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Effect of *Pseudomonas fluorescens* (IISR-6) and *Trichoderma harzianum* (P-26) on growth of black pepper (*Piper nigrum* L.) in the nursery

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Abstract

Experiments conducted to study the effect of *Pseudomonas fluorescens* (IISR-6) and *Trichoderma harzinaum* (P-26) on growth of black pepper (*Piper nigrum*) rooted cuttings in the nursery indicated that maximum height and leaf area of cuttings were obtained with application of *P. fluorescens* thrice, which was on par with raising cuttings in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice. Number of roots and biomass production were higher with combined application of *P. fluorescens* (thrice) and *T. harzianum* which was on par with application of *P. fluorescens* thrice.

Key words: black pepper, *Piper nigrum, Pseudomonas fluorescens, Trichoderma harzianum.*

Introduction

Black pepper (*Piper nigrum* L.) plants in the nursery are affected by many diseases caused by fungi, nematodes and viruses. Application of Bordeux mixture 1% or copper oxychloride 0.2% or carbendazim 0.2% at 15–30 days intervals during June-July has been suggested to control these diseases (Sarma 2000). Spurred by the ecological effects of chemical-based pest management strategies, focus is now shifted to eco-friendly practices, which maintain soil health. Combined application of *Trichoderma* sp. and vesicular arbuscular microrrhiza is reported to produce robust disease-free rooted black pepper cuttings in the nursery (Sarma 2000). Application of rhizobacteria and *T. harzianum* is also reported to significantly enhance growth of black pepper plants in the nursery (Anandaraj & Sarma 2003). *T. harzianum* and *Pseudomonas fluorescens* (IISR-6) promoted growth and vigour of black pepper, ginger and cardamom and suppressed soil-borne fungal pathogens in field conditions also (Jisha *et al.* 2002). In order to develop a healthy black pepper plantation, pathogen-free planting material is essential. In this research paper, the effect of application of *P. fluorescens* and *T. harzianum* on growth of rooted cuttings of three improved varieties of black pepper namely, Pournami, Subhakara and Panniyur-3 is described.

Materials and methods

The experiment was conducted at Peruvannamuzhi Farm of Indian Institute of Spices Research, Calicut, during June to September 2003. The potting mixture was prepared by mixing garden soil, farmyard manure and sand in 1:1:1 proportion and used for filling polythene bags of 20 cm x 10 cm

size. The mixture had a nutrient concentration of available nitrogen 688 ppm, phosphorous 54 ppm, potash 774 ppm and pH 6.4. Three high yielding black pepper varieties namely, Pournami, Subhakara (released from Indian Institute of Spices Research) and Panniyur-3 (released from Kerala Agricultural University) were used for the experiment. Single noded rooted cuttings collected from rapid multiplication nursery were used for planting in polythene bags and kept in nursery.

The biocontrol agents used included *P. fluorescens* (strain IISR-6) and *T. harzianum* (strain P-26). Both the biocontrol agents were obtained from Crop Protection Division of Indian Institute of Spices Research, Calicut. Lag phase cultures of the *P. fluorescens* raised in nutrient broth was inoculated to the molasses medium and incubated at 28^0C for 48 h at 150 rpm. Fifty ml of the diluted culture of *P. fluorescens* was added to the filled polythene bags which had 10^8 cfu ml^{-1} at the time of application. *T. harzianum* multiplied on sorghum grains and having a population of 10^8 cfu g^{-1} was mixed at the rate of 1g kg^{-1} of potting mixture and 500 g was used for filling in polythene bags for the corresponding treatment. The treatments included: Control (potting mixture); Potting mixture + Planting rooted cuttings dipped in *P. fluorescens;* Potting mixture + Application of *P. fluorescens* thrice (at the time of planting and after first and second month of planting); *T. harzianum* applied potting mixture + Dipping in *P. fluorescens*; *T. harzianum* applied potting mixture + Application of *P. fluorescens* thrice. The experiment was conducted in a two factor completely randomized design. The total number of replications was 4 and number of plants per treatment was 15.

