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RESEARCH PAPER

In-vitro evaluation of fungicides and bio-agents against *Fusarium solani* (Mart.) Sacc. inciting dry root rot/wilt of acid lime in northern Karnataka

SHIVALEELA SAJJAN[1], SYEDA SAMINA ANJUM[1], M. M. JAMADAR[1] AND R. B. NEGALUR[2]

[1]Department of Plant Pathology, [2]Department of Agronomy, College of Agriculture, Vijayapur - 586 101
University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India
E- mail: sajjanshivaleela333@gmail.com

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Abstract: Acid lime (*Citrus aurantifolia* Swingle) is one of the four commercially important citrus fruit crop grown in India among the citrus species. It belongs to the family *Rutaceae* and genus *Citrus aurantifolia* (Swingle). Among many soil borne diseases of acid lime, dry root rot/wilt is considered to be a severe disease caused by *Fusarium solani* which is prevalent in northern Karnataka. Present investigation was carried out under *In vitro* condition to know the efficacy of fungicides and bio-agents against *F. solani* for further evaluation under field condition benefitting the farmer's. Results revealed that all the tested fungicides significantly inhibited mycelial growth of tested pathogen over untreated control. Among the five systemic fungicides evaluated, Carbendazim 50 WP (98.37%) was superior over all other treatments followed by Propiconazole 25 EC (85.68%), Tebuconazole 250 EC (76.88%) and Hexaconazole 5 SC (68.92%). However, the least inhibition of mycelial growth was observed in Metalaxyl MZ 68WP (6.72%) at 500, 1000, 1500 ppm. Combi product hexaconazole 5% + captan 70% WP was significantly superior (74.55%) over all the other non-systemic fungicides evaluated. The next best treatment was found to be captan 50 WP (26.14%) at 1000, 2000, 2500 ppm concentrations. All the four bio-agents evaluated exhibited antifungal activity against *Fusarium solani.* It was noticed that, maximum reduction in colony growth was observed in *Trichoderma harzianum* (80.99%) followed by *Purpureocillium lilacinum* with 77.08 per cent inhibition. The least inhibition was observed in *Pseudomonas fluorescens* (39.82%).

Key words: Acid lime, Bio-agents, Dry root-rot/wilt, Fungicides, *In vitro,* Per cent inhibition

Introduction

Acid lime (*Citrus aurantifolia* Swingle) is one of the four commercially important citrus fruit crop grown in India among the citrus species. It belongs to the family *Rutaceae*. South East Asia is the origin of acid lime. It requires tropical and dry tropical climate, well drained soil with optimum temperature ranging from 20-30°C. The crop is grown in an area of 2,96,000 ha with a production of 33,97,000 t and a productivity of 11.47 t/ ha (Anon., 2018). Diseases cause serious problem to citrus cultivation which can be etiologically grouped under diseases caused by fungi, bacteria, viruses, viroids, phytoplasma and nematode. The crop is affected by many soil borne diseases like root-rot or gummosis, dry root rot and citrus decline.

In Andhra Pradesh the root rot disease incidence is reported to be 5 to 50 per cent and nearly 10 to 15 per cent of infected plants are being killed every year (Gopal *et al*., 2000 and Vijayakumar, 2001). Citrus dry root rot/wilt is a severe disease that is caused by *Fusarium solani* found to be prevalent in northern Karnataka. Symptoms of the disease include yellowing of leaves, gumming on surface of bark, drying of twig, partial wilting and finally death of whole plant. Considering this, present studies were carried out under *in vitro* condition to know the best effective chemical which would help in managing the disease.

Material and methods

a. In vitro evaluation of fungicides

The efficacy of five systemic fungicides (at the concentration of 500, 1000 and 1500 ppm) and four non-systemic fungicides (at the concentrations of 1000, 2000 and 2500 ppm) were tested *in vitro* by using poisoned food technique (Sharvelle, 1961). The fungicides used are as follows.

(a) Non-systemic Fungicides

Common name	Trade name
Captan 50WP	Captaf 50 WP
Hexaconazole 5% + captan 70% WP	Taqat 75 WP
Copperoxychloride 50 WP	Blue copper 50 WP
Mancozeb 75 WP	Indofil M-45 75 WP

(b) Systemic Fungicides

Common Name	Trade Name
Carbendazim 50WP	Bavistin 50 WP
Hexaconazole 5 SC	Contafplus 5 SC
Propiconazole 25EC	Tilt 25 EC
Tebuconazole 250 EC	Folicur 250 EC
Metalaxyl MZ 68 WP	Ridomil Gold 68 WP

The experiment was carried out in Completely Randomized Design (CRD) with four non-systemic and five systemic fungicides at above mentioned concentrations replicated four times. The observations were made after completion of incubation period of 10 days and the percent inhibition of mycelial growth was calculated by using Vincent's formula (1947).

$$\text{Inhibition per cent} = \frac{(C-T)}{C} \times 100$$

Where, I = Percent inhibition of mycelium
C= Growth of mycelium in control
T = Growth of mycelium in treatment

