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Full Length Research Paper

Evaluation of plant extracts and bioagents for the control of gummosis of mandarin orange (*Citrus reticulata* blanko) caused by *Phytophthora* species

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A study was conducted between 2010 and 2011 at Marathwada Krishi Vidyapeeth, Parbhani, to find out the efficacy of leaf extracts *viz* Neem (*Azadirachta indica*), Mehendi (*Lowsonia inermis*), Eucalyptus (*Eucalyptus cinarium*), Acacia (*Acacia catechu*), Glyricidia (*Glyricidia sepium*), Dhatura (*Dhatura stramonium*), Lantana (*Lantana camera*) and the antagonistic potential of bioagents *viz Trichoderma viride*, *Trichoderma hamatum, Trichoderma harzianum, Trichoderma lignorum, Gliocladium virens and Pseudomonas fluorescens* against *Phytophthora* spp. under *in vitro* conditions using 'poisoned food technique' and 'dual culture technique' respectively. *L. camera* was significantly more effective in inhibiting the mycelial growth (44.54%) of *Phytophthora citrophthora* and *D. stramonium* against *Phytophthora nicotianae*, inhibiting mycelial growth (57.78%), both at 5% concentration; whereas, among bioagents, *T. harzianum* was found significantly effective against both species inhibiting 84% mycelial growth of *P. citrophthora* and 91.86% of *P. nicotianae*.

Key words: Leaf extracts, bioagents, antifungal activity, gummosis, *Phytophthora*, mandarin orange.

INTRODUCTION

Mandarin orange (*Citrus reticulata* Blanko) is the chief commercial citrus cultivar in India (Singh, 2009). It is a major source of vitamin C. It shares about 65% of all citrus fruit produced in the country (Anonymous, 2003). In India, the area under mandarin orange is 214.8 thousand ha with production of 1443.1 thousand tonnes and productivity is 6.7 t/ha (Anonymous, 2009). In Maharashtra state, Vidarbha, Marathwada and Western Maharashtra are the major mandarin growing regions with an estimated production of 710.4 hundred tonnes of fruits and area under cultivation is 103.5 thousand h. (Anonymous, 2006). Over the years, some of the traditional citrus growing areas of India have shown steady decline in their harvest due to heavy infestation of diseases. Mandarin orange is known to be affected by many plant pathogens of which few are having economic importance. Among the major fungal diseases, gummosis caused by *Phytophthora* species has been recorded as a major constraint to sustain optimum production, and reduces 46% yield of plants annually (Menge, 1993). It is responsible for 10 to 30% of losses in citrus culture around the world (Timmer et al., 2000). Mortality up to 62.5% was reported in seedling of Coorg mandarin (Prasad and Rao, 1983). Like many other soil borne diseases, gummosis of mandarin orange is difficult to manage. Although several chemical fungicides are available for the management of plant diseases, a continuous, inappropriate, non-discriminative use of chemicals causes undesirable effects. Hence, attention is now being focused on developing environmentally safe, economic and effective alternatives for the management of plant diseases. Bioagents, of late, have been known to induce systemic resistance against several plant diseases. In view of this, some plant species and antagonistic bioagents were tested for the effective management of gummosis of mandarin orange.

MATERIALS AND METHODS

Source of the pathogen

The experiment was conducted between 2010 and 2011 at

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Table 1. *In vitro* effect of different botanicals on radial growth of *Phytophthora citrophthora*.

Common name	Scientific name	Colony diameter (mm) at 5% concentration	% inhibition
Neem	*Azadirachta indica*	34 (36.66)	10.92
Glyricidia	*Glyricidia maculata*	32 (34.44)	16.17
Lantana	*Lantana camera*	21.17 (27.37)	44.54
Eucalyptus	*Eucalyptus cinarium*	34.50 (35.94)	9.61
Acacia	*Acacia catechu*	33.67 (35.46)	11.79
Dhatura	*Dhatura stramonium*	33.00 (35.05)	13.55
Mehendi	*Lowsonia inermis*	32.83 (34.95)	13.99
Control	--	38.17 (38.14)	--
SE+		1.20	
CD at 5%		3.50	

Figures in parenthesis shows the angular transformed values.

the Department of Plant Pathology, College of Agriculture, Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra state. The experiment was carried out by using completely randomised design suggested by Panse and Sukhatme (1964) with three replication of each treatment. The trials were conducted separately for botanicals and bioagents. The diseased leaf samples were collected from different districts of Maharashtra and the pathogen were isolated on medium.