Urea (400 g), super phosphate (300 g) and potash (200 g) were dissolved in 100 l of water and 50 ml of the solution was applied 1 month after planting. Observations on growth parameters such as height, number of leaves and leaf area $plant^{-1}$ were recorded. Leaf area was estimated 3 months after planting using the empirical relation LA=0.6 x l x w, where LA=leaf area, l=length of leaf and w=width of leaf (Ibrahim *et al*. 1985). At the end of the experimental period (3 months), five plants per replication were destructively sampled and the number of roots and the dry weights of stem, leaves and roots were recorded to estimate the total biomass.

Results and discussion

Application of *P. fluorescens* thrice recorded maximum plant height, which was on par with raising cuttings in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice (Table 1).

Maximum number of leaves at 60 DAT was observed when *P. fluorescens* was applied thrice to the plants which was on par with raising cuttings in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice, dipping in *P. fluorescens,* dipping in *P. fluorescens* and *T. harzianum* (Table 2). At 90 DAT, raising plants in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice recorded maximum number of leaves which was on par with application of *P. fluorescens* thrice. Maximum leaf production was recorded by *var*. Subhakara which was on par with Panniyur-3.

Leaf area $plant^{-1}$ was significantly higher at 90 DAT in application of *P. fluorescens* thrice, which was on par with raising cuttings in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice. Number of roots at 90 DAT were significantly higher in raising cuttings in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice which was on par with application of *P. fluorescens* thrice and dipping in *P. fluorescens* and *T. harzianum* (Table 3). Among the varieties, maximum number of roots was observed in *var*. Pournami, which was on par with *var*. Subhakara.

Significantly higher biomass was found in raising cuttings in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice which was on par with application of *P. fluorescens* thrice (Table 4).

Table 1. Effect of *Pseudomonas fluorescens* and *Trichoderma harzianum* on height of black pepper cuttings

Treatment	Height of plants (cm) (60 DAT)				Height of plants (cm) (90 DAT)			
	Pournami	Subhakara	Panniyur-3	Mean	Pournami	Subhakara	Panniyur-3	Mean
Control	9.51	9.94	9.34	9.60	13.15	13.81	13.25	13.41
Dipping in Pf	10.38	12.21	12.66	11.80	15.65	18.17	15.50	16.50
Application of Pf thrice	13.34	13.50	14.24	13.70	18.50	20.13	20.90	19.84
Dipping in Pf + Th	9.49	11.45	11.59	10.87	14.00	17.08	15.75	15.60
Application of Pf thrice + Th	12.47	13.87	12.95	13.12	17.78	20.90	21.00	19.89
Mean	11.04	12.20	12.16		15.80	18.01	17.28	
CD (P=0.05) for treatments			1.80				3.21	
CD (P=0.05) for varieties			NS				NS	

DAT=Days after treatment; Pf=*Pseudomonas fluorescens* (IISR-6); Th = *Trichoderma harzianum* (P-26)

Table 2. Effect of *Pseudomonas fluorescens* and *Trichoderma harzianum* on production of leaves of black pepper cuttings

Treatment	No. of leaves plant^{-1} (60 DAT)				No. of leaves plant^{-1} (90 DAT)			
	Pournami	Subhakara	Panniyur-3	Mean	Pournami	Subhakara	Panniyur-3	Mean
Control	2.07	1.91	2.28	2.09	3.50	4.00	3.50	3.30
Dipping in Pf	2.00	2.75	3.00	2.60	3.25	3.50	4.00	3.58
Application of Pf thrice	2.75	2.75	3.00	2.90	4.00	4.50	4.50	4.30
Dipping in Pf + Th	2.00	3.00	2.75	2.60	3.25	4.00	3.50	3.58
Application of Pf thrice + Th	2.50	2.75	3.00	2.80	4.25	4.75	4.50	4.50
Mean	2.26	2.63	2.81		3.65	4.15	4.00	
CD (P=0.05) for treatments			0.47				0.66	
CD (P=0.05) for varieties			0.37				0.49	

DAT=Days after treatment; Pf=*Pseudomonas fluorescens* (IISR-6); Th=*Trichoderma harzianum* (P-26)

