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In vitro Evaluation of Microbial Antagonists, Botanicals and Fungicides Against *Phytophthora capsici* Leon. the Causal Agent of Foot Rot of Black Pepper*

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Abstract: Among the antagonists, *Trichoderma* isolates were most effective in inhibiting the growth of *P. capsici.* Least inhibition was noticed with *Pseudomonas* isolate followed by *Bacillus* sp. Among the ten plant extracts tested, garlic cloves extract was highly inhibitory to *P. capsici* and was followed by leaf extracts of duranta, eupatorium, neem and lantana. The least effective was tikki weed followed by adusoge. Out of ten fungicides tested *in vitro* Akomin, Melody duo, Ridomil and Secure at all three concentrations (0.1%, 0.2 % and 0.3 %), Aliette and Bordeaux mixture 0.3 per cent concentration were also highly inhibitory to the pathogen *P. capsici.* The least effective was Verita followed by Copper oxychloride.

Key words : Microbial antagonists, botanicals, fungicides, foot rot, black pepper

Introduction

Black pepper (*Piper nigrum* L.) the king of spices is a traditional, historic spice crop which has been under cultivation since ancient times in India. Black pepper is a woody climber and is a native of the Western Ghats of South India. The cultivation of black pepper is mainly confined to India, Brazil, Indonesia, Malaysia, Thailand, Sri Lanka and Vietnam. In India black pepper is being cultivated in Kerala (96%), Karnataka (3%) and to a lesser extent, in Maharashtra, Andhra Pradesh, Tamil Nadu and North Eastern regions (Anonymous, 2005).

In India, black pepper is being cultivated in an area of 2.2 lakh ha with a production of 70,000 tonnes. During 2005-06 India exported 28,750 tonnes of black pepper amounting to Rs 306.2 crores. In Karnataka, black pepper is cultivated in an area of 10,690 ha with a production of 2360 tonnes during 2005-06 (Anonymous, 2005). It is mainly cultivated in Kodagu, Uttara Kannada, Dakshina Kannada, Shimoga and Chikmagalore districts as mixed crop in plantations such as arecanut, cardamom, coffee and rubber whereas in Kerala it is being grown as pure a crop trained on *Erythrina indica* Lam. K. Indian black pepper is preferred in the international market due to its proper combination of pleasant flavour, taste, piperine content and essential oil. Black pepper is not only used as a condiment but also, widely used in culinary preparations, food processing, perfumery and as an important ingredient in most of the Ayurvedic medicine preparations.

In Uttara Kannada which is situated in upper region of Western Ghats, black pepper is mainly cultivated in moist valleys in arecanut plantations wherein arecanut trees serve as live standards for training black pepper. Due to epiphytotic appearance of *Phytophthora* foot rot disease of black pepper most of the gardens have been wiped out since 1978 (Sastry, 1982 and Dutta, 1984). A serious and most destructive disease of foot rot of back pepper caused by *Phytophthora capsici* was gaining most importance. Since, the pathogen is soil borne an attempt was made to evaluate antagonistic microorganism, botanicals and fungicides against *P. capsici* under laboratory condition.

Material and Methods

In vitro evaluation of native antagonistic microorganisms : Isolated fungal antagonists were evaluated by dual culture technique. The pathogen was inoculated on one side of the Petri plate filled with 20 ml of PDA and antagonist were inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing five days old cultures were used. In case of bacterial antagonist evaluation, bacterial antagonists were streaked in the plates and fungal discs were placed at one corner of the plates. After a period of incubation, when the growth in control plate reached maximum (90 mm diameter), the radial growth of the pathogen was measured. Per cent inhibition over control was worked out according to the equation given by Vincent (1927).

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

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

In vitro evaluation of botanicals: Aqueous leaf extracts of some of the plant species were used to study their efficacy to inhibit the growth of *Phytophthora capsici.* A known quantity of leaf samples was crushed by adding equal quantity of sterilized distilled water separately. Garlic clove extract was also prepared by the same way. The extracts were added with streptomycin sulphate to avoid bacterial contamination.