Determination of antifungal properties of the extracts and bioagents

Efficacy of leaf extracts *viz* Neem (*Azadirachta indica*), Mehendi (*Lowsonia inermis*), Eucalyptus (*Eucalyptus cinarium*), Acacia (*Acacia catechu*), Glyricidia (*Glyricidia sepium*), Dhatura (*Dhatura stramonium*), Lantana (*Lantana camera*) and the antagonistic potential of bioagents *viz Trichoderma viride*, *Trichoderma hamatum, Trichoderma harzianum, Trichoderma lignorum, Gliocladium virens and Pseudomonas fluorescens* against *Phytophthora* spp. under *in vitro* conditions were studied using 'poisoned food technique' and 'dual culture technique', respectively. For preparation of plant extracts, healthy and disease free leaf sample of selected plant species were brought to the laboratory and washed with sterile distilled water and then chopped into small bits with sterilized sharp knife. Each leaf sample of 100 g was separately ground and homogenized in mechanical grinder with equal quantity of sterile distilled water (1:1, w:v). The homogenate obtained was then strained though double layered muslin cloth and the filtrate was collected and filtered through Whatman No.1 filter paper using volumetric flasks (50 ml capacity). The clear leaf extracts obtained from the stock solution was 100%. An appropriate quantity of each leaf extract was incorporated separately in the molten and cooled potato dextrose agar (PDA) medium in conical flask (250 ml capacity) to get desired concentrations (5%) of each extract and autoclaved at 15 lbs pressure for 15 to 20 min. Plant extracts amended PDA was then poured (15 to 20 ml/plates) in sterilized Petri plates (90 mm diameter) under aseptic conditions. Three plates / treatment were maintained and each treatment with respective concentration was replicated thrice. On solidification of PDA in Petri plates, all treatment plates were inoculated or seeded aseptically by placing in the center with 5.0 mm uniform mycelial disc obtained form 7 days old culture of *Phytophthora* spp. multiplied on agar plates. Petri plates containing plain PDA without any plant extract were inoculated with 5 mm disc of the test pathogen and maintained as suitable untreated control. All the treatment (inoculated) and control Petri plates were then incubated at 28±2°C in BOD incubator for 7 days. The observations on area covered by pathogen were taken after 7 days and percent inhibition was calculated.

For evaluation of bioagents, autoclaved potato dextrose agar (PDA) was used as basal medium on which 5 mm mycelial disc of *Phytophthora* spp. and bio-agent were inoculated equidistantly from center and exactly opposite to each other. The Petri dishes were incubated at 28±1°C. The plates containing basal medium with disc of *Phytophthora* spp. placed at the centre served as control. The treatments were replicated thrice. Colony diameter in each treatment was measured in mm after control plates were fully covered with mycelial growth of the test pathogen and per cent growth inhibition of the test pathogen over control was worked out (Vincent, 1927) as follows:

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

Where C = growth of test fungus (mm) in control plate; T = growth of test fungus (mm) in treatment plates

Statistical analysis

The data obtained in the experiment (*in-vitro*) was statistically analyzed. The standard error (SE) and critical difference (CD) at P = 0.05 was worked out and results obtained was compared statistically.

RESULTS AND DISCUSSION

The results in Table 1 and Figure 1 indicate that all the botanicals tested were found inhibitory and caused significant inhibition of mycelial growth of *Phytophthora citrophthora* over untreated control.

