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Effect of bio-agents and growth stimulators on rooting and survival of orthotropic shoots in black pepper (*Piper nigrum* L.)

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Abstract
A field experiment was carried out during *Rabi* season, 2021-2022, Venkataramannagudem, West Godavari district, Andhra Pradesh. The experiment was laid in Factorial Completely Randomized Block Design with three replications. The first factor consists of three levels of bio-agents *viz*., Arbuscular mycorrhizal fungi (AMF) @ 5 grams, *Azospirillum* @ 5 grams and no bio-agent and the second factor consists of eight levels of growth stimulators viz., NAA 500 ppm, IBA 500 ppm, NAA 250 ppm + IBA 250 ppm, Common sugar solution 2%, Common sugar solution 3%, Mono Ammonium phosphate (MAP) 1%, Mono ammonium phosphate (MAP) 2% and fresh water dip. *Azospirillum* @ 5 grams recorded the maximum values for most of the growth characters. AMF @ 5 grams recorded the more values for root characters. In case of growth stimulators, IBA 500 ppm recorded the maximum values for all growth characters. Among the treatment combinations, *Azospirillum* @ 5 grams + IBA 500 ppm treatment recorded the best values for most of the growth characters and *Azospirillum* @ 5 grams + (NAA 250 ppm + IBA 250) ppm recorded the higher values for root characters.

Keywords: Bio-agents, growth stimulators

Introduction

Black pepper (*Piper nigrum* L.), is a flowering vine in the family Piperaceae, cultivated for its fruit, known as a pepper corn, which is usually dried and used as a spice and seasoning. The fruit is a drupe (stone fruit), about 5 mm in diameter (fresh and fully mature), dark red in colour and contains a stone which encloses a single seed. Pepper corns and the ground pepper described simply as pepper, or more precisely as black pepper (cooked and dried unripe fruit), green pepper (dried unripe fruit) or white pepper (ripe fruit seeds). The crop is native to Malabar coast of India, having chromosome number 2n = 52. Malabar pepper is extensively cultivated in Kerala, Karnataka and in other tropical regions.

The pepper plant is a perennial woody vine growing up to 4 m (13 ft.) in height on supporting trees, poles, or trellises. It is a spreading vine, rooting readily where trailing stems touch the ground. The leaves are alternate, entire, 5 cm to 10 cm long and across. The flowers are small, produced on pendulous spikes 4 cm to 8 cm long at the leaf nodes, the spikes lengthening up to 7 cm to 15 cm as the fruit matures. It is the most important spice of India and world due to its day to day usage. It is therefore rightly considered as King of spices.

The commercial method of propagation of pepper is by using runner shoots which develop at the base of the vine. The vines planted with runner shoots in general start flowering from third year onwards or later. In such bushes, the fruiting invariably starts at a height of two to three feet from the base of the bush. But the vines from the terminal orthotropic shoots are found to give better growth and the large number of fruit bearing lateral branches, starting right from the base of the vine which ensures full coverage of the standard with fruiting branches and enable the plant to be more productive (Sarma *et al*., 2013) [9].

Thus it has been proven beyond doubt that terminal orthotropic shoots can be used for planting after rooting in nurseries, for getting the higher yield. Further, the orthotropic shoots will be totally free from soil contamination compared to runner shoots which are often contaminated with soil leading to *Phytophthora* infection (Sarma *et al*., 2013) [9].

In rooting of black pepper cuttings, a number of factors such as type of cuttings, pre- treatment to cuttings, environmental conditions during rooting, rooting medium etc. are influence the rooting and regeneration of plants from cuttings. Plant growth regulators and bio-agents can

play an important role for root induction in the large number of crops including vine crops like black pepper. However, there exists a lot of contradiction with regard to optimum concentration and period of auxins treatments and bio-agents. Use of auxins like IBA, NAA, etc. and bio-agents has been found effective to induce rooting in cuttings of crops like pepper.

This aspect has also been identified as one of the priority areas for research in black pepper. Hence the present investigation was aimed to know the effect of bio-agents and growth stimulators on rooting and survival of orthotropic shoots of black pepper (*Piper nigrum* L.).

