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Efficacy of bioagents and fungicides against *Phytophthora nicotianae* infecting Crossandra

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Abstract

Phytophthora nicotianae causing a polycyclic soil borne disease infects a wide range of host plants belonging to different families all over the world. The foot and root rot disease of crossandra is a major setback for its cultivation in the country, wherever the crop is grown, causes huge loss to flower industry. Due to soil borne nature of the disease, management is a challenging process. Therefore, the bio-efficacy of the fungicides and bio-agents against *Phytophthora nicotianae* causing the root rot disease of crossandra was studeed. Ten different novel fungicides and bio-agents were screened against root rot disease under in-vitro condition. Of these, most of the systemic fungicides were found to completely inhibit radial growth of the fungal mycelium, whereas in case of bioagents, *Trichoderma harzianum* (84.19%) and *Streptomyces viridobrunneus* (81.44) was found to be most effective in reducing the mycelial growth inhibition of *Phytophthora nicotianae*.

Keywords: Crossandra, Bio-agents, *Phytophthora nicotianae*, Fungicides

Crossandra is an important ornamental flower in Southern India. It is extensively grown in Karnataka, Tamil Nadu and Andhara Pradesh states of India. The flowers are commonly used for hair adornments alone or in combination with jasmine. It is an evergreen perennial shrub bearing flowers with different colours (orange, yellow, red and bluish flowers) throughout the year, but commercially orange colour flowers of locally available crossandra which are highly susceptible to many diseases were most exploited. Of these phytophthora root rot, fusarium wilt, sclerotium rot and alternaria leaf blight are the major fungal pathogens. Phytophthora root rot usually occur after onset of monsoon and causes 50-80 percent disease incidence. The pathogen mainly infects the collar region and roots with toppling of seedlings and plants. In nursery the infection results in damping-off which is aggravated by soil moisture. In field, the plants exhibit symptoms of root rot and collar rot, which is favoured by high soil moisture in the field leading to black lesions on the main stem. Soil and infected plants debris serves as primary source of inoculums for spread of the disease under field condition. Based on the morphological characteristics, the pathogen was identified as *Phytophthora nicotianae* infecting crossandra in Karnataka (Ramachandran, 1992, 1993) and disease incidence was recorded to be 42.22 to 96.47 per cent in 1998-1999 (Ramachandran, *et al*., 2000). *P. nicotianae* is a ubiquitous pathogen causing root rot and collar rot in many horticultural crops been well documented (Erwin and Ribeiro, 1996). The pathogen was soil borne in nature and hence early infection to the root goes undetected till foliar symptoms (Lamour, *et al*., 2003) causing huge loss to the crossandra growers. The literature showed that the management of Phytophthora root rot disease using various fungicides and bioagents in different crops has been well documented (Wagner and Kaminski, 2008). Whereas, in case of crossandra, the information regarding root rot management is very scantly. In this context, the experiment was conducted to know the efficacy of new fungicides and bioagents for integrated approach and pre-planting management of Phytophthora root rot in crossandra.

MATERIALS AND METHODS

The fungal pathogen causing foot and root rot disease of crossandra was isolated from infected stem and root samples collected from crossandra experimental plot at IIHR, Bengaluru. The fungus was isolated by standard plant pathological procedures as described by Erwin and Ribeiro, (1996) on specific media amended with Pimaricin, Ampicillin, Rifamficin, Pentachloronitrobenzene (PCNB) and Hymexazol (RPARH) medium. After isolation the pathogen was incubated at 25°C. The pure culture of the fungus was further transferred to PDA plates and maintained until further use.

Morphological and Molecular characterization

The Species identification was performed with the support of tabular cues given by Waterhouse *et al*., 1963; Martin *et al*., 2012. Based on the cues, the pathogen was identified as *Phytophthora nicotianae* with following morphological characteristics that include colony growth, sporangia, oogonia, antheridia, chlamydospores, hyphal swellings and aggregations using light microscope (Nikon Eclipse 50i) at 400× magnification as for species descriptions

literature. The images were imported to ImageJ (National Institutes of Health, USA) and analyzed by setting the scale at 3.6 pixel μm^{-1}.

For molecular characterization, the pure culture of the fungus was grown on potato dextrose broth at 25 ± 2 °C for 7 days. The fungal mycelium was harvested by filtration through Whatman No.1 filter paper and washed with sterile distilled water and dried, Two grams of dried mycelium were used for total genomic DNA isolation by following modified protocol of CTAB method (Doyle and Doyle, 1990). The genomic DNA isolated was subjected to PCR amplification using universal ITS primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The PCR amplification was carried out as previously described by Sonavane and Venkataravanappa (2017). The amplified ITS region was cloned and sequenced commercially. The nucleotide sequence (ITS) analysis showed that the fungi isolated from the foot and root rot disease of crossandra is closely (> 97% identity) related to *Phytophthora nicotianae* and the consensus sequence was submitted in NCBI database under accession number MW486624.