Amongst seven aqueous leaf extracts tested (at 5% concentration), Lantana recorded least mean colony diameter (21.17 mm) and highest mean mycelial growth inhibition (44.54%). Glyricidia ranked second, followed by Mehendi, Dhatura, Acacia, Neem, and Eucalyptus in inhibiting the *P. citrophthora*. The mean colony diameter recorded by Glyricidia, Mehendi, Dhatura, Acacia, Neem,

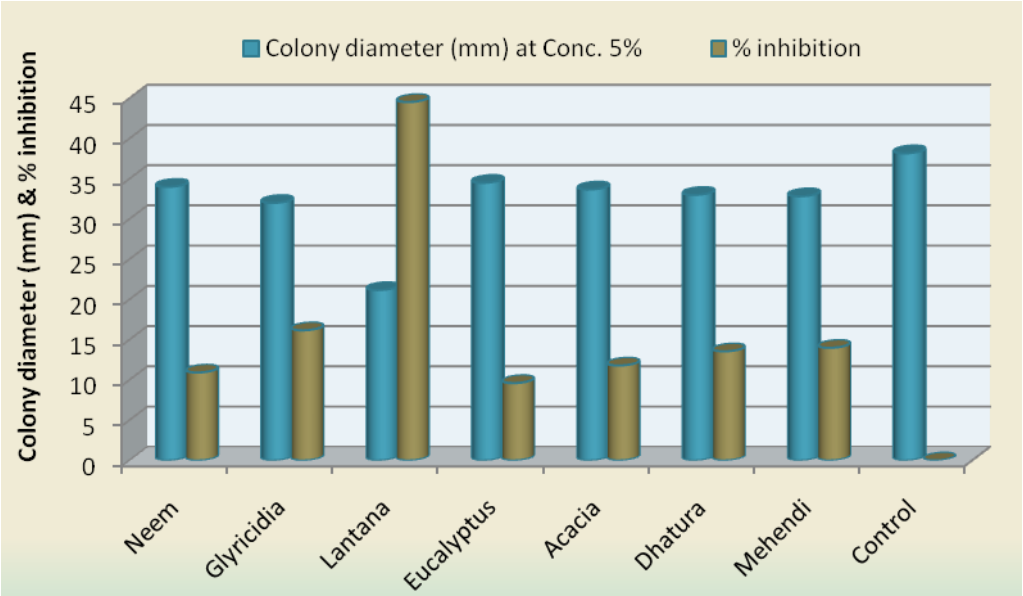

Figure 1. *In vitro* effect of different botanicals on radial growth of *Phytophthora nicotianae*.

Table 2. *In vitro* effect of different botanicals on radial growth of *Phytophthora nicotianae*.

Common name	Scientific name	Colony diameter (mm) at 5% concentration	% inhibition
Neem	*Azadirachta indica*	87 (68.86)	3.33
Glyricidia	*Glyricidia maculate*	81 (64.24)	10.00
Lantana	*Lantana camera*	74 (59.79)	18.00
Eucalyptus	*Eucalyptus cinarium*	84 (67.08)	6.67
Acacia	*Acacia catechu*	45 (42.41)	50.00
Dhatura	*Dhatura stramonium*	38 (38.54)	57.78
Mehendi	*Lowsonia inermis*	80 (63.42)	11.11
Control	-	90 (71.56)	--
SE±		1.09	
CD at 5%		3.2	

Figures in parenthesis shows the angular transformed values.

and Eucalyptus were 32, 32.83, 33, 33.67, 34, and 34.50 mm, and mean mycelial growth inhibition were 16.17, 13.99, 13.55, 11.79, 10.92, and 9.61% respectively. Eucalyptus was found least effective and caused mycelial inhibition in about 9.61%. Statistically, the treatment of Lantana was significantly superior over the other botanicals and control whereas all other treatments were at par with each other.