Material and Methods

A research trial was carried out during rabi season, 2021-2022 at a farmer's garden, Kalavalapalli village, Chagallu mandal, West Godavari district, Andhra Pradesh. The experiment was laid out in Factorial Completely Randomised Block Design with three replications. The first factor consists of three levels of bio-agents viz., Arbuscular mycorrhizal fungi (AMF) @ 5 grams, *Azospirillum* @ 5 grams and no bio-agent and the second factor consists of eight levels of growth stimulators viz., NAA 500 ppm, IBA 500 ppm, NAA 250 ppm + IBA 250 ppm, Common sugar solution 2%, Common sugar solution 3%, Mono Ammonium phosphate (MAP) 1%, Mono ammonium phosphate (MAP) 2% and fresh water dip. The experiment included 24 treatment combinations comprising of three levels of bio-agents and 8 levels of growth stimulators.

Results and Discussion

The data on growth parameters are presented in (Table) showed that application of bio-agents and growth stimulators significantly influence the growth and yield parameters of black pepper.

Among bio-agents, treatment with *Azospirillum* @ 5 grams recorded the minimum number of days for first sprouting (22.88 days) and 50% sprouting (25.95 days) and maximum values for the percent survival (65.98%), height of the sprouted shoot (11.01 cm), no. of leaves (1.72), leaf length (7.60 cm), leaf breadth (4.61 cm), leaf area (22.15 cm^2) and AMF @ 5 grams recorded the best values for more no. of roots (6.05) and length of the longest root (9.58 cm).

Significant differences were observed in various growth stimulators, among all IBA 500 ppm treatment was recorded the minimum number of days for first sprouting (18.77 days) and 50% sprouting (22.45) and highest values for per cent survival (68.84%), height of the sprouted shoot (12.45 cm), no. of leaves (2.29), leaf length (8.01 cm), leaf breadth (5.19 cm), leaf area (25.04 cm^2), petiole length (3.41 cm) and intermodal length (3.71 cm and 5.01 cm), number of roots (7.88) and length of the longest root (12.79 cm).

Interactions between bio-agents and growth regulators was found significant with respect to growth characters. The treatment combination (*Azospirillum* @ 5 grams + IBA 500 ppm (B_2G_2) recorded the minimum number of days for first sprouting (18.23 days) and days to 50% sprouting (21.73 days) and higher values in terms of growth characters viz., height of the sprouted shoot (14.07 cm), no. of leaves (2.80), leaf length (8.59 cm), leaf breadth (5.54 cm), leaf area (27.37 cm^2), petiole length (4.07 cm) and intermodal length (5.67 cm). Whereas, higher values for number of roots (6.05) and length of the longest root (9.58 cm) were recorded by AMF @ 5 grams + IBA 500 ppm (B_1G_2).

The results in present study corroborated with the findings of Subramanian *et al*., (2003) [12] who inferred that *Azospirillum* application helps to fix nitrogen in soil and also produce growth promoting substances like GA and cytokinins and antifungal substances, which could be the reason for enhanced vegetative growth of the cuttings. Okon (1985) [7] revealed that *Azospirillum* inoculated plants has the ability to absorb the nutrients at faster rate from soil solution than inoculated plants.

Enhanced mineral uptake (Nair and Chandran, 2001) [5] and phyto-hormone producing ability of *Azospirillum* might have enhanced growth and establishment of rooted cuttings. Earlier findings revealed that *Azospirillum* inoculation could improve ion uptake and contributed to the significant elevation of plant growth (Lin *et al*., l983; Nath and Korla, 2000 and Molla *et al*., 2001) [3, 6, 4].

Kumari *et al*. (2010) [2] observed that hormonal treatment (IBA) significantly increased sprouting in *Jatropa curcas* cuttings. This is because auxins positively influence cell enlargement, bud formation and root initiation and also promote the production of other hormone in conjunction with cytokinins (Osborne and McManus, 2005) [8]. Similar trend was noticed by Srivastava *et al*. (2008) in kiwi fruit leafless cuttings and Thota (2012) [13] in fig (*Ficus Caria*) cuttings. The promotory effect of IBA on rooting and shooting of stem cuttings has been reported by several workers. Chalapathi *et al*. (2001) [1] reported superior sprouting and survival percentage in stevia cuttings treated with IBA 500 ppm.

Earlier findings of Sharma (2009) [10] revealed that treatment with IBA 500 ppm increases the shoot height in *Premna integrifolia* hard wood cuttings. The increase in the length of the shoot with the increase in concentration of IBA was also noted by Shivanna *et al*. (2006) in Jeevanthi (*Leptadenia reticulata*).