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List of native antagonistic microorganisms tested against *Phytophthora capsici* were as follows

Sl. No.	Antagonistic microorganisms
1	*Bacillus* sp. (Siddapur isolate)
2	*Pseudomonas* sp. (Siddapur isolate)
3	*Pseudomonas* sp. (Yellapur isolate)
4	*Pseudomonas* sp (Edahalli isolate)
5	*Trichoderma* sp. (Gudnapur isolate)
6	*Trichoderma* sp. (Siddapur isolate)
7	*Trichoderma* sp. (Yellapur isolate)
8	*Trichoderma* sp. (Mundigesara isolate)
9	*Trichoderma* sp. (Edahalli isolate)
10	*Trichoderma harzianum* (ARS, Sirsi)
11	*Trichoderma harzianum* (UAS, Dharwad)

Required quantity of individual botanicals was added separately into molten and cooled potato dextrose agar so as to get the desired concentrations of 5 or 10 per cent. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates and the mycelium disk of 5 mm size from five days old cultures of *P. capsici* was cut out by a sterilized cork borer and one such disc was placed at the center of each agar plate. Control was maintained without adding any botanicals to medium. Four replications were maintained for each concentration and plates were incubated at room temperature for seven days and radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the plant extracts was expressed as percent inhibition of mycelial growth over control, which was calculated by using the formula of Vincent (1927) as stated earlier.

The plant species selected for the evaluation of leaf extract for their efficacy to inhibit the growth of fungus were given below:

Sl. No.	Common name	Scientific name	Plant parts used
1	Adusoge	*Adathoda vesica* Nees	Leaf
2	Clerodendron	*Clerodendron inerme* G	Leaf
3	Duranta	*Duranta plumeri* Jacq.	Leaf
4	Eupatorium	*Chromolaena odorata* King.	Leaf
5	Garlic (cloves)	*Allium sativum* Linn.	Cloves
6	Glyricidia	*Glyricidia maculata* L.	Leaf
7	Lokkisoppu	*Vitex nigundo* L.	Leaf
8	Lantana	*Lantana camera* L.	Leaf
9	Neem	*Azadirachta indica* A.Juss	Leaf
10	Tridax	*Tridax procumbens* Linn.	Leaf

In vitro evaluation of fungicides: The efficacy of nine fungicides at the concentrations of 0.1, 0.2 and 0.3 per cent was assayed *in vitro* using poisoned food technique (Falck, 1907). The list of fungicides evaluated are given below.

Required quantity of individual fungicide was added separately into molten and cooled potato dextrose agar so as to get the desired concentrations of the fungicides. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates.

	Fungicides	
Sl. No.	Trade name / Common name	Chemical name
1	Akomin	Potassium phosphonate
2	Aliette 80 WP	Fosetyl-Al 80% WP
3	Bordeaux mixture	Copper sulphate + Calcium hydroxide
4	Blitox 50% WP	Copper oxychloride
5	Melody duo 66.75 WP	Iprovalicarb 5.5% + Propineb 61.25%
6	Profiler 71.04 WDG	Fluopicolide – 4.44% + Fosetyl – 66.66% WDG
7	Ridomil MZ 72 WP	Metalaxyl 8% + Mancozeb 64% WP
8	Secure 60 WDG	Fenamidone 10% + Mancozeb 50%
9	Verita 71 WDG	Fenamidone 4.44% + Fosetyl 66.66%

Mycelium disk of five mm size from five days old cultures of *P. capsici* was cut out by a sterilized cork borer and one such disc was placed at the center of each agar plate. Control treatment was maintained without adding any fungicide to the medium. Three replications were maintained for each concentration. After incubation for nine days at room temperature, radial growth was measured when fungus attained maximum growth in control plates. The efficacies of the fungicides were expressed as percent inhibition of mycelial growth over control, which was calculated by using the formula of Vincent (1927) as stated earlier.

