Plant Archives Vol. 26, No. 1, 2026 pp. 2254-2258 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.312

EFFECT OF BIO-AGENTS ON GROWTH, ANATOMICAL AND PHYSIOLOGICAL PERFORMANCE OF ORTHOTROPIC CUTTINGS IN BLACK PEPPER (*PIPER NIGRUM* L.)

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(Date of Receiving : 02-01-2026; Date of Revision : 26-02-2026; Date of Acceptance : 10-03-2026)

ABSTRACT

Black pepper (*Piper nigrum* L.) is propagated through orthotropic cuttings. The present study evaluated the influence of bio-agents on growth, anatomical and physiological attributes of black pepper cuttings under net house conditions. Three treatments were tested: arbuscular mycorrhizal fungi (AMF @ 5 g per cutting), Azospirillum (@ 5 g per cutting) and an untreated control. Bio-agent application significantly enhanced vegetative growth at 60 days after transplanting (DAT). Azospirillum recorded the highest shoot height (6.95 cm), number of leaves (1.24), leaf length (5.02 cm), leaf breadth (3.60 cm) and leaf area (12.53 cm²), showing clear superiority over the control. Petiole length (2.41 cm) and internodal length (3.13 cm) were also significantly improved. Shoot girth and number of branches, however, did not differ significantly among treatments. Anatomical characteristics were positively influenced by Azospirillum, which exhibited greater lamina thickness (0.27 mm) and stomatal frequency (88.75). At 90 DAT, physiological performance was markedly enhanced under Azospirillum treatment, with maximum total dry matter production (2.68 g), chlorophyll content (19.95 SPAD units) and relative water content (68.75%), whereas root girth remained non-significant. Overall, Azospirillum @ 5 g per cutting consistently improved vegetative vigor, leaf structural traits and physiological efficiency, indicating its potential as an effective bio-inoculant for enhancing nursery establishment and quality planting material production in black pepper.

***Keywords*:** Black pepper (*Piper nigrum* L.), bio-agents, growth, anatomical and physiological attributes.

Introduction

Black pepper (*Piper nigrum* L.) belonging to the family Piperaceae is one of the most important spice crops of India and the world (Reshma *et al.*, 2022), widely valued for its dried fruits (peppercorns) used as a spice, seasoning and traditional medicine. It is native to the Malabar coast of India with a chromosome number of 2n = 52, and is extensively cultivated in Kerala, Karnataka, Tamil Nadu and other tropical regions. Black pepper is popularly known as the “King of Spices” due to its universal culinary demand and high export value (Damanhouri & Ahmad, 2014; Hu *et al.*, 2019). The pungency of black pepper is mainly attributed to the alkaloid piperine, which also contributes to its medicinal importance.

India is one of the major producers, consumers and exporters of black pepper globally (Kweho & Sharma). The area under cultivation in India is about 137 thousand hectares with a production of 66 thousand tonnes (National Horticulture Board, 2019–20). Despite its economic significance, availability of quality planting material remains a major constraint in expanding productivity and sustaining commercial cultivation.

Black pepper is commercially propagated through vegetative means, mainly by runner shoots developing at the base of the vine. However, vines raised from runner shoots generally start flowering only after the third year, and fruiting begins at a height of two to three feet above the base. In contrast, vines developed from terminal orthotropic shoots exhibit better early growth, produce a greater number of fruit-bearing lateral branches from the basal region and ensure better coverage of the standard, thereby resulting in improved productivity (Sarma *et al*., 2013) Furthermore, orthotropic shoots are comparatively free from soil contamination, whereas runner shoots often carry soil-borne pathogens such as Phytophthora, leading to infection and poor establishment (Anandaraj, 2000).