Required quantity of individual fungicide was added separately into molten and cooled Potato Dextrose media so as

to get the desired concentration of fungicides. Later 20 ml of the poisoned medium was poured into sterile Petri plates. Mycelial discs of five mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium. Each treatment was replicated four times. Then such plates were incubated 28±1ºC (at room temperature) for eight days and radial colony growth was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by using the above mentioned Vincent formula (1947).

b. *In vitro* evaluation of bio- agents:

Efficacy of four bio-agents was carried out in CRD against the identified pathogen *F. solani* through dual culture technique replicated four times. The zone of inhibition of test fungus by the antagonist was measured and per cent inhibition of growth of pathogen was calculated using the formula.

$$\text{Inhibition per cent} = \frac{(C-T)}{C} \times 100$$

Where, I = Percent inhibition of mycelium
C= Growth of mycelium in control
T = Growth of mycelium in treatment

Treatments

T_1: *Trichoderma harzianum*
T_2: *Pseudomonas fluorescens*
T_3: *Purpureocillium lilacinum*
T_4: *Bacillus subtilis*

Four bio-control agents *viz*., *Trichoderma harzianum*, *Purpureocillium lilacinum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against pathogen *F. solani* under *in vitro* conditions using dual culture technique. Both bio-control agents and test fungus were cultured on Potato Dextrose Agar medium in order to get fresh and active growth of fungus.

About 20 ml of sterilized and cooled Potato Dextrose Agar media was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal bio-control agents, mycelial discs of test fungus was inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at one end of the Petri plates and mycelial discs of the fungus was placed at the other side of the Petri plate. The plates were incubated at 28±1°C and diameter of growth of fungus was recorded in treatment. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

Results and discussion

1.Mycelial inhibition by fungicides

Results revealed that, all the tested fungicides significantly inhibited mycelial growth of tested pathogen over untreated control. Further, the per cent mycelial growth inhibition increased with increase in concentrations of the fungicides tested.

It was clear that, there was a significant difference among the systemic fungicides (Table 1) in inhibiting the growth of *F. solani*. Among the five systemic fungicides evaluated, carbendazim 50 WP (98.37%) was superior over all other treatments followed by propiconazole 25 EC (85.68%), tebuconazole 250 EC (76.88%) and hexaconazole 5 SC (68.92%) in inhibiting the mycelia growth of pathogen *F. solani*. However, the least inhibition was observed in metalaxyl MZ 68WP (6.72 %).

Systemic fungicide carbendazim 50 WP was successful in inhibiting the growth of *F. solani* (97.07 %) and (98.03 %) at 500 and 1000 ppm respectively and complete inhibition (100.00 %) was recorded at 1500 ppm concentration. Propiconazole 25 EC was highly effective at 1500 ppm (96.64 %) concentration followed by 1000 ppm (83.20%) and 500 ppm (77.21%) concentrations. Fungicide Tebuconazole 250 EC showed inhibition of 76.10, 76.89 and 77.67 per cent and hexaconazole 5 SC showed inhibition of 66.03, 69.42 and 71.31 per cent at 500, 1000 and 1500 ppm concentrations, respectively.

The least fungal inhibition was observed in metalaxyl MZ 68 WP *i.e.* 4.98, 5.97 and 9.20 per cent at 500, 1000 and 1500 ppm concentrations. Among the different concentrations of systemic fungicides tested, significantly highest inhibition was recorded at 1500 ppm followed by 1000 ppm and 500 ppm concentrations of the fungicides.

Table 1. *In vitro* evaluation of systemic fungicides against *Fusarium solani*

Fungicides	Per cent Inhibition Concentrations(ppm)			
	500	1000	1500	Mean
Carbendazim 50 WP	97.07 (80.14)	98.03 (81.94)	100.00 (90.00)	98.37 (82.66)
Hexaconazole 5 SC	66.03 (54.35)	69.42 (56.43)	71.31 (57.61)	68.92 (56.12)
Propiconazole 25 EC	77.21 (61.48)	83.20 (65.80)	96.64 (79.44)	85.68 (67.77)
Tebuconazole 250 EC	76.10 (60.73)	76.89 (61.27)	77.67 (61.80)	76.88 (61.26)
Metalaxyl MZ 68 WP	4.98 (12.90)	5.97 (14.15)	9.20 (17.65)	6.72 (15.02)
Mean	64.28 (53.29)	66.70 (54.76)	70.96 (57.39)	67.31(55.13)
S.Em±	CD at 1%			
Fungicides	1.12	3.19		
Concentration	0.86	2.47		
Fungicides × concentration	1.93	5.53		

*Figures in parenthesis indicate angular transformed values

Table 2. *In vitro* evaluation of non-systemic fungicides against *Fusarium solani*

