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Effect of *Azospirillum* sp. and nutrients on yield of black pepper (*Piper nigrum* L.)

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Abstract

An experiment was conducted at Peruvannamuzhi (Kerala) to study the effect of *Azospirillum* sp. along with nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), boron (B) and molybdenum (Mo) on yield of black pepper (*Piper nigrum*). Maximum fresh yield (2207 g vine^{-1}) was recorded in the treatment *Azospirillum* sp. + 50% recommended N + Mg followed by application of NPK alone. Significantly higher N, K and Mg content in the soil and N and Ca content in the leaf was observed in the treatment, 50% recommended N + Mg. Application of *Azospirillum* sp. increased the population of bacteria and a final population of 10^6 x 10^5 cfu (colony forming units) in the soil was recovered in the treatment 50% recommended N + Mg (0.2 kg ha^{-1}) compared to uninoculated control.

Keywords: *Azospirillum*, black pepper, nutrients, *Piper nigrum,* yield.

Introduction

The productivity of black pepper (*Piper nigrum* L.) is low in India (281 kg ha^{-1}) due to many factors among which non-adoption of scientific crop management practices is a major factor. The increasing cost of fertilizers and environment degradation by using fertilizers have created considerable interest on bio-fertilizers. *Azospirillum* sp. inoculation improved rooting of black pepper var. Panniyur-1 in the nursery (Govindan & Chandy 1985). Application of *Azospirillum* sp. and chemical fertilizers as 100 g nitrogen (N), 40 g phosphorus (P) and 140 g potassium (K) in addition to 15 kg farmyard manure vine^{-1} resulted in highest berry yield of black pepper at Pechiparai, Tamil Nadu (Kanthaswamy *et al.* 1996). However, information on the effect of *Azospirillum* sp. with other nutrients such as calcium (Ca), magnesium (Mg), zinc (Zn), boron (B) and molybdenum (Mo) on yield of black pepper is lacking. Hence a study was initiated with the objective to evaluate the performance of *Azospirillum* sp. in combination with major, secondary and micro nutrients on yield of black pepper.

Materials and methods

The experiment was conducted at Indian Institute of Spices Research (IISR), Experimental Farm, Peruvannamuzhi (Kerala) during 2001-06 on the black pepper variety Subhakara. The soil had a initial

nutrient status of organic carbon (1.8%), available P (3.6 ppm), K (415 ppm), Ca (554 ppm) and Mg (95 ppm) with a pH of 5.5. Lime (calcium hydroxide) was applied @ 500 kg ha^{-1} for improving the pH of the soil.

Six-month-old rooted black pepper plants were planted in the basins of two year old support tree *Ailanthus* sp. during June 2001. During the first year, 1/3rd of recommended fertilizer 140: 55: 270 g $plant^{-1}$ in the form of urea, superphosphate and potash was given. The treatments consisted of two main plot (A-with *Azospirillum* sp. and B-without *Azospirillum* sp.) and five sub plot treatments, namely, farmyard manure (FYM) 10 kg (T1); 50% Recommended N + 10 kg FYM (T2); 50% Recommended N + Zn, B, Mo (T3); 50% Recommended N + Mg (T4); and NPK alone, control (T5). The experiment was laid out in a split plot design, with four replications and six plants $treatment^{-1}$ and with a spacing of 3 m x 3 m per plot. Other nutrients such as lime 500 g, magnesium sulphate 200 g, zinc sulphate 30 g, borax 10 g and sodium molybdate 2.5 g were applied to the vines as per treatments. Chemical fertilizers were given in two splits, in June and October. *Azospirillum* sp. strain isolated from black pepper garden of the Experimental Farm was multiplied in agar medium under laboratory conditions (Govindarajan & Thangaraju 1998). The inoculum was mixed with FYM to get a final inoculum potential of 10^8 cfu g^{-1} and applied to the plants twice during May and October in a year. The plants were irrigated during summer and plant protection measures were followed as per the package of practices recommendation of IISR, Calicut.

Soil samples were drawn during November from 0-15 cm depth, and analyzed for pH, N, P, K, Ca and Mg and for counting the population of *Azospirillum* sp. Leaf samples were collected by the procedure suggested by Dewaard (1969) during May. The leaf N, P and K, Ca and Mg were estimated by the method suggested by Singh *et al.* (2001). The available soil N was determined by Kjeldahl method (Subbiah & Asija 1956); P was determined by Bray method (Bray & Kurtz 1945). K, Ca and Mg were determined by atomic absorption spectrophotometer (Hesse 1994). *Azospirillum* sp. population in the fresh soil was counted during May by the method suggested by Govindarajan & Thangaraju (1998). During 2005 and 2006, yield and yield parameters were recorded and data was analyzed statistically (Panse & Sukhatme 1985).