Table 2 and Figure 2 indicate that all the botanicals tested against *Phytophthora nicotianae* were found inhibitory and caused significant inhibition of mycelial growth of pathogen over control. Amongst seven aqueous leaf extracts tested (at 5% concentration), Dhatura recorded least mean colony diameter (38 mm) and highest mean mycelial growth inhibition (57.78%). This was followed by Acacia, Lantana, Mehendi, Glyricidia, Eucalyptus, and Neem which recorded mean colony diameter of 45, 74, 80, 81, 84, and 87 mm and mean mycelial growth inhibition of 50, 18, 11.11, 10, 6.67, and 3.33% respectively. Neem was found least effective and caused minimum inhibition (3.33%) of the test pathogen. Statistically, Dhatura was significantly superior over all other botanicals and control. Acacia, Lantana, and Mehendi significantly differed from each other. Mehendi and Glyricidia were statistically at par. Glyricidia and Eucalyptus were also at par with each other. Eucalyptus and Neem were at par with each other but Mehendi and Glyricidia were variably significant over Neem. Jadeja (2003) found turmeric, garlic, Dhatura, parthenium and Neem as effective phytoextracts against the fungal growth of *Phomopsis citri*. Karegowda et al. (2009) studied the effect of plant leaf extracts (10%) of 25

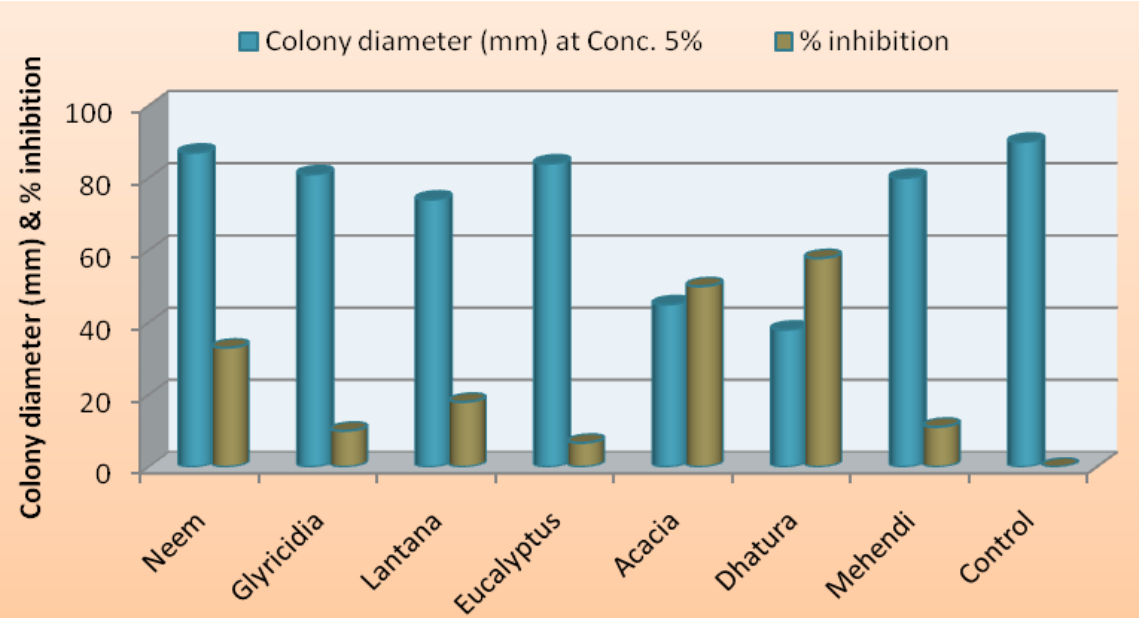

Figure 2. *In vitro* effect of different botanicals on radial growth of *Phytophthora nicotianae*.

Table 3. *In vitro* evaluation of different bioagents on radial growth of *Phytophthora citrophthora*.

Bioagents	Area covered (cm^2)		% inhibition
	Pathogen	Antagonists	
Trichoderma viride	5.42 (13.37)	58.22	79
Trichoderma hamatum	4.71 (12.56)	58.93	81.75
Trichoderma harzianum	4.13 (11.57)	59.51	84
Trichoderma lignorum	5.42 (13.43)	58.22	79
Gliocladium virens	6.84 (14.88)	55	73.50
Pseudomonas fluorescens	4.71 (12.23)	58.93	81.75
Control	25.81(30.53)	--	--
SE±	1.06		
CD at 5%	3.15		

Figures in parenthesis shows the angular transformed values.

plant *species* on *Phytophthora parasitica* var. *nicotianae*, the causal agent of black shank disease of tobacco *in vitro*. They reported that the *A. indica*, *D. innoxia*, and *L. camera* showed 18, 35.3, and 62.7% inhibition as compare to control, respectively. A similar result was found by Al-Azeez and Nezam (2009) and Yanar et al. (2011).

