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In vitro evaluation of fungicides, biocontrol agents and botanicals for their bioefficacy against root rot of acid lime (*Citrus aurantifolia* Swingle) in northern Karnataka

Acid lime is believed to have originated in south-east Asia. It is grown mainly in Mexico, India, Brazil, US (Florida) and countries with tropical climate. Acid lime (*Citrus aurantifolia* Swingle) is one of the four commercially important citrus fruits grown in the country, besides orange, mandarin and grape fruit. Karnataka ranks fifth in production of acid lime with 2.83 lakh tonnes and seventh in area accounting to 12,150 ha. Among different crops, acid lime is one of the most remunerative fruit crops which is grown on commercial scale in Vijayapur district in an area of 6,499 ha with a production of 0.16 MT and productivity of 25 t/ha (Anon., 2013). Even though there is a gradual increase in both area and production of acid lime in the district over the years, but still the growers are facing few problems related to production of acid lime. Studies on effective management of root rot of acid lime Fusarium solani (Mart.) Sacc. are scanty. Keeping in the view the importance of acid lime and severity of the disease, the present investigation was carried out to know the effective fungicides, biocontrol agents and botanicals against the root rot of acid lime and results were documented in this paper.

The efficacy of seven systemic fungicides such as carbendazim, propiconazole, hexaconazole, thiophanate methyl, carboxin + thiram a combiproduct, metalaxyl MZ and fosetyl-Al (at the concentration of 0.05, 0.1 and 0.15 per cent) and five non-systemic fungicides *viz.* mancozeb, captan, copper oxychloride, Bordeaux mixture and chlorothalonil (at the concentrations of 0.1, 0.2 and 0.25 per cent) were assayed *in vitro* following poisoned food technique (Sharvelle, 1961) and antagonistic potential of nine biocontrol agents *viz. Trichoderma harzianum* Rifai, *T. viride* Pers. *T. virens* Miller, *T. asperellum* Samuel, *T. harzianum* (local), *Paecilomyces lilacinus* (Thom.) Samson, *Verticillium lecani* Zimmerman, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn Emend Pras against *F. solani* under *in vitro* conditions were studied using dual culture technique. The efficacy of botanicals such as *Allium cepa* L. *Bougainvillea spectabilis* L. *Allium sativum* L. *Pongemia pinnata* L. *Tridax procumbens* L. *Lantana camera* L. *Durantha repens* L. *Azadirachta indica* A. Juss, *Parthenium hysterophorus* L. and *Capsicum annum* L. under *in vitro* conditions using poisoned food technique (Nene and Thapliyal, 1973).

For evaluation of fungicides required quantity of individual fungicide was added separately into molten and cooled Richards' agar 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 5 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 thrice. Then such plates were incubated 28±1^{0}C (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 formula given by Vincent (1947).

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

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

For evaluation of fungal biocontrol 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 middle of the Petri plates and mycelial discs of the fungus was placed at the centre of the Petri plate. The plates were incubated at 28±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. 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).

For preparation of botanicals, fresh plant materials were collected and washed first in tap water and then in distilled water. 100 grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution. Five and ten ml of stock solution was mixed with 95 and 90 ml of sterilized molten PDA medium, respectively so as to get 5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile Petri plates, mycelium of five mm size discs form periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed on the center of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 28±1°C temperature and radial growth was taken when maximum growth occurred in the control plates. The efficacy of plant products or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using Vincent (1947) formula.

Among the systemic fungicides evaluated (Table 1) under *in vitro* conditions, the highest and significant inhibition (96.83%) was obtained by carbendazim at different concentrations. Further the fungicide formulation evaluated at 0.1 and 0.15 per cent concentrations completely inhibited (100%) the growth of *F. solani*. Following this other fungicide formulations *viz.* propiconazole (86.12%), thiophanate methyl (73.62%) and hexaconazole (68.49%) were also found significantly efficient in inhibition of the fungal pathogen. While the combi-product carboxin + thiram was moderate (51.30%) and the least inhibition of the pathogen was recorded in fosetyl-Al (2.86%) and metalaxyl MZ (6.12%) across different concentrations. Though there are no effective fungicides for

Table 1. *In vitro* evaluation of systemic fungicides against *F. solani*, an incitant of root rot of acid lime

