In vitro Biological Management of Fusarium Wilt of Patchouli Caused By *Fusarium solani*

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SUMMARY

Patchouli is an important aromatic plant used as a low calorie sweetener. Fusarium wilt caused by *Fusarium solani* is an important disease and is a major constraint in patchouli cultivation. Evaluation of the biocontrol agents indicated that maximum inhibition of mycelial growth of *F. solani* (78.11%) was noticed in *Trichoderma harzianum* (Dharwad isolate). Among botanicals tested neem seed kernel extract was more effective (74.86%) inhibiting the growth of *F. solani*.

Key words : Patchouli, *Fusarium solani*, *Trichoderma harzianum*, Neem seed kernel extract

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Patchouli (*Pogostimon patchouli* Pellet.) belonging to family Lamiaceae, is a hardy perennial herb adapted to hot and humid climatic conditions (Maheshwari *et al.*,1993). A commercially important aromatic patchouli oil is extracted from the leaves of this plant. Patchouli oil is a natural fixative used in the aromatic industry. It is well known to blend with other essential oils like vetiver, sandalwood, geranium and lavender etc. It is used in a wide range of toilet soaps, scents, body lotions etc. Patchouli is attacked by many pathogens causing wilts, blight and root knot. The wilt caused by *Fusarium solani* (Mart.) Sacc. was found predominant in Karnataka. Fungicidal sprays are generally recommended for the control of this disease. But extensive use of chemicals leads to serious environmental problems, development of resistance and it may also affect the quality of the crop as many people consume it. Therefore, it becomes necessary to look for economically better and safer means of disease control.

MATERIALS AND METHODS

In vitro evaluation of plant extracts:

Fresh plant materials were collected and washed first in tap water and then in distilled water. One hundred g 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.

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1982). Five and ten ml of stock solution was mixed with 95 and 90 ml of sterilized molten Potato dextrose agar(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 Petriplates. Mycelial discs (five mm) of *Fusarium solani* were cut out by sterile cork borer and one such disc was placed at the centre of each agar plate. Eleven plant extracts *viz.*, *Azadirachta indica* A. Juss, *Allium sativum* L., *Bougainvillea spectabilis* L., *Cassia occidentalis* L., *Clerodendron inerme* Gaerth. *Durantha repens* L., *Eucalyptus globes* Labill, *Glyricidia maculata* L., *Parthenium hysterophorus* L., *Pongemia pinnata* L., *Ocimum sanctum* L. were evaluated at 5 and 10% concentrations. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27±1^{0}C and radial growth was taken when maximum growth occurred in control plates. The efficacies were expressed as per cent inhibition, which was calculated by using the formula suggested 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

In vitro evaluation of biocontrol agents:

Six biocontrol agents such as *Trichoderma harzianum* Rifai, *Trichoderma koningii* Oudern, *Trichoderma virens* Miller, *Trichoderma viride*, Pers. ex S.F.Gray, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn emend Prers were tested against *Fusarium solani*. Both biocontrol agents and test fungus were cultured on Potato dextrose agar in order to get fresh and active growth of fungus. The cultures of antagonistic microorganisms used in the present study were obtained from the Project Directorate of Biological Control (PDBC) Bangalore and native isolate of Dharwad, Karnataka state.

Twenty ml of sterilised and cooled Potato dextrose agar was poured into sterile Petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, a mycelial disc of test fungus was inoculated at one end of the Petriplate 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 Petriplates and mycelial discs of the fungus was placed at the centre. The plates were incubated at 27±1^{0}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 given by Vincent (1947).

Table 1 : Effect of plant extracts on inhibition of mycelial growth of *Fusarium solani*