Rooting and establishment of black pepper cuttings are influenced by several factors including type of cutting, pre-treatment methods, rooting medium and environmental conditions. Among these, application of bioagents such as Arbuscular Mycorrhizal Fungi (AMF) and Azospirillum are known to improve nutrient uptake, enhance microbial activity and produce growth-promoting substances, thereby supporting better establishment of cuttings (Subramanian *et al*., 2003; Thanuja *et al*., 2002).

Although several studies have reported the beneficial role of bioagents in rooting of black pepper cuttings, there exists considerable variation and contradiction regarding the optimum combinations and treatments for maximum growth and nursery performance. Therefore, identification of suitable bioagents for improving early growth of orthotropic cuttings is regarded as an important priority area in black pepper nursery research.

Hence, the present investigation entitled “Effect of Bio-agents on Growth, Anatomical and Physiological Performance of Orthotropic Cuttings in Black Pepper (*Piper nigrum* L.)” was undertaken to evaluate the role of AMF and Azospirillum in enhancing nursery establishment and production of quality planting material.

Materials and Methods

The present investigation was carried out during February 2022 to July 2022 at Kalavalapalli village, West Godavari district, Andhra Pradesh, under net house conditions at the Horticultural Research Station, Kovvur, Dr. Y.S.R. Horticultural University, during the academic year 2021–2022. Black pepper (*Piper nigrum* L.) variety IISR-Thevam was propagated through four-node orthotropic semi-hardwood cuttings collected from healthy, pest- and disease-free mother vines.

The cuttings were planted individually in polythene bags (20 × 15 cm) filled with a sterilized potting mixture consisting of soil, sand and farmyard manure in the ratio of 2:1:1, enriched with Trichoderma to suppress soil-borne pathogens. Regular irrigation and intercultural operations were followed to ensure proper establishment of the cuttings.

The experiment was laid out in a factorial completely randomized design (FCRD) with three replications. Bio-agent treatments consisted of arbuscular mycorrhizal fungi (AMF @ 5 g per pit), Azospirillum (@ 5 g per pit) and an untreated control (no bio-agent). The inoculants were applied at the time of planting to ensure effective colonization at the basal portion of the cuttings. Although the overall experiment included growth stimulator treatments, the present manuscript reports only the main effect of bio-agents, with means pooled across growth stimulator treatments.

Biometric observations were recorded at 60 days and 90 days. Parameters such as shoot height, shoot girth, number of leaves, leaf length, leaf breadth, leaf area, petiole length, internodal length, leaf lamina, stomatal frequency and number of branches were assessed. Rooting and physiological traits including root girth, chlorophyll content (SPAD units), relative water content (%) and total dry matter production were recorded at 90 days after planting.

The data recorded on various parameters were subjected to statistical analysis following the procedure outlined by Fisher (1926) for a factorial completely randomized design (FCRD). The significance of differences among treatments was tested using the F-test. Wherever the F-test indicated significant effects, critical difference (CD) values were calculated at the 5% level of significance to compare treatment means and determine statistical superiority or parity among the treatments.

Results and Discussion

The results are presented in Tables 1–3. Bio-agents significantly influenced growth, anatomical and physiological parameters of black pepper orthotropic cuttings. The significance of treatment differences was determined using Critical Difference (CD) at 5% level. CD represents the minimum difference required between two treatment means for them to be considered statistically different at $P = 0.05$. When the difference between means exceeds the CD value, the treatments are considered significantly different.

Growth parameters

Bio-agent application significantly enhanced vegetative growth at 60 DAT (Table 1). Azospirillum @ 5 g (B2) recorded the maximum shoot height (6.95 cm), which was significantly superior to AMF@ 5 g (B1) (6.30 cm) and control (B3) (5.18 cm), as the mean difference exceeded the CD (0.05) value of 0.36. Similarly, number of leaves was significantly higher in B2 (1.24) compared to B1 and B3 (1.08 each). Leaf growth parameters were markedly improved under Azospirillum treatment. B2 registered maximum leaf length (5.02 cm), leaf breadth (3.60 cm) and leaf area (12.53 cm²), which were significantly higher than control (3.97 cm, 2.57 cm and 6.48 cm², respectively), since the observed differences exceeded the respective CD values (0.26, 0.12 and 0.69). Petiole length (2.41 cm) and internodal length (3.13 cm) were also significantly higher in B2 than control. However, shoot girth and number of branches did not differ significantly, as indicated by non-significant CD values.

