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Growth and nutrient uptake of black pepper (*Piper nigrum* L.) varieties in nursery as influenced by the application of *Pseudomonas fluorescens* and *Trichoderma harzianum*

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ABSTRACT

An experiment was conducted at Indian Institute of Spices Research, Calicut during the months of June to September 2003 to find out the effect of *Trichoderma harzianum* and *Pseudomonas fluorescens* on growth and nutrient uptake of black pepper varieties in greenhouse conditions. Observations on plant growth parameters were taken three months after raising the rooted cuttings in polythene bags. Maximum biomass production and uptake of nutrients nitrogen and phosphorous was recorded by the treatments where combined application of *Pseudomonas fluorescence* (IISR 6) and *Trichoderma harzianum* (P 26) were carried out. Among the varieties, maximum biomass production and nutrient uptake was observed in Panniyur3.

Key words: Black pepper, *Piper nigrum*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, nutrient uptake.

INTRODUCTION

Black pepper (*Piper nigrum* L.) known as the king of spices, is an important foreign exchange earner for the country. It is grown for its dried fruits especially favoured for its pungency, flavour, and spicy taste and is used in many food preparations. In addition to this, it is used as medicine and as a component of many traditional ayurvedic drugs. Though the crop originated in Western Ghats of India, productivity is very low, only 315 kg ha^{-1}. Several reasons were attributed to the declining trend in productivity, among them non-availability of quality planting materials is a major one. Black pepper is propagated mainly by rooted cuttings raised in nursery . In the nursery, diseases caused by *Phytophthora capsici, Rhizoctonia solani, Sclerotium rolfsii* and viruses causes substantial losses to the rooted cuttings. Prolonged use of chemicals is undesirable in view of higher maintenance cost, pollution of the environment and consequent health hazards. Ecofriendly method of crop management is widely accepted. For this, identification of potential biological control agents which could control disease and promote growth, has been given priority in the prevailing organic cultivation. Work done for controlling *Phytophthora* diseases in black pepper nursery revealed that combined application of *Trichoderma harzianum* and *Arbuscular mycorrhizal* fungi found to reduce the disease incidence and produced robust disease free cuttings [3].

Application of *Pseudomonas fluorescens* and *Trichoderma harzianum* were recommended for promoting plant growth and suppressing diseases caused by pathogens in black pepper, ginger and cardamom [6]. Growth promotion in black pepper by the application of *P. fluorescens* was observed [12, 2]. Biofertilizers could be used as an alternative remedy to meet the nutrient requirement of the crops. Therefore, the present study was undertaken to study the effect of *P. fluorescens* and *Trichoderma harzianum* on growth and nutrient content of black pepper cuttings under green house condition at Indian Institute of Spices Research, Calicut.

MATERIALS AND METHODS

The study was conducted at Peruvannamuzhi farm of Indian Institute of Spices Research, Calicut during the months of June to September, and 2003.The potting mixture consisted of garden soil, farmyard manure and sand in 2:1:1 proportion with a nutrient composition of available nitrogen 688 ppm, phosphorous 54 ppm, potash 774 ppm and pH 6.4. Single noded rooted cuttings collected from rapid multiplication nursery were used for planting in polythene bags filled with soil, farmyard manure and sand in the ratio 2:1:1. The plants were allowed to grow for three months in polythene bags. 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 of *P. fluorescens* culture raised in nutrient broth was used to inoculate the molasses medium and incubated at 28°C 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 having a population of 10^8 cfu g^{-1} was mixed at the rate of 1 g kg^{-1} of potting mixture and 500 g of this mixture was used for filling in polythene bags for the corresponding treatment. Factorial combinations of three black pepper varieties and five application methods formed the treatments for the experiment. 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. *flurescens* thrice. The varieties used were Subhakara, Pournami and Panniyur-3 .The experiment was conducted in completely randomized design with 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 1st & 2nd month after planting to black pepper plants.

