Madras Agric. J., 94 (1-6) : 51-54 January-June 2007

https://doi.org/10.29321/MAJ.10.100632

Efficacy of various antagonistic microflora against chilli powdery mildew caused by *Leveillula taurica* (Lev.) Arn.

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Abstract: Chilli powdery mildew incited by *Leveillula taurica* is one of the most serious diseases in Tamil Nadu. Among the antagonistic microflora, maximum reduction in conidial germination was recorded by the culture filtrates of *Trichoderma viride, T.harzianum* and *Pseudomonas fluorescens* under *in vitro* condition. These antagonistic microflora were highly effective in reducing disease severity in the greenhouse and field. In greenhouse, *T.harzianum* recorded maximum disease reduction of 66.0 per cent, followed by *T.viride* (48.9 per cent) and *P.fluorescens* (39.9 per cent). Same trend was observed in field also, where 54.9, 47.8 and 36.1 per cent disease reduction were recorded respectively.

Key words: Chilli, powdery mildew, Leveillula taurica, antagonistic microflora, biocontrol, management.

Introduction

Chilli *(Capsicum annuum* L), an important spice crop is cultivated commercially in many parts of Tamil Nadu. Indian chilli is exported to over 90 countries and has become a good foreign exchange earner. During 1996-'97, India produced 9.45 lakh tonnes of dried chilli from an area of 9,565 lakh ha spread over 23 states. Andhra Pradesh ranked first in India both in area and production with 2.04 lakh ha. producing 320 thousand tonnes (Peter, 1999). In Tamil Nadu, the area under chilli cultivation is 76,138 ha with a production of 55,266 tonnes (Anonymous, 2000-2001). One third of the chilli production is reduced annually by field and storage fungus (McEwen, 1978). The diseases caused by fungal pathogens led to heavy yield losses in the field as well as in storage. Among the fungal diseases, powdery mildew incited by *Leveillula taurica* (Lev.) Arn. is one of the most destructive diseases and caused yield loss upto 10-15 per cent (Cerkauskas *et al.,* 1999). For the management of chilli powdery mildew, researches mainly concentrated only on fungicides which had success only to a limited extent. Large scale utilization of chemical fungicides led to residual toxicity in fruits. Environmentally safe, long lasting and eco-friendly non-chemical methods are in-need for effective plant disease management presently. Hence, the present study was aimed to select suitable antagonistic microflora for the management of chilli powdery mildew.

Materials and methods

In vitro evaluation of antagonistic microflora

Various antagonistic microflora namely *Trichoderma viride* Per.ex.S.F.Gray., *T.harzianum* Rifai., *Pseudomonas fluorescens* Migula., *Bacillus subtilis* Cohn emend. Prazmowski., *Chaetomium globosum* Kunze., and *Gliocladium virens* Corda. were collected from Department of Plant Pathology, TNAU Coimbatore and were tested

against *L.taurica* for their inhibitory effect on conidial germination.

Bacterial /fungal cultures initiated from two month old slant cultures incubated at 4°C, were first transferred to 9 cm Petri dishes containing potato - dextrose agar / nutrient agar media and incubated for two weeks in the dark at 24°C. Then, three 5mm plugs taken from the margin of actively growing colonies were inoculated into 150ml conical flask containing 75 ml of potato- dextrose /nutrient broth. Ten days after incubation, the culture filtrates were obtained, separately by filtering through Buchner funnel using Whatman No.l filter paper. The filtrates were centrifuged at 2000 rpm for 10 min at 6°C. The supernatants collected were used for assay (Bateman, 1964; Belanger *et al.,* 1994).

Conidia of *L.taurica* harvested from the chilli leaves, maintained in the greenhouse were mounted on cavity slides in a drop of water. Uniform smear was made and the conidia were fixed on the cavity slides by alternate drying. Slides with fixed spores were soaked separately, in 1ml of culture filtrates of various antagonistic microflora. They were then incubated in moist chamber made from large Petri dishes. Wet cotton pads were placed on backside of the slides to help the condensation of moisture (Gupta and Singh, 1984). The slides were incubated at room temperature for ten days. Water without culture filtrates served as control and sulphur 0.25% was used as standard check. Each treatment was replicated thrice. Ten days after incubation the number of germinated and ungerminated conidia were counted in ten randomly selected microscopic fields per slide (Gupta and Singh, 1984).