Table 1 : Effect of bio-agents on growth parameters of black pepper at 60 DAT

Bio-agents (B)	Shoot height (cm)	Shoot girth (cm)	No. of leaves	Leaf ength (cm)	Leaf breadth (cm)	Leaf area (cm²)	Petiole length (cm)	Internodal length (cm)	No. of branches
B1	6.3	0.17	1.08	4.4	3.19	10.13	2.11	2.95	1
B2	6.95	0.18	1.24	5.02	3.6	12.53	2.41	3.13	1
B3	5.18	0.14	1.08	3.97	2.57	6.48	1.84	2.34	1
SEm±	0.13	0.01	0.03	0.09	0.04	0.24	0.03	0.02	0.01
CD (0.05)	0.36	NS	0.08	0.26	0.12	0.69	0.09	0.05	NS

Footnote: B1 – Arbuscular mycorrhizal fungi (AMF) @ 5 g, B2 – Azospirillum @ 5 g, B3 – No bio-agent (Control), NS – Not significant at 5% level, Values are mean ± SE. DAT = Days after treatment.

The enhanced vegetative growth under Azospirillum may be attributed to biological nitrogen fixation and production of growth-promoting substances such as auxins and gibberellins, which stimulate cell division and elongation (Subramanian *et al*., 2003). Similar improvements in black pepper cuttings due to Azospirillum inoculation were reported by (Nimisha, 2014).

Anatomical parameters

Anatomical traits were significantly influenced by bio-agents (Table 2). Azospirillum recorded higher leaf lamina thickness (0.27 mm) compared to control (0.19 mm), exceeding the CD value (0.01). Stomatal frequency was also significantly higher in B2 (88.75) than control (65.75), surpassing the CD (1.37). Increased lamina thickness and stomatal density indicate improved leaf structural development and potential enhancement in gas exchange efficiency.

Table 2 : Effect of bio-agents on anatomical parameters of black pepper at 60 DAT

Bio-agents (B)	Leaf lamina thickness (mm)	Stomatal frequency
B1	0.24	85.38
B2	0.27	88.75
B3	0.19	65.75
SEm±	0.00	0.48
CD (0.05)	0.01	1.37

Footnote: B1 – Arbuscular mycorrhizal fungi (AMF) @ 5 g, B2 – Azospirillum @ 5 g, B3 – No bio-agent (Control), NS – Not significant at 5% level, Values are mean ± SE.

Improvement in anatomical features under bio-inoculation has been associated with better nutrient uptake and physiological regulation (Ramakrishnan & Selvakumar, 2012). Enhanced chlorophyll and structural development under biofertilizer application have been documented earlier (Patidar & Mali, 2004; Selvakumar & Thamizhiniyan, 2011).

Fig. 1: Root parameters as influenced by of bio-agents

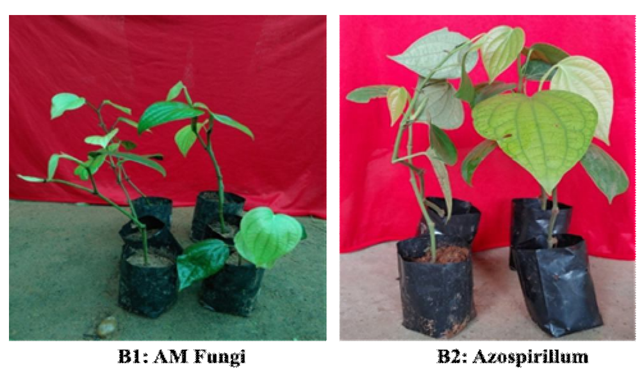

Fig. 2: Growth parameters as influenced by bio-agents.