At the end of the growing period, five plants per replication were destructively sampled. The dry weight of stem, leaves and roots were recorded separately and added together to estimate the total biomass. Three plants of each variety per replication per treatment were used for nutrient estimation. The leaf sample wiped with tissue paper was kept in a hot air oven at 70 ± 5°C for 48 hours. The dried leaves were ground to a fine powder and passed through 72-mesh sieve. For the estimation of plant nutrients elements, 0.5g samples were digested by nitric acid and Perchloric acid mixture (2:1). Phosphorus was determined by Vanadomolybdo Phosphoric yellow colour method in nitric acid medium and Potassium was determined by flame photometer [5]. Total uptake of nutrients was obtained by multiplying the percent quantity of the nutrient in each part with total dry weight of the plant and by adding together.. The data were subjected to statistical analysis [8].

RESULTS AND DISCUSSION

There was significantly higher biomass production in plants treated with biocontrol agents than control. Combined application of *P. fluorescens* and *T. harzianum* showed beneficial effect on growth. Significantly higher biomass production was observed for the treatment raising of rooted black pepper cuttings in *T. harzianum* applied potting mixture and application of *P. fluorescens* thrice that was at par with application of *P. fluorescens* thrice (Table-1). Among the varieties maximum biomass was produced by Panniyur-3, that was at par with Subhakara and minimum in Pournami.

Effect of P. fluorescens and T. harzianum on nutrient uptake of black pepper varieties

The results regarding uptake of NPK per plant recorded were found to be significant (Fig1,2,3).The treatment, application of *P. fluorescens* thrice + *T. harzianum* recorded maximum nitrogen uptake followed by application of *P. fluorescens* thrice. Among the varieties, significant higher uptake of nitrogen was recorded by Panniyur-3 followed by Subhakara.

Table 1. Effect of *P.fluorescens* and *T. harzianum* on biomass of black pepper rooted cuttings

Treatment	Biomass (g) (90 DAT)			
	Pourn-ami	Subha-kara	Panni-yur-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		
CD IA(P=0.05)		NS		

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

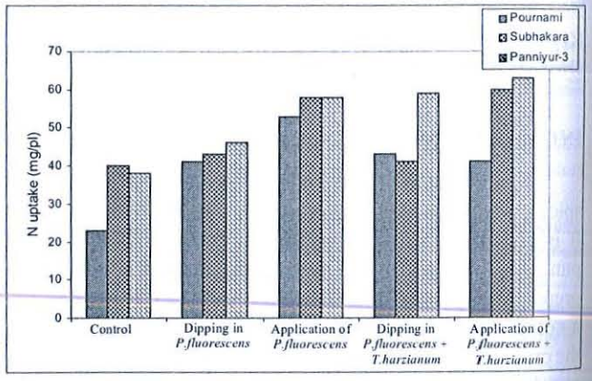

Figure 1. Effect of *P.fluorescens* and *T.harzianum* on uptake of nitrogen in black pepper varieties

Uptake of total phosphorous in the plants varied from 3.40 g/ plant to 11.94 and maximum Phosphorous uptake was recorded by the treatment having combined application of *P. fluorescens* thrice and *Trichoderma*. The percent increase of uptake of phosphorous over control was worked out for all the treatments. The values were higher for the treatment application of *P. fluorescens* + *T. harzianum* (57) and application of *P. fluorescens* thrice (47) compared to the treatments dipping in *P. fluorescens* and dipping in *P. fluorescens* + *T. harzianum* . Uptake of phosphorous was not influenced by various treatments in all the varieties.