Table 1: Effect of bio-agents and growth stimulators on no. of days taken for first sprouting in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	22.07	18.40	19.55	26.10	24.87	26.83	30.53	31.87	25.03
B_2	21.43	18.23	18.27	21.10	21.10	23.44	29.57	29.87	22.88
B_3	25.10	19.67	23.13	28.77	29.33	30.17	31.50	32.47	27.52
Mean of G	22.87	18.77	20.32	25.32	25.10	26.81	30.53	31.40	

	SEm±	CD (0.05)
B	0.35	0.99
G	0.57	1.62
B X G	0.99	2.81

Table 2: Effect of bio-agents and growth stimulators on no. of days taken for 50% sprouting in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	25.39	22.42	23.21	28.47	29.20	28.19	35.70	36.00	28.57
B_2	23.23	21.73	21.82	23.83	25.78	25.87	32.13	33.20	25.95
B_3	26.73	23.20	24.93	29.07	30.87	34.07	35.87	37.87	30.33
Mean of G	25.12	22.45	23.32	27.12	28.62	29.38	34.57	35.69	
	SEm±				CD (0.05)				
B	0.31				0.87				
G	0.50				1.42				
B X G	0.87				2.46				

Bio-agents (B)
B_1- Arbuscular mycorrhizal fungi (AMF) @ 5 grams
B_2- Azospirillum @ 5 grams
B_3- No bio-agent

Growth stimulators (G)
G_1- NAA 500 ppm
G_2- IBA 500 ppm
G_3- NAA 250 ppm + IBA 250 ppm
G_4- Common sugar solution 2%
G_5- Common sugar solution 3%
G_6- Mono Ammonium Phosphate (MAP) 1%
G_7- Mono Ammonium Phosphate (MAP) 2%
G_8- Fresh water dip

Table 3: Effect of bio-agents and growth stimulators on per cent survival (%) in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	60.95	74.59	71.93	53.33	53.42	37.14	33.33	36.22	52.61
B_2	68.61	85.25	83.65	60.48	60.80	56.22	53.89	54.15	65.38
B_3	30.00	46.67	43.33	36.67	23.33	20.00	20.00	26.67	30.83
Mean of G	53.19	68.84	66.30	50.16	45.85	37.79	35.74	39.01	
	SEm±				CD (0.05)				
B	1.23				3.49				
G	2.00				5.70				
B X G	3.47				9.87				

Table 4: Effect of bio-agents and growth stimulators on height of the sprouted shoot (cm) in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	11.20	11.88	11.46	10.20	9.50	9.83	8.90	6.84	9.98
B_2	10.85	14.07	13.65	10.60	9.80	10.10	9.79	9.20	11.01
B_3	9.70	11.39	10.67	8.70	7.99	8.50	7.80	6.77	8.94
Mean of G	10.58	12.45	11.93	9.83	9.10	9.48	8.83	7.61	
	SEm±				CD (0.05)				
B	0.27				0.76				
G	0.44				1.24				
B X G	0.76				2.15				

Bio-agents (B)
B_1- Arbuscular mycorrhizal fungi (AMF) @ 5 grams
B_2- Azospirillum @ 5 grams
B_3- No bio-agent

Growth stimulators (G)
G_1- NAA 500 ppm
G_2- IBA 500 ppm
G_3- NAA 250 ppm + IBA 250 ppm
G_4- Common sugar solution 2%
G_5- Common sugar solution 3%
G_6- Mono Ammonium Phosphate (MAP) 1%
G_7- Mono Ammonium Phosphate (MAP) 2%
G_8- Fresh water dip

Table 5: Effect of bio-agents and growth stimulators on number of leaves in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	1.59	2.09	2.06	1.50	1.34	1.00	1.00	1.00	9.98
B_2	1.66	2.80	2.33	2.04	1.93	1.00	1.00	1.00	11.01
B_3	1.00	1.97	1.16	1.18	1.23	1.00	1.00	1.00	8.94
Mean of G	1.42	2.29	1.85	1.57	1.50	1.00	1.00	1.00	
	SEm±				CD (0.05)				
B	0.07				0.19				
G	0.11				0.31				
B X G	0.19				0.53				