Results and Discussion

In vitro evaluation of antagonistic micro organisms against P. capsici : Per cent mycelial inhibition of *P. capsici* under *in vitro* condition ranged from 2.02 to 58.41 per cent. Significant with highest inhibition of radial growth of mycelia of *P. capsici* was recorded with *Trichoderma* sp. of Mundigesara isolate T_8 (58.41%) which was on par with (T_7) *Trichoderma* sp. of Yellapur isolate (56.76%). *Trichoderma* sp. of Gudnapur (52.71%), Edahalli (52.27%) and Siddapur (50.53%) have also recorded a considerable level of inhibition of mycelial growth of *P. capsici* which were on par with each other.

Trichoderma harzianum from Agricultural Research Station (Pepper)(ARS), Sirsi (with inhibition 49.02%) and *T. harzianum* from University of Agricultural Sciences, Dharwad (UASD) (48.38%) were also significantly superior to control and were on par with each other. Least inhibition of mycelial growth of *P. capsici* was recorded in *Pseudomonas* sp. of Yellapur isolate (2.02%). Among bacterial antagonists, highest mycelial inhibition was recorded in *P. fluorescens* of Edahalli village (22.92%) followed by *Pseudomonas* sp. (Siddapur) (21.09%) which were on par with each other (Table 1).

In vitro evaluation of botanicals against P. capsici : Among the ten plant extracts tested, highest per cent mycelial inhibition of *P. capsici* was observed in garlic cloves extract at five per cent concentration (28.87%), followed by lantana (27.33%), eupatorium (25.47%) and neem (24.07%). Plant extracts of Duranta (18.06%) and Lakkisoppu (18.40%), Adusoge (7.73%) and Glyricidia (6.89%) were at par with each other in relation to

Table 1. *In vitro* evaluation of native microbial antagonists against *Phytophthora capsici*

Sl. No.	Microbial antagonists	Per cent mycelial inhibition
1	*Bacillus* sp. (Siddapur)	9.62(18.06)
2	*Pseudomonas* sp. (Siddapur)	12.96(21.09)
3	*Pseudomonas* sp. (Yellapur)	0.37(2.02)
4	*Pseudomonas fluorescens* (Edahalli)	15.19(22.92)
5	*Trichoderma* sp.(Gudnapur)	63.33(52.71)
6	*Trichoderma* sp. (Siddapur)	59.62(50.53)
7	*Trichoderma* sp.(Yellapur)	69.99(56.76)
8	*Trichoderma* sp.(Mundigesara)	72.59(58.41)
9	*Trichoderma* sp. (Edahalli)	62.59(52.27)
10	*Trichoderma harzianum* (ARS, Sirsi)	57.04(49.02)
11	*Trichoderma harzianum* (UASD)	55.92(48.38)
	S.Em. ±	0.659
	CD at 1%	2.608

* Figures in parenthesis are arcsine transformed values

relation to inhibition of *P. capsici* and no inhibitory effect was recorded in Tikki weed at five per cent concentration.

At higher concentration (10%) also, cloves extract of garlic recorded highest inhibition of radial growth of mycelia (36.58%) and was followed by duranta (31.07%), eupatorium (30.83%), neem (30.10) and lantana (30.09%) which were on par with each other. Least mycelial inhibition was recorded in Adusoge (21.09%) and Tikki weed (22.61%). Though all the plant extracts recorded the inhibition of radial growth of mycelia of *P. capsici* to certain extent, their inhibitory effect on radial growth of mycelia increased with increase in concentration. Garlic cloves extract recorded highest inhibition at both the concentration and found most effective (Table 2).