Fungicides	Per cent inhibition of mycelial growth			
	Concentration (%)			Mean
	0.05	0.1	0.15	
Carbendazim 50WP	90.51(72.09)*	100.00(90.00)	100.00(90.00)	96.83(84.03)
Hexaconazole 5EC	64.14(53.24)	70.37(57.03)	70.96(57.45)	68.49(55.90)
Propiconazole 25EC	78.81(62.61)	82.44(65.25)	97.11(84.29)	86.12(70.71)
Thiphanate methyl 70WP	61.55(51.69)	78.22(62.23)	81.11(64.25)	73.62(59.39)
Carboxin 37.5% + Thiram 37.5% 75WP	34.29(35.81)	52.88(46.65)	66.74(54.78)	51.30(45.74)
Metalaxyl MZ 68WP	3.69(10.96)	6.22(14.41)	8.51(16.91)	6.12(14.09)
Fosetyl-Al 80WP	0.37(2.07)	2.74(9.33)	5.48(13.38)	2.86(8.46)
Mean	47.62(41.21)	56.12(49.27)	61.41(54.43)	55.05(48.33)
	Fungicides (F)	Concentration(C)		F X C
S.Em±	0.97	0.63		1.68
C.D. at 1%	3.73	2.44		6.46

* Figures in parenthesis indicate angular transformed values

the management of root rot disease of lime however, soil drenching of fungicides like carbendazim and captafol have been reported to be effective against *F. solani,* causing dry root rot of acid lime (Vijayakumar, 2001 and Gopal *et al.,* 2008).

Significant differences were observed in the per cent inhibition of mycelial growth of *F. solani* with non systemic fungicides (Table 2). Chlorothalonil at 0.25 per cent was found effective (50.41%) inhibition as compared to other non-systemic chemicals while mancozeb at 0.25 per cent (24.56%) and captan (22.74%) were moderately inhibitive. The least inhibition (7.07%) of fungal growth was observed in copper oxychloride at all concentrations tested. No inhibition of fungal growth was observed in Bordeaux mixture. Among the different concentrations significantly highest inhibition was recorded at 0.25 per cent followed by 0.2 per cent and 0.1 per cent concentrations of the fungicides. Chlorothalonil gave best control (67%) in case of *Fusarium* spp. infection of okra (Hema *et al.,* 1991) and *F. solani* causing root-rot of egg plant (Hundoo *et al., 1992).*

The antagonists significantly reduced the growth of *F. solani* (Table 3) either by competition (over growing) or by antibiosis (exhibiting inhibition zones). It was noticed that maximum reduction in colony growth was observed in *Trichoderma harzianum* (82.29%) which was significantly superior over other bio agents tested. The next best bio agent was *Paecilomyces lilacinus* (80.74%) which was on par with *T. viridae* (78.37%) and *T. asperellum* (76.33%), *T. harzianum* local (73.96%) and *T. virens* (72.74%) which were on par with each other. The least inhibition was observed in *Pseudomonas fluorescens* (36.33%) followed by *Bacillus subtilis* (44.29%) and *Verticillium lecani* (51.92%). Soltani *et al.* (2005) reported that an isolate of *T. harzianum* was best against Fusarium wilt of potato; similar results were reported by Kavitha *et al.* (2004) in case of dry root rot of acid lime.

Table 2. *In vitro* evaluation of non-systemic fungicides against *F. solani,* an incitant of root rot of acid lime

Fungicides	Per cent inhibition of mycelial growth			
	Concentration (%)			Mean
	0.10	0.20	0.25	
Mancozeb 75WP	11.55(18.78)*	26.96(31.19)	36.07(36.86)	24.56(28.94)
Copper oxychloride 50WP	3.03(9.94)	6.85(15.12)	11.33(19.59)	7.07(14.88)
Bordeaux Mixture**	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
Captan 50WP	17.40(23.91)	23.18(27.87)	27.62(31.70)	22.74(27.83)
Chlorothalonil 75WP	40.07(39.27)	52.74(46.56)	58.44(49.85)	50.41(45.23)
Mean	14.41(18.38)	21.94(24.14)	26.69(27.60)	21.01(23.37)
	Fungicides (F)	Concentration (C)		FXC
S.Em±	1.51	1.17		2.62
C.D. at 1%	5.91	4.58		10.25

