxychloride (4.35%). Among the tested three concentrations 0.3 per cent of all fungicide was significantly found superior to 0.2 and 0.1 per cent concentrations. The results are in conformity with Sahana *et al* [5]. Who reported that copper oxy chloride least effective at 50, 100 and 150 ppm and they noticed increase in mycelial inhibition with increase in the concentration.

Among six systemic fungicides tested, cent per cent inhibition of mycelia growth of *S. rolfsii* was observed in propiconazole, difenconazole, thiophanate methyl and triadimefon followed by hexaconazole (83.41%). The least per cent inhibition of fungus was recorded in carbendazim (54.33%) at 0.05 per cent concentration (Table 2). Among the tested three concentrations, 0.15 per cent concentration of all fungicides showed cent per cent inhibition of mycelial growth of fungus and which was found significantly superior to 0.1 and 0.05 per cent. This inhibition by triazole fungicides may be due to their interference with the ergosterol biosynthesis. Similar findings were reported by Suryawanshi *et al.* [6], Basamma [7] and Hegde *et al* [8]. Where they observed mycelia inhibition by propiconazole and difenconazole against *S. rolfsii*.

Among the four combi products tested, cent per cent inhibition of growth of *S. rolfsii* was observed in all four i.e., tricyclazole 18% + mancozeb 62%, carbendazim 12% + mancozeb 63%, thiram 37.5 % + carboxin% and tebuconazole 50% + trifloxystrobin 25% at all the three concentrations tested (Table 3). Combi product have different mode of action and avoids the development of resistance to systemic fungicides. Effectiveness of carboxin fungicide against *S. rolfsii* was reported by Prabhu [9], Kulkarni [10] and Basamma [7]. In addition Hegde *et al* [8]. And Hegde *et al.* [11] reported that carbendazim + mancozeb, carboxin + thiram, tricyclazole + mancozeb are highly effective against *S. rolfsii*.

**Table 1:** *In vitro* evaluation of non-systemic fungicides against *S. rolfsii*

Fungicides	Per cent inhibition of mycelia growth			
	Concentrations (%)			Mean
	0.1	0.2	0.3	
Mancozeb 75 WP	36.00 (36.89)*	49.26 (44.60)	100.00(90.05)	61.75
Captan 50 WP	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00
Chlorothalonil 75 WP	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00
Copper oxychloride 50 WP	0.00(0.00)	0.00(0.00)	13.07(21.21)	4.36
Control	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00
	Fungicide (F)	Concentration(C)	FxC	
S. Em±	0.04	0.04	0.07	
CD at 1%	0.16	0.14	0.28	

\* Figures in parenthesis are arcsine transformed values

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

Fungicides	Per cent inhibition of mycelia growth			
	Concentrations (%)			Mean
	0.05	0.10	0.15	
Carbendazim 50WP	54.33 (47.51)*	100.00 (90.05)	100.00 (90.05)	84.78
Hexaconazole 5EC	83.41 (66.00)	96.85 (79.83)	100.00 (90.05)	93.42
Difenconazole 25EC	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
Thiophanate methyl 70WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
Triadimefon 25WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
Propiconazole 25EC	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	Fungicide (F)	Concentration (C)	FxC	
S. Em±	0.05	0.04	0.09	
CD at 1%	0.20	0.14	0.34	

\* Figures in parenthesis are arcsine transformed values

**Table 3:** *In vitro* evaluation of combi products against *S. rolfsii*

Fungicides	Per cent inhibition of mycelia growth			
	Concentrations (%)			Mean
	0.1	0.2	0.3	
Carboxin 37.5% + Thiram 37.5%	100.00 (90.05)*	100.00 (90.05)	100.00 (90.05)	100.00
Tricyclazole 18% + Mancozeb 62%	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
Carbendazim 12% + Mancozeb 63%	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
Tebuconazole 50% + Trifloxystrobin 25%	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00

\* Figures in parenthesis are arcsine transformed values

## Conclusion

Among the systemic fungicides evaluated *in vitro* against *S. rolfsii*, propiconazole, thiophanate methyl, triadimefon and difenconazole were found to be highly effective at all the concentrations with cent per cent inhibition of mycelial growth. In case of nonsystemic fungicides captan and chlorothalonil were found to be highly effective. While least per cent inhibition was recorded in mancozeb (61.75%) and copper oxy chloride (4.36%). All the four combi fungicides used in the study were very effective with cent per cent inhibition of mycelial growth at all the concentrations tested. By this study it was observed that the triazoles and triazole containing combi products were found to be effective even at low concentrations under lab conditions against *Sclerotium rolfsii*. Whereas, the contact fungicide mancozeb was found inhibitive only at higher concentrations.

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