Efficacy of fungicides against P. capsici: The data on different fungicides screened *in vitro* at three concentrations and their

Table 2. In vitro evaluation of plant extracts against *Phytophthora capsici*

Sl. No.	Common name	Botanical name	Plant parts used	Per cent mycelial inhibition Concentrations 5%	10%	Mean
1	Adusoge	*Adathoda vesica* Nees	Leaf	1.85 (7.73)	12.96 (21.09)	7.40 (14.41)
2	Baad	*Clerodendron inerme* G.	Leaf	12.96 (21.06)	22.96 (28.61)	17.96 (24.85)
3	Duranta	*Duranta plumeri* Jacq.	Leaf	9.62 (18.06)	26.66 (31.07)	18.14 (24.56)
4	Eupatorium	*Chromolaena odorara* King.	Leaf	18.51 (25.47)	26.29 (30.83)	22.40 (28.15)
5	Garlic	*Allium sativum* Linn.	Cloves	23.33 (28.87)	35.55 (36.58)	29.44 (32.72)
6	Glyricidia	*Glyricidia maculata* L.	Leaf	1.48 (6.89)	21.85 (27.86)	11.66 (17.37)
7	Lakkisoppu	*Vitex nigundo* L.	Leaf	9.99 (18.40)	21.11 (27.33)	15.55 (22.87)
8	Lantana	*Lantana camera* L.	Leaf	21.11 (27.33)	25.18 (30.09)	23.14 (28.71)
9	Neem	*Azadiracta indica* A.Juss	Leaf	16.66 (24.07)	25.18 (30.10)	20.92 (27.09)
10	Tikki weed	*Tridax procumbens* Linn.	Leaf	0.00 (0.00)	14.81 (22.61)	7.40 (11.31)
			Mean	16.17	26.02	21.09

	S.Em. ±	CD at 1 %
Plant extract (P)	0.366	1.393
Concentration(C)	0.156	0.954
Interaction (P X C)	0.517	1.970

* Figures in parenthesis are arcsine transformed values

per cent mycelial inhibition over control are presented in table 3. The cent per cent mycelial inhibition was recorded with fungicides, viz. Akomin, Melody duo, Ridomil MZ 72 WP and Secure at all the three concentrations (0.1%, 0.2% and 0.3%), Aliette and Bordeaux mixture also recorded cent per cent inhibition of radial growth of mycelia of *P. capsici* at 0.3 per cent concentration. Mean minimum per cent mycelial inhibition was observed with fungicides Verita (51.25%) and Copper oxychloride (60.26%). However, all the fungicides screened *in vitro* against *P. capsici* at three different concentrations were significantly superior to control and significantly differed with each other (Table 3).

Biological control of plant diseases would help in preventing increase of pathogen population and also health hazards because of use of various synthetic chemicals. Biological control through the use of antagonistic microorganisms is a potential, non-chemical means of controlling plant diseases by reducing inoculum level of pathogen. The present investigation assessed the antagonistic effect of

Table 3. Bioassay of fungicides against *Phytophthora capsici* causing foot rot of black pepper

Sl. No.	Fungicides	Per cent mycelial inhibition			Mean
		Concentrations			
		0.1 %	0.2 %	0.3%	
1	Akomin	100	100	100	100
		(89.96)	(89.96)	(89.96)	(89.96)
2	Aliette 80 WP	74.08	78.15	100	84.08
		(59.37)	(62.11)	(89.96)	(70.48)
3	Bordeaux mixture	66.30	72.22	100	79.51
		(54.49)	(58.17)	(89.96)	(67.54)
4	Blitox COC 50% WP	71.11	77.41	79.30	75.94
		(57.46)	(61.60)	(62.92)	(60.26)
5	Melody duo 66.75 WP	100	100	100	100
		(89.96)	(89.96)	(89.96)	(89.96)
6	Profiler 71.04 WDG	77.41	84.44	85.56	82.47
		(61.60)	(66.74)	(66.75)	(65.33)
7	Ridomil MZ 72 WP	100	100	100	100
		(89.96)	(89.96)	(89.96)	(89.96)
8	Secure 60 WDG	100	100	100	100
		(89.96)	(89.96)	(89.96)	(89.96)
9	Verita 71 WDG	49.63	64.44	68.52	60.86
		(44.77)	(53.37)	(55.85)	(51.25)
	Mean	63.75	66.19	72.62	67.57
		S.Em. ±	CD at 1%		
Fungicide (F)		0.129	0.486		
Concentration(C)		0.068	0.254		
Interaction (F X C)		0.224	0.842		