Greenhouse evaluation

Chilli cv.K2 was sown in peat trays (10 x 20 x 40cm). Four weeks after germination, the seedlings were transplanted to mud pots (dia. 10cm) at the rate two seedlings per pot filled with gardenland soil. Seedlings in pots were maintained in insect-proof cage. At the time of flowering, the plants, were sprayed with promising antagonistic microflora. This was followed by spraying of conidial suspension of *L.taurica* (5 x 10^4 conidia per ml) on the lower surface of the leaves. Spraying of sulphur 0.25 per cent was used as standard check. Untreated plants served as control. Four plants were used for each treatment, replicated thrice in a complete randomized block design. After 15 days of inoculation, disease intensity was assessed by examining the leaves from each treatment at random, using a 0-4 scale, where 0 = no disease; 1 = 1-10 per cent leaf area affected; 2=11 -25 per cent leaf area affected, 3=26-50 per cent leaf area affected and 4=>50 per cent leaf area affected (Reuveni *et al.,* 1998). The per cent disease index was calculated by using the formula:

$$PDI = \frac{\text{Sum of all individual ratings}}{\text{Total number of leaves assessed}} \times \frac{100}{\text{Maximum disease category}}$$

Field evaluation

Promising antagonistic microflora under greenhouse condition *viz., T.viride, T.harzianum, C.globosum* and *P.fluorescens* were further evaluated in the field.

A field trial was conducted from Dec. 2002 to April 2003 at Agricultural College and Research Institute, Madurai. Chilli seedlings

Table 1. Efficacy of antagonistic microflora against *Leveillula taurica*

Sl.No	Treatments	*In vitro* evaluation		Greenhouse evaluation		Field evaluation		Yield (t/ha)
		Spore germination (%)	Percent spore inhibiton over control	Percent disease index*	Disease reduction over control (%)	Per cent disease index*	Disease reduction over control (%)	
1.	*Trichoderma viride*	34.86(36.18)	59.71	31.80 (34.32)	48.90	30.74(33.67)	47.80	7.57
2.	*Trichoderma harzianum*	34.71 (36.09)	59.88	21.16(27.38)	66.00	26.67(31.09)	54.86	7.69
3	*Pseudomonas fluorescens*	46.56(43.02)	46.19	37.40(37.70)	39.90	37.77(37.92)	36.06	7.48
4.	*Bacillus subtills*	54.21 (47.41)	37.34	51.85(46.06)	16.67	—		—
5.	*Cheatomium globosum*	47.54(43.59)	45.95	40.74 (39.66)	34.52	48.51 (44.15)	17.88	7.36
6.	*Gliocladium virens*	54.28 (47.45)	37.27	—	—	—	—	—
7.	Wettable sulphur	8.42(16.87)	90.77	8.51 (16.96)	86.32	9.61 (18.06)	83.72	7.70
8.	Control (distilled water)	86.52 (68.46)		62.22 (52.07)		59.03 (50.20)		5.64
	CD (P=0.05)	3.8		3.91		3.66		0.03

* Mean of three replications
Values in parenthesis are Arcsine transformed values.

cv.K2 were transplanted in ridges and furrows, three meter long and spaced at 15cm apart. Plots were laid out in a randomized block design with three replications. Talc based commercial products of *T.viride, T.harzianum, C.globosum* and *P.fluorescens* were prepared as per the method of Vidhyasekaran and Muthamilan (1995). The spraying was started as soon as disease symptom appeared and subsequent second spray was given at 15-days interval. Spraying of sulphur (W.P) 0.25 per cent concentration served as standard check. Water spray treated as control. Disease intensity was assessed at weekly intervals upto final harvest as described earlier. Total yield from the individual treatments was recorded after the final harvest.

Result and discussion

Maximum reduction in conidial germination was recorded with the culture filtrates of *T.harzianum, T.viride,* and *P.fluorescens,* where the per cent reduction in conidial germination was 46-60 per cent compared to control (Table 1). These treatments were also promising in reducing disease intensity in greenhouse and field. In greenhouse, *T.harzianum* recorded maximum disease reduction of 66.0 per cent; followed by *T.viride* (48.9 per cent) and *P.fluorescens* (39.9 per cent). Same trend was observed in field also, where 54.9, 47.8 and 36.1 per cent disease reduction were recorded respectively. These treatment recorded maximum yield of 7.5-7.7 tonnes/ha.

There was strong evidence that natural biological control provides protection against many foliar diseases in the field. The drastic reduction of powdery mildew disease severity due to foliar application of *Trichoderma* spp. and *P.fluorescens*, might be due to the production of antibiotic substances. It has been well established that *Trichoderma* spp. produced both non-volatile and volatile (Dennis and Webster, 1971) antibiotics *viz.*, sesquiterpene (trichodermin) and peptide (acetaldehyde) which were active against both fungal and bacterial plant pathogens. *P.fluorescens* was also well known potential antagonists producing antibiotic substance *viz.*, pseudobactin (Meyer and Abdallah, 1978; Teintze *et al.*, 1981; Jeyarajan *et al.*, 1994).

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