Collection of fungal and bacterial bio-agents

The pure cultures of the bioagents were obtained from Dept. of microbiology, ICAR-Indian Institute of Horticultural Research, Bengaluru (Table 1) and were cultured in their respective media and screened Phytophthora by dual culture plate method.

Evaluation of bioagents against *P. nicotianae*

The antagonistic activity of bioagents was tested against *P. nicotianae* by following dual culture technique (Dennis and Webster, 1971) for fungal bioagents using PDA as the medium. The culture plugs (7 days old) were placed in opposite end of the petriplates and each treatment was replicated four times for each bioagent with suitable control incubated at 25±2 °C for 7 days. The radial growth of the pathogen was recorded and the percent inhibition was calculated by using following formula (Vincent, 1927).

$R = T_0 - T_1 / T_0 X 100$

R= Per cent growth reduction of test pathogen

T_0= Radial growth of test pathogen in control (mm)

T_1= Radial growth of test pathogen in treatment (mm)

The antagonistic activity of the six bacterial biocontrol agents was tested against *P. nicotianae*. A gentle superficial streak was made at one side of the sterilized petriplate on Nutrient Agar. 9 mm mycelial disc of *P. nicotianae* was placed on the opposite side of the petridish perpendicular to the bacterial streak. Three replications were maintained for each bacterial antagonists and a control was maintained by inoculating the pathogen alone containing NA medium. The plates were incubated at 25±2ÚC for seven days. The per cent reduction over control was calculated by using the formula mentioned above. In vitro evaluation of actinomycetes was also carried out by following the same method described for bacterial bioagents on Kenknight media.

***Evaluation of fungicides against P. nicotianae*:**

Twelve systemic fungicides were assayed for their efficacy against *P. nicotianae* under in vitro condition by poisoned food technique at 3 concentrations (500 ppm, 1000 ppm and 1500 ppm) (Mortan and Straube, 1955). Three replications were maintained for each concentration. Required quantity of fungicides were thoroughly mixed in PDA before pouring in 90mm sterilized petriplates and allowed to solidify. The plates were inoculated at centre with 5 mm mycelia disc of 7 days old culture of *P. nicotianae*. Control without fungicide in media was also maintained. The inoculated petriplates were incubated at 25±2°C. The colony diameters were measured after 10 days when the control plates were full of fungal growth. Per cent inhibition of growth was calculated by using formula given by Vincent (1927).

I = C-T/C X 100

I = Percent inhibition of mycelia growth

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

RESULTS AND DISCUSSION

Evaluation of bioagents against *P. nicotianae*:

Ten bioagents belonging to different groups were evaluated against *P. nicotianae* by using dual culture technique (Fig. 1 and Table 1). The results revealed that only few bioagents exhibited fungistatic/antifungal activity against *P. nicotianae* and significantly inhibited its growth, over untreated control. Of the bioagents/antagonists tested, *Trichoderma harzianum* (84.19%), *Trichoderma viride* (82.85%) and *Streptomyces viridobrunneus* (81.44%) was found most effective in mycelial growth inhibition of *Phytophthora*. Whereas none of the bacterial bioagents were found to be inhibit the mycelial growth of the pathogen. The results are in accordance with the findings of Singh and Islam, (2010) they reported that *T. harzianum* (0034H) as best treatment in its efficacy to suppress mycelial growth of *P. nicotianae* under in vitro studies infecting tobacco. Similarly *T. harzianum* and

T. viride were most effective in suppressing mycelial growth of different species of *Phytophthora* infecting citrus (Benfradj *et al*., 2016,). Further, it was also showed that *T. harzianum* and *T. viride* were most effective in reducing the growth of the *P. nicotianae* compared to *Pseudomonas flourscens* and *Bacillus subtilis* (Sharma *et al*., 2018).

Abbasi *et al.,* (2020) demonstrated the antifungal activity of *Streptomyces rochei* and *S. vinaceusdrappus* against *Phytophthora capsici* causing pepper blight. Loliam, *et al.,* 2012 reported *Streptomyces rubrolavendulae* to be most effective in inhibition mycelial growth of the *Phytophthora infestans* causing seedling damping off in tomato and chilli seedlings.