Plant extracts	Per cent inhibition of mycelial growth		
	Concentration (%)		Mean
	5	10	
Bougainvillea	31.96	39.16	35.57
	(34.44)*	(38.76)	(36.60)
Cassia	19.16	20.77	19.97
	(25.98)	(27.12)	(26.54)
Clerodendron	32.16	46.13	39.15
	(34.56)	(42.80)	(38.66)
Durantha	28.46	34.10	31.28
	(35.26)	(35.74)	(34.00)
Garlic	56.50	57.36	56.93
	(48.76)	(49.26)	(49.02)
Eucalyptus	48.50	52.86	50.68
	(44.17)	(46.66)	(45.42)
Glyricidia	20.43	31.93	26.18
	(26.88)	(34.43)	(30.65)
Neem seed kernel	62.00	74.86	68.43
extract	(51.97)	(59.94)	(55.95)
Parthenium	43.00	52.73	47.81
	(40.99)	(46.59)	(43.79)
Pongamia	30.40	45.43	37.92
	(33.47)	(42.40)	37.93)
Tulsi	35.06	43.53	39.30
	(36.33)	(41.30)	(38.81)
Mean control	37.06	45.35	
	(34.21)	(40.47)	
	Botanicals	Concentration	B x C
	(B)	(C)	
S.E.±	0.14	0.05	0.19
C.D. (P=0.01)	0.53	0.19	0.72

* Figures in parenthesis indicate arc sin transformed values

RESULTS AND DISCUSSION

In vitro evaluation of plant extracts:

In the present study, all the plant extracts tested at both the concentrations were significantly effective in inhibiting the growth of *Fusarium solani*. Irrespective of the concentrations, neem seed kernel extract proved to be the most effective botanical and recorded the maximum inhibition of growth (68.43%) which was significantly superior to all other plant extracts. Garlic (56.93%) and eucalyptus (50.68%) were next best followed by parthenium (47.81%). Tulsi (39.30%) and clerodendron (39.15%) were at par to each other extracts in order of supenoxty in comparison to control were pongamia (37.92%), bougainvillea (35.57%) and durantha (31.28%) which were which different significantly to each other. Least inhibition was observed with glyricidia (19.97%).

Among the two concentrations, the plant extracts at 10 per cent were significantly superior to five per cent. In the interaction between plant extract and concentration, neem seed kernel extract showed significant increase in inhibition of mycelial growth at 10 per cent concentration (74.86%) compared to 5 per cent concentration (62.00%) followed by garlic (57.36%) and eucalyptus (52.86%) and which were significant from each other. Glyricidia at five per cent (20.43%) and cassia at five per cent (19.16) were least effective in inhibiting the mycelial growth of *F. solani*.

These reports are in consonance with earlier workers who have reported the fungitoxic properties of plants like neem seed kernel extract and eucalyptus against *F. oxysporum* in gladiolus (Sumitra, 2006), *Allium sativum* against *F. solani* in ginger and turmeric (Shalini, 2006).

In vitro evaluation of bioagents:

Among the six bioagents evaluated against *F. solani*,

Table 2 : Effect of biocontrol agents on inhibition of mycelial growth of *Fusarium solani*

Biocontrol agents	Per cent inhibition of mycelial growth
Bacillus subtilis	57.02 (49.06)*
Pseudomonas fluorescens	65.10 (53.82)
Trichoderma harzianum (Dharwad isolate)	78.11 (62.13)
Trichoderma koningii	75.63 (60.45)
Trichoderma virens	70.16 (56.92)
Trichoderma viride	74.92 (59.83)
S. E.±	0.28
C.D. (P=0.01)	1.16

* Figures in parenthesis indicate arc sin transformed values

Trichoderma harzianum (Dharwad isolate) inhibited maximum mycelial growth (78.11%) followed by *T. konigii* (75.63%) which was significantly superior to all other bioagents tested. Next best was T. *viride* (74.92%) being at par to *T. koningii*. However, *B. subtilis* (57.02%) and *Pseudomonas fluorescens* (65.10%) were least effective in inhibiting mycelial growth of the pathogen. Results are in accordance with Kempf and Wolf (1989), Sivan and Chet (1986) and Mishra *et al.* (2004).

The results of dual culture technique on *Fusarium solani* revealed that all the five fungal antagonists significantly reduced the growth of *Fusarium solani* either by over growing or by exhibiting inhibition zones, except the bacterial bioagents. *T. harzianum* was more effective antagonist which in addition to secreting antibiotic non-volatile compounds, also causes rapid death of the pathogen by mycoparasitism and lysis and could be therefore selected as a potential biocontrol agent against the pathogen. (Robinson and Park, 1966; Dennis and Webster, 1971).

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