Results and discussion

Various nutrient schedules influenced the length of spikes, number of berries and yield (Table 1). Yield parameters and yield were higher in plots in which *Azospirillum* sp. was applied. Maximum number of spikes was recorded in the treatment involving 50% Recommended N + Mg followed by 50% Recommended N + Zn + B + Mo. Maximum length of spikes, was recorded in the treatment NPK alone that was on par with all the treatments except 50% Recommended N + FYM 10 kg. Maximum number of berries $spike^{-1}$ was recorded in 50% Recommended N + FYM 10 kg followed by 50% Recommended N + Mg. Significantly higher yield was registered in the treatment, 50% Recommended N + Mg followed by NPK application alone (control).

The higher yield in the treatment 50% Recommended N + Mg may be due to presence of significantly higher number of spikes and number of berries and also due to increased availability of soil nutrients like N, K and Mg (as evident from Table 2) and also due to the production of phytohormones like IAA, GA and cytokinin like substances by *Azospirillum* sp. (Govindan & Purushothaman 1984; Summer 1990).

Nutrient status in the soil was improved by the application of *Azospirillum* sp. (Table 2). Available N content in the soil was significantly higher in 50% Recommended N + Mg followed by 50% Recommended N + Zn + B + Mo. Maximum Bray P was recorded in NPK alone followed by 50% Recommended N +10 kg FYM. Available K content, was maximum in NPK alone. Maximum Ca content was recorded in the treatment NPK

Table 1. Effect of *Azospirillum* sp. and nutrients on yield characters and yield of black pepper (mean of 2 years)

Treatment	Number of spikes			Length of spikes (cm)			Number of berries spike^{-1}			Fresh yield (g vine^{-1})		
	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean
T_1	18.75	15.00	16.80	8.73	7.25	7.90	32.40	40.00	36.20	1154.5	630.0	892.0
T_2	16.73	19.75	18.30	7.55	7.20	7.40	48.70	45.10	46.90	111.5	550.0	831.0
T_3	23.00	21.00	22.30	8.58	7.18	7.90	46.72	40.20	43.50	845.0	612.5	729.0
T_4	29.25	21.50	25.40	8.77	7.78	8.30	46.80	44.03	45.40	2207.1	1275.4	1741.0
T_5	21.00	19.50	20.30	8.83	7.58	8.20	34.06	38.00	36.00	1950.0	935.0	1442.0
Mean	20.62	19.40		8.49	7.39		41.70	41.50		1461.0	790.0	
CD (MP) (P=0.05)		2.52			0.41			1.39			109.0	
CD (T) (P=0.05)		3.60			0.57			0.55			153.50	
CD IA (P=0.05)		NS			0.61			6.54			164.00	

AZO=*Azospirillum* sp.; WOA=Without *Azospirillum* sp.; MP=Main plot; T=Treatment; IA=Interaction.

T_1 = FYM 10 kg; T_2 = 50% Recommended N + 10 kg FYM; T_3 = 50% Recommended N + Zn, B, Mo; T_4 = 50% Recommended N + Mg; T_5 = NPK alone, control.

Table 2. Nutrient status in the soil after application of *Azospirillum* sp. and nutrients (mean of 2 years)

Treatment	Nitrogen (mg kg^{-1})			Phosphorous (mg kg^{-1})			Potassium (mg kg^{-1})			Calcium (mg kg^{-1})			Magnesium (mg kg^{-1})		
	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean
T_1	122.80	103.00	112.9	26.88	17.10	21.9	238.25	217.50	227.9	520.8	536.8	528.8	184.6	230.8	207.7
T_2	131.95	130.50	131.2	34.46	13.78	24.1	242.75	213.45	228.1	833.8	609.0	721.4	308.4	180.4	244.3
T_3	134.25	133.00	133.6	24.50	10.79	12.7	226.0	247.48	236.8	652.5	529.3	610.9	298.3	222.6	260.4
T_4	140.66	127.30	133.9	26.50	13.64	15.1	338.50	281.25	309.9	497.8	403.8	450.8	367.0	310.5	338.9
T_5	126.30	132.20	119.8	42.00	34.93	38.5	313.40	295.00	304.3	850.8	529.8	690.3	261	231.8	246.5
Mean	131.16	121.21		26.86	18.05		271.80	251.90		679.1	521.7		283.9	235.2	
CD (MP) (P=0.05)	0.81			3.40			15.54				91.60			31.40	
CD (T) (P=0.05)	4.90			4.80			21.00				129.50			44.30	
CD (IA) (P=0.05)	5.30			5.16			23.49				144.30			47.40	

AZO=*Azospirillum* sp; WOA=Without *Azospirillum* sp; MP=Main plot; T=Treatment; IA=Interaction.