Table 1. *In vitro* studies on efficacy of Bioagents against *Phytophthora nicotianae*

Treatments	Mycelial growth Inhibition (%)
Trichoderma harzianum	84.19 (66.61)
Trichoderma viride	82.85 (65.57)
Bacillus subtillis	14.42 (22.33)
Bacillus pumilus	16.26 (23.79)
Bacillus amyloliquefaciens	17.07 (24.42)
Streptomyces viridobrunneus	81.44 (64.51)
B. aryabhattai	16.94 (24.32)
P. taiwanensis	17.32 (24.60)
Streptomyces bulli	17.42 (24.68)
Mean	38.66 (37.87)
S.Em.±	0.71
CD at 1%	2.89

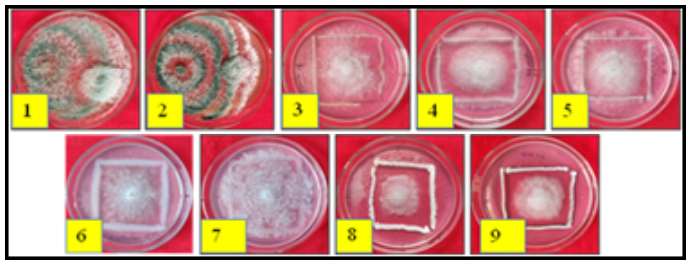

Fig. 1: *In vitro* studies on efficacy of Bioagents against *Phytophthora nicotianae*

1. *T. harzianum*, 2. *T. viride*, 3. *B. subtilis*, 4. *B. pumilus*, 5. *B. amyloliquefaciens*, 6. *B. aryabhattai*, 7. *P. taiwanensis*, 8. *S. bulli*, 9. *S. viridobrunneus*

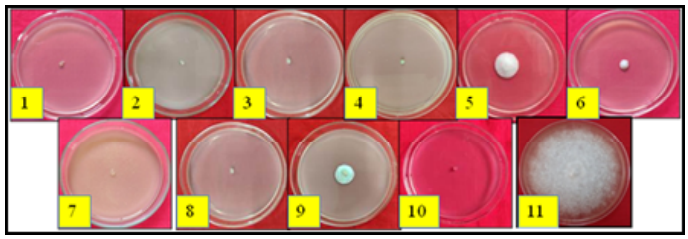

Fig. 2: *In vitro* studies on efficacy of Fungicides against *Phytophthora nicotianae*

1. Fosetyl-Al 80 WP, 2. Cymoxanil 50 WP, 3. Dimethomorph 50 WP, 4. Metiram 70 WG, 5. Chlorothalonil 75 WP, 6. Hexconazole 5 EC, 7. Mancozeb 75 WP, 8. Azoxystrobin 23 SC, 9. Captan 50 WP, 10. Krilaxyl 35 WS, 11. Control

Evaluation of different fungicides against *P. nicotianae*

A total ten systemic fungicides with three different concentrations (@ 500 ppm, 1000 ppm and 1500 ppm) were evaluated in vitro by poisoned food technique (Fig.2 and Table 2). The result showed that most of the fungicides were found to be significantly inhibited the radial growth and sporulation of *P. nicotianae*. Of these, fosetyl-Al 80 WP, cymoxanil 50 WP, dimethomorph 50 WP, metiram 70 WG,

Table 2. *In vitro* studies on efficacy of Fungicides against *Phytophthora nicotianae*

Fungicides	Percent inhibition			
	Concentration (%)			
	0.05	0.1	0.15	Mean
Fosetyl 80 WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Cymoxanil 50 WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Dimethomorph 50 WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Metiram	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Chlorothalonil 75 WP	73.09 (58.78)	75.40 (60.30)	74.69 (59.82)	74.39 (59.63)
Hexaconazole 5 EC	72.83 (74.51)	77.14 (80.30)	73.96 (75.81)	79.64 (76.87)
Mancozeb 75 WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Azoxystrobin 23 SC	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Captan 50 WP	86.23 (68.25)	82.81 (65.54)	85.51 (67.66)	84.85 (67.15)
Metalaxyl 35 WS	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Mean	95.81 (77.36)	95.50 (77.79)	95.38 (77.63)	95.35 (77.59)
	Fungicides (F)	Concentration (C)	F x C	
SEm±	16.60	4.13	0.47	
CD 1 %	2.24	1.37	3.38	

mancozeb 75 WP, azoxystrobin 23 SC and krilaxyl 35 WS were found to be the effective in completely inhibiting the radial growth of *P.nicotianae* followed by chlorothalonil 75 WP (74.39%), Hexconazole 5 EC (79.64%) and captan 50 WP (84.85%). Similarly Vawdrey, *et al*., 2015 showed that chlorothanol and meriram + pyraclostrobin combination was significantly reduced the percentage of *Phytophthora* fruit rot in papaya. Rende *et al.,* (2012) also showed that azoxystrobin and trifloxystrobin were most effective in controlling pepper phytophthora blight (80%) caused by *Phytophthora capsici*. Further the fungicidal activity of dimethomorph on mycelia growth, sporulation, sporangia and zoospore cyst germination in *Phytophthora* and *Perenospora* has been well documented (Keinath, 2007).

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