Table 3. Effect of *Pseudomonas fluorescens* and *Trichoderma harzianum* on leaf area and production of roots of black pepper cuttings

Treatment	Leaf area plant^{-1} (90 DAT) (cm^2)				No. of roots of plant^{-1} (90 DAT)			
	Pournami	Subhakara	Panniyur-3	Mean	Pournami	Subhakara	Panniyur-3	Mean
Control	182.9	175.6	146.3	168.4	13.75	11.25	10.00	11.60
Dipping in Pf	198.5	199.5	182.2	193.4	16.25	13.26	13.50	14.30
Application of Pf thrice	284.8	325.4	266.6	292.2	21.25	16.25	15.00	17.50
Dipping in Pf + Th	168.8	230.8	193.3	197.6	19.50	16.00	15.00	16.80
Application of Pf thrice + Th	216.2	310.9	289.7	272.2	19.00	19.25	14.50	17.58
Mean	210.2	248.5	215.6		17.95	15.20	13.60	
CD (0.05) for treatments			69.49				3.47	
CD (P=0.05) for varieties			NS				2.69	

DAT=Days after treatment; Pf=*Pseudomonas fluorescens* (IISR-6); Th=*Trichoderma harzianum* (P-26)

Table 4. Effect of *Pseudomonas fluorescens* and *Trichoderma harzianum* on biomass of black pepper cuttings

Treatment	Biomass (g) (90 DAT)			
	Pournami	Subhakara	Panniyur-3	Mean
Control	2.29	2.39	2.50	2.39
Dipping in Pf	2.66	2.90	3.08	2.87
Application of Pf thrice	3.28	3.79	3.56	3.54
Dipping in Pf + Th	3.02	2.92	3.41	3.12
Application of Pf thrice + Th	3.30	3.68	3.83	3.61
Mean	2.90	3.10	3.30	
CD (P=0.05) for treatments		0.51		
CD (P=0.05) for varieties		NS		

DAT=Days after treatment; Pf=*Pseudomonas fluorescens* (IISR-6); Th=*Trichoderma harzianum* (P-26)

Glick (1995) reported that phosphorus solubilisation, biological nitrogen fixation, improvement of other plant nutrients uptake and phytohormone production like indole acetic acid (IAA) are some mechanisms that directly influence plant growth by plant growth promoting rhizobacteria. The role of IAA for better and profuse rooting in various plants is well documented (Hartman & Kester 1972). Increased feeder root production and absorptive surface area in black pepper plants due to *P. fluorescens* has been reported (Anandaraj & Sarma 2003). In the rhizhosphere, production of ethylene results in inhibition of root elongation. In plants treated with rhizobacteria, the production of ethylene is inhibited by aminocyclo propane carboxylic acid deaminase which results in production of ammonia instead of ethylene. This results in rapid elongation of roots (Kloepper 2003). Repeated application of *P. fluorescens* might have increased the auxin pool near to the root zone resulting in increased root and biomass production. Significant uptake of nitrogen, potassium and enhanced nutrient mobilization was also recorded due to application of *P. fluorescens* in black pepper (Paul *et al.* 2001). Shanthi *et al.* (2003) reported that soil application of *P. fluorescens* resulted in higher plant height, number of leaves, root length and root weight compared to rhizome dip in banana.

T. harzianum is also capable of increasing the uptake of nutrients by secreting enzymes that solubilize insoluble nutrients (Altomare *et al.* 1999; Harman *et al*. 2004). *P. fluorescens* and *T. harzianum* are compatible, synergistic and enhance biomass production and disease suppression when applied to soil (Saju *et al.* 2003). Mixtures of compatible organisms would be an added advantage over single species application (Saju *et al.* 2003; Thankamani *et al*. 2003). Thus, although the treatments raising black pepper cuttings in *T. harzianum* applied potting mixture plus application of *P. fluorescens* thrice and application of *P. fluorescens* thrice were on par for growth parameters, the treatment *T. harzianum* plus *P. fluorescens* is preferred as this ensures greater protection of the root system against *P. capsici* when it is transplanted into the field.

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