T_1 = FYM 10 kg; T_2 = 50% Recommended N + 10 kg FYM; T_3 = 50% Recommended N + Zn, B, Mo; T_4 = 50% Recommended N + Mg; T_5 = NPK alone, control.

alone followed by 50% Recommended N + 10 kg FYM (Table 2). Mg content was significantly higher in 50% Recommended N + Mg followed by 50% Recommended N + FYM 10 kg.

Leaf nutrient status showed pronounced changes and significantly higher N in the leaves was observed in the treatment 50% Recommended N + Mg that was on par with 50% Inorganic N + 10 kg FYM (Table 3). Maximum Ca content was observed in the treatment 50% Recommended N + Mg followed by 50% Recommended N + Zn + B + Mo.

Among the recommended nutrients, application of Mg increased the yield of black pepper. The same treatment had higher N as well as Ca content in leaves. Magnesium is one of the components in the chlorophyll molecule which helps in chlorophyll synthesis and higher chlorophyll levels may enhance light interception resulting in higher yield.

Application of *Azospirillum* sp. significantly increased the population of *Azospirillum* sp. in the soil (Table 4). 50% Recommended N + Mg recorded higher population followed by 50% Recommended N + Zn + B + Mo. Interaction effect was also significant with respect to application of *Azospirillum* sp. Application of lime increased pH of the soil (Table 4) which varied from 5.9 to 6.7. Maximum pH was observed in 50% Recommended N + Mg in *Azospirillum* sp. applied plots.

Deficiency of Ca and Mg and Al toxicity are major yield limiting factors in black pepper growing soils of low pH (Sadanandan 2000). Application of lime increased the pH of soil which in turn must have increased *Azospirillum* sp. population as evident from Table 4. This may be the reason for increased population found in the treatment combination 50% Recommended N + Mg. The beneficial effects due to application of Ca and Mg has also been reported earlier in black pepper (IISR 1993).

Table 3. Nutrient content in leaves of black pepper after application of *Azospirillum* sp. and nutrients

Treatment	Nitrogen (%)			Phosphorus (%)			Potassium (%)			Calcium (mg kg^{-1})			Magnesium (mg kg^{-1})		
	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean	AZO	WOA	Mean
T_1	2.50	2.52	2.51	0.19	0.12	0.15	2.46	1.37	1.90	1.37	1.07	0.52	0.16	0.12	0.12
T_2	2.75	2.42	2.59	0.17	0.16	0.16	2.42	1.90	2.10	1.23	1.05	0.51	0.15	0.11	0.13
T_3	2.62	2.53	2.58	0.19	0.13	0.15	2.50	1.47	2.00	1.63	1.60	0.58	0.17	0.14	0.14
T_4	2.83	2.61	2.72	0.17	0.18	0.18	2.40	1.95	2.20	1.90	1.57	0.58	0.18	0.17	0.18
T_5	2.42	2.43	2.43	0.18	0.12	0.14	2.30	2.17	2.30	1.40	1.30	0.58	0.19	0.10	0.13
Mean	2.62	2.50		0.18	0.14		2.4	1.77		0.15	1.30	0.55	0.17	0.13	
CD (MP) (P=0.05)	0.08			0.13			0.13			0.07			NS		
CD (T) (P=0.05)	0.19			NS			NS			0.16			NS		
CD (IA) (P=0.05)	0.27			0.05			0.42			0.22					

AZO=*Azospirillum* sp; WOA=Without *Azospirillum* sp; MP=Main plot; T=Treatment; IA=Interaction.
T_1 = FYM 10 kg; T_2 = 50% Recommended N + 10 kg FYM; T_3 = 50% Recommended N + Zn, B, Mo; T_4 = 50% Recommended N + Mg; T_5 = NPK alone, control.

Table 4. *Azospirillum* sp. population and pH of soil after application of inoculum and nutrients

Treatment	*Azospirillum* sp. population (10^5 cfu)			pH		
	AZO	WOA	Mean	AZO	WOA	Mean
T_1	76	24.5	50.2	5.9	5.8	5.8
T_2	80	24.8	52.4	6.0	5.9	5.9
T_3	93	21.5	57.3	6.0	5.9	5.9
T_4	106	23.0	64.5	6.7	6.6	6.6
T_5	90	18.6	54.3	6.2	6.3	6.3
Mean	89	21.8				
CD (MP) (P=0.05)	9.7					
CD (T) (P=0.05)	13.7					

AZO=*Azospirillum* sp; WOA=Without *Azospirillum* sp; MP=Main plot; T=Treatment; IA=Interaction.
T_1 = FYM 10 kg; T_2 = 50% Recommended N + 10 kg FYM; T_3 = 50% Recommended N + Zn, B, Mo; T_4 = 50% Recommended N + Mg; T_5 = NPK alone, control.

In conclusion, application of *Azospirillum* sp. in combination with 50% Recommended N + Mg, superphosphate and potash resulted in higher yield over application of recommended dose of fertilizer in black pepper.

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