* Figures in parenthesis are arcsine transformed values

different native antagonists by dual culture technique. Maximum reduction in radial growth of *P. capsici* was observed in *Trichoderma* sp. of Mundigesara isolate which was at par with *Trichoderma* sp. of Yellapur isolate and both were significantly superior to all other fungal and bacterial isolates tested. Next best isolate was *Trichoderma* sp. of Gudnapur isolate, it was on par with Edahalli and Siddapur isolates. Bacterial isolates recorded minimum inhibition of *P. capsici. Trichoderma* sp. showed more mycelial inhibition of the pathogen compared to bacterial antagonists. This could be obviously attributed to several possibilities of existence of microbial interactions such as higher competitive ability, stimulation, antibiosis by these *Trichoderma* native isolates over test pathogen. This has been reported by many workers (Porter, 1924; Ghaffer, 1969 and Naik and Sen, 1995). The antagonism of *Trichoderma* spp. against many fungi is mainly due to production of acetaldehyde (Robinson and Park, 1966). This may also be the reason for antagonistic effect of native isolates of *Trichoderma* against *P. capsici*. Filippi *et al*. (1989) reported antagonistic nature of *Bacillus subtilis* and *Pseudomonas* sp.

Similar results wherein the efficacy of *Trichoderma* spp. and *Pseudomonas* sp. against the pathogen *P. capsici* was previously reported by Anandaraj *et al.* (1995), Jahagirdar (1998), Jubina and Girija (1998), Anith *et al.* (2001) and Rajan *et al.* (2002). In the present investigation, except Tikki weed (*Tridax procumbens*) at 5 per cent concentration, all the plant extracts tested at both 5 and 10 per cent concentrations were significantly effective in reducing the growth of *P. capsici.* Garlic clove extract (36.58%) at 10 per cent concentration proved to be most effective botanical. This was followed by duranta, eupatorium, neem and lantana at 10 per cent concentration, which were at par with each other. At 5 per cent concentration also, among all the botanicals tested, garlic clove extract (28.87%) proved to be the most effective botanical.

In the present investigation, contrary to the reports of Hegde (1983) and Subramanian (1993), neem (*Azadirachta indica*) also proved as one of the effective botanicals against *P. capsici*. The present investigation of various botanicals inhibiting the growth of *P. capsici* are in line with findings of Anandaraj and Leela (1996) and Indian Institute of Spice Research (Anon., 2003).

The present investigation revealed that cent per cent inhibition of mycelial growth of *P. capsici* was recorded with Akomin, Melody, Ridomil MZ 72 WP and Secure at 0.1, 0.2 and 0.3 per cent concentrations. Aliette and Bordeaux mixture also recorded cent per cent mycelial inhibition at 0.3 per cent concentration. Jahagirdar (1998) reported the fungicidal nature of Akomin, a plant tonic generally being recommended for plantation crops. The laboratory evaluation of Ridomil against *Phytophthora parasitica* var. *nicotianae* revealed significant reduction in growth and sporulation of fungus at 0.1, 0.2, 0.3

and 0.4 per cent concentration. Sastry (1982) reported Bordeaux mixture (1%), Blitox and Metalaxyl which were found effective in inhibiting the growth and sporangial formation of *P. capsici* and *P. meadii.* Similar reports in *in vitro* screening were reported by Ramachandran and Sarma (1990), Ramachandran *et al.* (1990). The present investigation results are also in line with the findings of Subramanyam (1993), Jahagirdar (1998) and Veena and Sarma (2000).

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