* Figures in parenthesis indicate angular transformed values

**[Bordeaux Mixture was manually prepared using copper sulphate ($CuSO_4$) and lime [$Ca(OH)_2$] that was dissolved separately and later mixed together to get proportionate concentrations of 0.5% (5000 ppm), 1.0% (10000 ppm) and 2.0% (20000 ppm) for evaluation]

Table 3. *In vitro* evaluation of bio control agents against *F. solani*, an incitant of root rot of acid lime

Biocontrol agents	Per cent inhibition of mycelial growth
Trichoderma viridae Pers.	78.37 (62.29)*
Trichoderma harzianum Rifai.	82.29 (65.18)
Trichoderma virens Miller.	72.74 (58.54)
Trichoderma asperellum Samuel.	76.33(60.88)
Trichoderma harzianum (Local)	73.96 (59.31)
Paecilomyces lilacinus (Thom) Samson.	80.74 (64.21)
Verticillium lecani Zimmerman.	51.92 (46.10)
Bacillus subtilis (Cohn Emend) Pers.	44.29 (41.71)
Pseudomonas fluorescens Migula	36.33 (37.05)
S.Em±	1.93
C.D. at 1%	7.88

* Figures in parenthesis indicate angular transformed values

Allium sativum at 10 per cent (60.37%) was found significantly superior (Table 4) over all other botanicals. Next best was A. cepa at 10 per cent (49.25%) followed by *Durantha repens* at 10 per cent (49.18%), *A. sativum* at 5 per cent (44.22%) and *Azadirachta indica* (41.33%) at 10 per cent. *Pongamia pinnata* and *Bougainvillea spectabilis* were least effective in inhibiting the growth of the pathogen. Among the different concentrations significantly highest inhibition was recorded at 10 per cent (60.37%) followed by 5 per cent (44.22 %) concentrations of the botanicals. Shivapuri *et al*. (1997) recorded that the ethanol extracts of *Allium cepa* L., *A. sativum L., Lantana camara* L., *Polyalthia longifolia* Benth and Hook, *Tagetes erecta* L., *Vinca rosea* L. and *Withania somnifera* showed fungitoxic effect against *F. solani.* Sreedevi (2007) reported neem seed kernel extract, *Eucalyptus* and garlic at 10 per cent showed maximum inhibition of *F. solani*.

The *in vitro* evaluation indicated that, carbendazim (96.83% inhibition) and chlorothalonil (50.41% inhibition) among the systemic and non systemic fungicides respectively were significantly most effective against the root rot pathogen *(Fusarium solani)* in acid lime. Also the biocontrol agent *Trichoderma harzianum* and *Allium sativum* @10 per cent; among the botanicals were significantly effective in inhibiting (60.37%) mycelial growth of *F. solani.*

Table 4. *In vitro* evaluation of botanicals against *F. solani*, an incitant of root rot of acid lime

Botanicals	Per cent inhibition of mycelial growth		
	Concentration (%)		Mean
	5%	10%	
Onion (*Allium cepa* L.)	33.55(35.37)*	49.25(44.49)	41.40(39.93)
Bougain villea (*Bougainvillea spectabilis* L.)	14.37(22.26)	38.14(38.08)	26.25(30.17)
Garlic (*Allium sativum* L.)	44.22(41.66)	60.37(50.99)	52.29(46.32)
Durantha (*Durantha repens* L.)	34.51(35.95)	49.18(44.52)	41.85(40.24)
Tridax/Deer foot (*Tridax procumbens* L.)	24.00(29.17)	31.40(34.00)	27.70(31.58)
Lantana (*Lantana camara* L.)	25.11(29.65)	38.88(38.31)	32.00(33.98)
Honge (*Pongamia pinnata* L.)	15.40(22.95)	18.29(25.14)	16.85(24.04)
Neem (*Azadirachta indica* A. Juss)	21.11(26.49)	41.33(39.99)	31.22(33.24)
Parthenium (*Parthenium hysterophorus* L.)	16.74(19.96)	18.22(25.18)	17.48(22.57)
Chilli (*Capsicum annum* L.)	21.70(27.62)	35.77(36.59)	28.74(32.10)
Mean	25.07(29.10)	38.08(37.72)	31.57(33.41)
	Botanicals (B)	Concentration(C)	B x C
S.Em± 2.35	1.05	3.32	
C.D. at 1%	9.01	4.03	12.75

* Figures in parenthesis indicate angular transformed values

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