In case of bioagents, the data from Table 3 and Figure 3 indicate that all the bioagents tested were inhibitory to *P. citrophthora* and significantly superior over untreated control.

Amongst bioagents tested, *Trichoderma harzianum* grew fast and caused 84% inhibition of mycelial growth of pathogen which was followed by *Pseudomonas fluorescens* (81.75%), *Trichoderma hamatum* (81.75%), *Trichoderma viride* (79%), *Trichoderma lignorum* (79%), and *Gliocladium virens* (73.50%). Significantly least area covered by pathogen against bioagents was found in *T. harzianum*, about 4.13 cm^2, followed by *P. fluorescens* and *T. hamatum* which ranked second in least area covered (71 cm^2), *T. viride* (5.42 cm^2), *T. lignorum* (5.42 cm^2), and *G. virens* (6.84 cm^2). Statistical analysis revealed that all treatments were at par with each other, that is, *P. fluorescence* and *T. hamatum*, and *T. viride and T. lignorum* showed the same activity against the pathogen.

Table 4 and Figure 4 reveal that all the bioagents tested were inhibitory to *P. nicotianae* and were significantly superior over control.

Amongst the six bioagents evaluated, *T. harzianum*

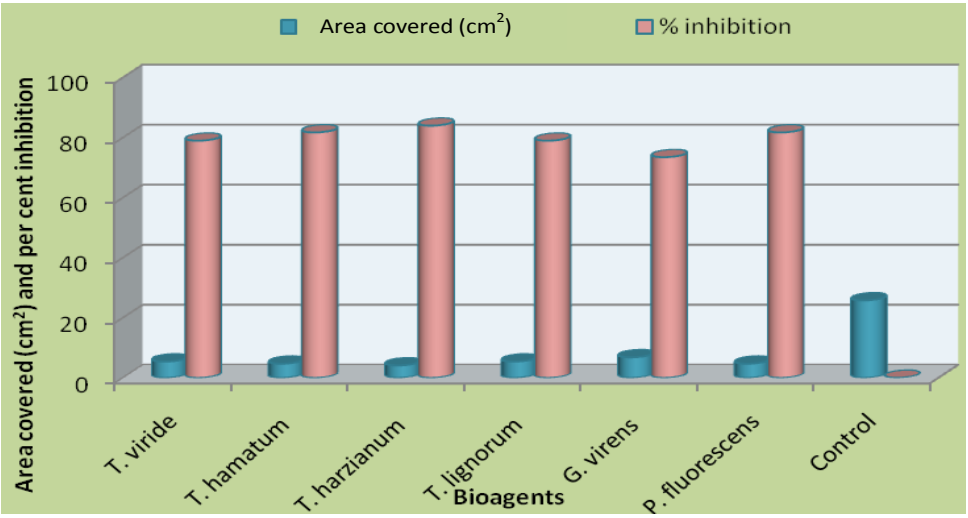

Figure 3. *In vitro* effect of different bioagents on radial growth of *Phytophthora citrophthora*.

Table 4. *In vitro* evaluation of different bioagents on radial growth of *Phytophthora nicotianae*.

Bioagents	Area covered (cm^2)		% inhibition
	Pathogen	Antagonists	
Trichoderma viride	11.90 (20.17)	51.74	81.30
Trichoderma hamatum	9.07(17.53)	54.47	85.75
Trichoderma harzianum	5.18(13.16)	58.46	91.86
Trichoderma lignorum	12.14(20.39)	51.5	80.92
Gliocladium virens	12.72 (20.90)	50.92	80.01
Pseudomonas fluorescens	11.37(19.65)	52.27	82.23
Control	63.64 (52.91)	--	--
SE±	0.26		
CD at 5%	0.74		

Figures in parenthesis shows the angular transformed values.