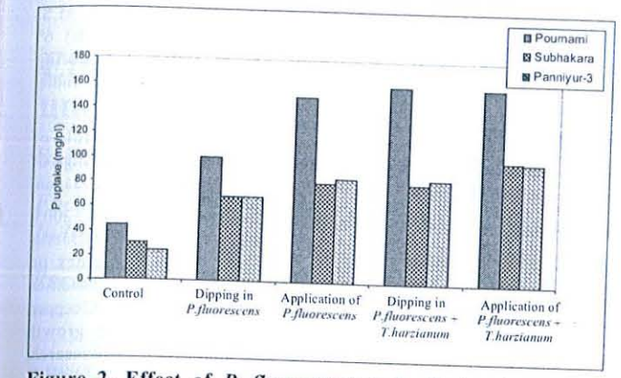

Figure 2. Effect of *P. fluorescens* and *T .harzianum* on uptake of phosphorous in black [pepper varieties]

Uptake of potassium per plants varied from 23.44 to 77 mg. Higher uptake of potassium was recorded by the treatment application of *P. fluorescens* thrice that was at par with the treatment combined application of *P. fluorescens* thrice + *T. harzianum* , and minimum uptake of potassium was recorded in control. The percent increase in uptake of potassium over control was higher for the treatments application of *P. fluorescens* +*T. harzianum* (234) and application of *P. fluorescens* thrice (225) compared to the treatments dipping in *P. fluorescens* (110) and dipping in *P. fluorescens* + *T. harzianum* (164). With regard to K uptake, Panniyur-3 and Pournami varieties were at par and minimum K uptake was recorded in Subhakara.

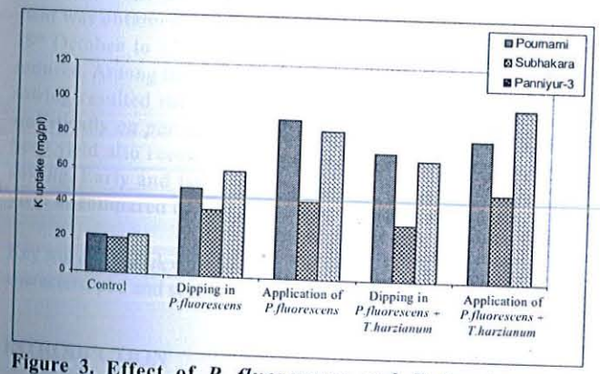

Figure 3. Effect of *P. fluorescens* and *T. harzianum* on uptake of potassium in black pepper varieties

Growth of black pepper plants can be attributed to higher amount of nutrients uptake and to the absence of disease causing organisms in the rhizosphere. Growth was significantly higher when *T.harzianum and P.fluorescens* applied together to the plants. *Trichoderma* is also capable of increasing the nutrients by secreting enzymes that solubilize insoluble nutrients [1]. The antagonistic effect of *T.harzianum* on the growth and proliferation of *Phytophthora capsici* in plant rhizosphere and reduction in foot rot disease in black pepper has been reported [9, 2,10] . Increased feeder root production and absorptive surface area in black pepper plants due to *P.fluorescens* has been reported [2]. In the present study, nutrient uptake rate was higher for the treatment combined application of *P.fluorescens* and *T. harzianum*. Enhanced nutrient mobilization effected in the rhizosphere of black pepper with collective effect of *P.fluorescens* and *T. harzianum* might have increased photosynthesis and plant vigour [7]. A higher nitrogen concentration with plant is likely to result in higher rate of photosynthesis [4]. Mixtures of compatible organisms would be an added advantage over single species application [10].

In the present study, higher biomass production was observed for Panniyur-3 and Subhakara. Enhanced root production observed in Panniyur 3 might have increased higher nutrient uptake, photosynthesis that would have resulted in production of higher biomass. Increased uptake of nutrients by these varieties in this study proved higher nutrient use efficiency.

CONCLUSION

Application of *P.fluorescens* thrice along with *Trichoderma harzianum* in rooted cuttings of black pepper plants resulted in better nutrient uptake and increased biomass production. Nutrient use efficiency of the variety Panniyur-3 was higher compared to the varieties Subhakara and Pournami.

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