Table 6: Effect of bio-agents and growth stimulators on leaf length (cm) in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	7.14	7.73	7.68	7.32	7.63	6.87	5.85	5.81	7.00
B_2	7.28	8.59	8.46	7.30	7.96	7.29	7.10	6.82	7.60
B_3	6.21	7.69	6.90	5.10	6.44	5.70	5.20	5.02	6.03
Mean of G	6.88	8.01	7.68	6.57	7.34	6.62	6.05	5.88	
	SEm±				CD (0.05)				
B	0.09				0.26				
G	0.15				0.42				
B X G	0.25				0.73				

Bio-agents (B)
B_1- Arbuscular mycorrhizal fungi (AMF) @ 5 grams
B_2- Azospirillum @ 5 grams
B_3- No bio-agent

Growth stimulators (G)
G_1- NAA 500 ppm
G_2- IBA 500 ppm
G_3- NAA 250 ppm + IBA 250 ppm
G_4- Common sugar solution 2%
G_5- Common sugar solution 3%
G_6- Mono Ammonium Phosphate (MAP) 1%
G_7- Mono Ammonium Phosphate (MAP) 2%
G_8- Fresh water dip

Table 7: Effect of bio-agents and growth stimulators on leaf length (cm) in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	4.45	5.10	5.02	3.82	4.63	3.83	2.84	2.71	4.05
B_2	4.95	5.54	5.36	3.78	5.03	4.58	3.86	3.79	4.61
B_3	3.15	4.92	3.75	2.43	3.30	2.85	2.64	2.10	3.14
Mean of G	4.18	5.19	4.71	3.34	4.32	3.75	3.11	2.87	
	SEm±				CD (0.05)				
B	0.05				0.15				
G	0.08				0.24				
B X G	0.14				0.41				

Table 8: Effect of bio-agents and growth stimulators on number of roots in black pepper.

Bio-agents (B)	Growth stimulators (G)								Mean of B
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
B_1	5.80	8.77	8.54	6.07	6.83	5.98	3.31	3.05	6.05
B_2	6.50	8.37	8.45	5.70	6.22	5.69	3.07	3.01	5.88
B_3	5.55	6.50	5.70	3.10	3.03	2.98	2.71	2.89	4.06
Mean of G	5.95	7.88	7.56	4.96	5.36	4.88	3.03	2.98	
	SEm±				CD (0.05)				
B	0.19				0.54				
G	0.31				0.88				
B X G	0.54				1.53				

Bio-agents (B)
B_1- Arbuscular mycorrhizal fungi (AMF) @ 5 grams
B_2- Azospirillum @ 5 grams
B_3- No bio-agent

Growth stimulators (G)
G_1- NAA 500 ppm
G_2- IBA 500 ppm
G_3- NAA 250 ppm + IBA 250 ppm
G_4- Common sugar solution 2%
G_5- Common sugar solution 3%
G_6- Mono Ammonium Phosphate (MAP) 1%
G_7- Mono Ammonium Phosphate (MAP) 2%
G_8- Fresh water dip

Table 9: Effect of bio-agents and growth stimulators on length of the longest root (cm) in black pepper.

Bio-agents (B)		Growth stimulators (G)								Mean of B
		G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	
	B_1	10.87	13.42	12.93	9.14	7.49	8.54	7.20	7.02	9.58
	B_2	10.85	13.19	12.80	9.13	7.33	7.97	7.18	6.85	9.41
	B_3	7.57	11.77	9.43	5.85	5.75	5.52	5.67	4.94	7.06
Mean of G		9.76	12.79	11.72	8.04	6.86	7.34	6.68	6.27	
		SEm±				CD (0.05)				
B		0.12				0.35				
G		0.20				0.58				
B X G		0.35				1.00				

Bio- agents (B)
B_1- Arbuscular mycorrhizal fungi (AMF) @ 5 grams

Growth stimulators (G)
G_1- NAA 500 ppm

B_2- Azospirillum @ 5 grams
B_3- No bio-agent

G_2- IBA 500 ppm
G_3- NAA 250 ppm + IBA 250 ppm
G_4- Common sugar solution 2%
G_5- Common sugar solution 3%
G_6- Mono Ammonium Phosphate (MAP) 1%
G_7- Mono Ammonium Phosphate (MAP) 2%
G_8- Fresh water dip

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