Fungicides	Per cent inhibition Concentrations(ppm)			
	1000	2000	2500	Mean
Captan 50 WP	16.38(23.88)	26.84(31.20)	35.20(36.39)	26.14(30.75)
Hexaconazole 5%+ captan 70% WP	74.00(59.34)	74.55(59.70)	75.10(60.07)	74.55(59.70)
Copper oxychloride 50 WP	3.58(10.90)	5.35(13.37)	8.39(16.84)	5.77(13.90)
Mancozeb 75 WP	13.46(21.53)	25.22(30.14)	28.33(32.16)	22.34(28.20)
Mean	26.86(31.21)	32.99(35.05)	36.76(37.32)	32.20(34.57)
S.Em±	CD at 1%			
Fungicides	1.13	3.26		
Concentration	0.98	2.82		
Fungicides× concentration	1.96	5.65		

*Figures in parenthesis indicate angular transformed values

Similar results were reported in case of *F. oxysporum* f. sp. *gladioli* (Sumitra, 2006), wilt of patchouli caused by *F. solani* (Sreedevi, 2007), *F. solani* causing dry root rot of acid lime (Gopal *et al*., 2008) and Saraswati and Jamadar (2015). Padvi *et al*. (2018) also evaluated eight different fungicides at different concentrations *viz*. 500, 1000, 2000 and 2500 ppm against *Fusarium solani*. The highest average mycelial growth inhibition (each 100 %) was recorded with carbendazim and SAAF. These were followed by the fungicides *viz*. propiconazole (79.09 %).

Significant differences were recorded in per cent inhibition of mycelial growth of *F. solani* with non-systemic fungicides (Table 2). Combi product of hexaconazole 5% + captan 70% WP was significantly superior (74.00%, 74.55% and 75.10%) over all the other non systemic fungicides evaluated in different concentrations *viz,* 1000, 2000 and 2500 ppm. The next best treatment was found to be captan 50 WP with inhibition of 16.38, 26.84, and 35.20 per cent which was significantly superior over mancozeb 75 WP with 13.46, 25.22 and 28.33 per cent inhibition at 1000, 2000 and 2500 ppm concentrations, respectively.

The least fungal inhibition was observed in copper oxychloride of 3.58, 5.35 and 8.39 per cent at 1000, 2000 and 2500 ppm concentrations, respectively. Among the different concentrations tested, significantly highest inhibition was recorded at 2500 ppm followed by 2000 and 1000 ppm concentrations of the non-systemic fungicides.

These results are in agreement with Sreedevi (2007) who found mancozeb at 0.2 and 0.3 per cent effective against *F. solani* followed by propineb at 0.3 per cent.

Table 3. *In vitro* evaluation of bio-agents against *Fusarium solani*

Bio-agents	Per cent mycelial inhibition
Trichoderma harzianum	80.99(64.15)
Pseudomonas fluorescens	39.82(39.12)
Purpureocillium lilacinum	77.08(61.40)
Bacillus subtilis	45.62(42.48)
S.Em±	0.72
C.D. at 1%	2.24

*Figures in parenthesis indicate angular transformed values

Kumar and Bhat (2021) also reported SAAF and TAQAT (hexaconazole 5% + captan 7%) as effective in inhibiting mycelial growth of the pathogen *F. oxysporu*m f. sp. *lycopersici* infecting tomato plants.

2. Mycelial inhibition by Bio-agents

The results revealed that, the antagonists significantly reduced the growth of *F. solani* either by competition (over growing) or by antibiosis (exhibiting inhibition zones).

All the four bio-agents evaluated exhibited antifungal activity against *Fusarium solani* (Table 3) and significantly inhibited its mycelial growth over untreated control. It was noticed that, maximum reduction in colony growth was observed with bio-agent, *Trichoderma harzianum* (80.99%) which was significantly superior over other bio-agents tested. The next best bio agent was *Purpureocillium lilacinum* with 77.08 per cent inhibition. The least inhibition (39.82 %) was observed in *Pseudomonas fluorescens*.

These results are in accordance with Karima and Nadia (2012) who reported the antagonistic activity of *Trichoderma harizianum* (71.9%) against *F. solani* under *In vitro* condition.

Saraswati and Jamadar (2015) reported *Trichoderma harzianum* (82.29%) effectively inhibited the growth of *F. solani* causing wilt of acid lime followed by *Purpureocillium lilacinum* (80.74%).

Conclusion

The results of *In vitro* evaluation revealed that, among the systemic fungicides, carbendazim 50 WP (98.37%) was highly effective in inhibiting the growth of *F. solani* followed by propiconazole 25 EC (85.68%) and tebuconazole 25 EC (76.88%). Among the non-systemic fungicides, combi product TAQAT (hexaconazole 5% + captan 70%) was found significantly superior (74.55%) followed by captan 50 WP (26.14%) in inhibiting *F. solani.* Among the different fungal and bacterial bio-agents tested against *F. solani*, maximum reduction in colony growth of the test pathogen was observed in *Trichoderma harzianum* (80.99%), followed by *Purpureocillium lilacinum* with inhibition of 77.08 per cent. The least inhibition was observed in *Pseudomonas fluorescens* (39.82%) followed by *Bacillus subtilis* (45.62%).

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