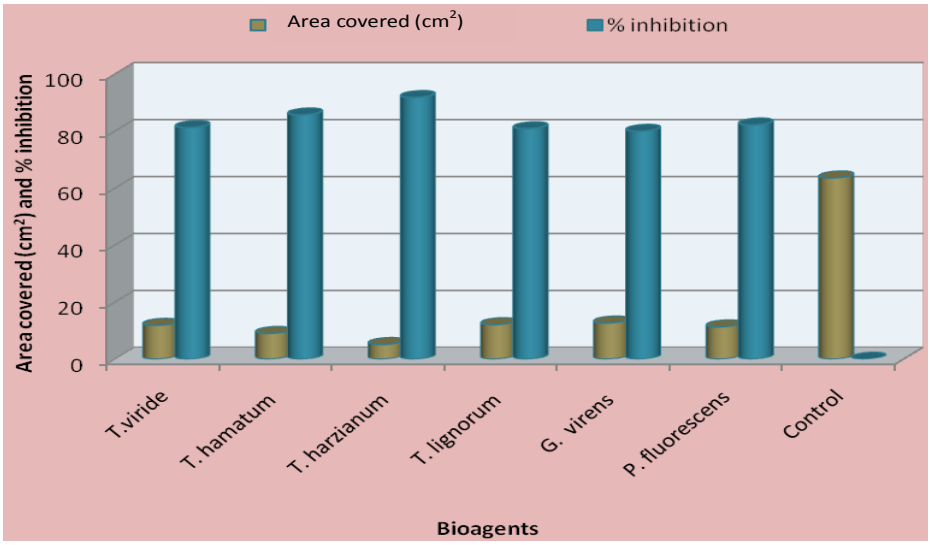

Figure 4. *In vitro* effect of different bioagents on radial growth of *Phytophthora citrophthora*.

grew fast and caused 91.86% inhibition of mycelial growth of pathogen which was followed by *T.hamatum* (85.75%), *P. fluorescens* (82.23%), *T. viride* (81.30%), *T. lignorum* (80.92%), and *G. virens* (80.01%). Significantly least area covered by pathogen against bioagents was recorded in *T. harzianum*, about 5.18 mm, followed by *T. hamatum* (9.07 cm^2), *P. fluorescens* (11.37 cm^2), *T. viride* (11.90 cm^2), *T. lignorum* (12.14 cm^2) and *G. virens* (12.72 cm^2).

Statistically, *T. harzianum* was found significantly superior over all other treatment. The treatment of *T. hamatum* and *P. fluorescens* significantly differed from each other. *P. fluorescens, T. viride, T. lignorum,* and *G. virens* were at par with each other. *P. fluorescens* and *T. viride* were found significantly different from *G. virens*.

The antagonism of *Trichoderma* spp. against many fungi is mainly due to production of acetaldehyde (Dennis and Webster, 1971). This may also be the reason for antagonistic effect of *Trichoderma* against *Phytophthora* spp. Casale (1990) postulated hyperparasitism as the mode of action of *Trichoderma*, as *Trichoderma* coiled around hyphae of *P. cinnamomi*. Subramanyam (1993) reported that both *T*. *viride* and *T. harzianum* overgrew and suppressed the growth of *P*. *capsici*. Karegowda et al. (2009) found that in dual cultures, *T. harzianum* engulfed the test fungus within 7 days of incubation thus indicating that the bioagent used in the study was highly antagonistic to pathogen. Similar results were found in the present investigation against *P. citrophthora* and *P. nicotianae*. Findings of the present investigation are in agreement with several investigators who observed the significant reduction of the growth of anthracnose pathogen *Colletotrichum dematium* in different crops in the presence of *T. harzianum* (Moon et al., 1988; Sundar et al., 1995; D'Souza et al., 2001; Rajathilagam and Kannabiran, 2001).

Conclusions

The efficacy of the individual component of an integrated measure against the target pathogen should be clearly understood to develop an integrated control strategy. The results of the current laboratory evaluation of plant extracts and *Trichoderma* isolates against *Phytophthora* spp. of mandarin orange will fulfill the prerequisite criteria for the selection of appropriate dose of plant extract and specific antagonist fungal isolate to develop an eco-friendly integrated disease management of causing root rot, collar rot and gummosis of mandarin orange in the field.

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