Root and physiological parameters

Root and physiological parameters at 90 DAT further supported the positive role of bio-agents (Table 3). Total dry matter production was significantly higher in Azospirillum-treated cuttings (2.68 g) compared to control (1.93 g), as the difference exceeded CD (0.26). Chlorophyll content was significantly higher in B2 (19.95 SPAD) than control (15.78 SPAD), exceeding CD (0.22). Relative water content was also significantly improved in B2 (68.75%) compared to control (57.22%), with differences greater than CD (3.87).

Root girth did not show significant variation among treatments. The increased dry matter and physiological characters under Azospirillum may be due to improved nutrient assimilation and metabolic activity. Similar enhancement of physiological traits due to Azospirillum inoculation has been reported (Fasciglione *et al*., 2012).

Table 3 : Effect of bio-agents on root and physiological parameters of black pepper at 90 DAT

Bio-agents (B)	Root girth (cm)	Total dry matter (g)	Chlorophyll (SPAD)	Relative water content (%)
B1	0.13	2.23	18.83	64.64
B2	0.14	2.68	19.95	68.75
B3	0.11	1.93	15.78	57.22
SEm±	0.01	0.09	0.08	1.36
CD (0.05)	NS	0.26	0.22	3.87

Footnote: B1 – Arbuscular mycorrhizal fungi (AMF) @ 5 g, B2 – Azospirillum @ 5 g, B3 – No bio-agent (Control), NS – Not significant at 5% level, Values are mean ± SE. DAT = Days after treatment.

Overall, Azospirillum @ 5 g consistently exhibited superior performance in most growth, anatomical and physiological parameters compared to AMF and control, indicating its potential in improving nursery establishment of black pepper cuttings.

Conclusion

Bio-agents significantly influenced vegetative growth, anatomical development and physiological performance of orthotropic cuttings in black pepper. Among the treatments, Azospirillum @ 5 g per cutting proved superior by recording higher shoot growth, improved leaf development, enhanced lamina thickness and stomatal frequency, increased dry matter production, higher chlorophyll content and improved relative water content.

Therefore, Azospirillum @ 5 g can be recommended as an effective bio-agent for improving nursery performance and production of quality planting material in black pepper (*Piper nigrum* L.).

References

Anandaraj, M. (2000). Diseases of black pepper. *Black pepper, Piper nigrum*, 239–252.

Carina, V. T., Emilia, M. M., Miguel, U. S., Ortiz, D. R., Manjunatha, B., Thangaswamy, S., & Mulla, S. I. (2016). Effect of Arbuscular mycorrhizal fungi (AMF) and Azospirillum on growth and nutrition of banana plantlets during acclimatization phase. *Journal of Applied Pharmaceutical Science*, **6**(6), 131–138.

Cruz Romero, W., Barrios Díaz, J. M., Rodríguez Mendoza, M. d. l. N., Espinoza Victoria, D., & Tirado Torres, J. L. (2016). Production of vegetable seedlings with Azospirillum sp. and foliar spray with bee honey. *Revista mexicana de ciencias agrícolas*, **7**(1), 59–70.

Damanhouri, Z. A., & Ahmad, A. (2014). A review on therapeutic potential of Piper nigrum L. *Black Pepper): The King of Spices*. *Med. Aromat. Plants*, *3*(3), 161.

Fasciglione, G., Casanovas, E. M., Yommi, A., Sueldo, R. J., & Barassi, C. A. (2012). Azospirillum improves lettuce growth and transplant under saline conditions. *Journal of the Science of Food and Agriculture*, **92**(12), 2518–2523.

Hu, L., Xu, Z., Wang, M., Fan, R., Yuan, D., Wu, B., Wu, H., Qin, X., Yan, L., & Tan, L. (2019). The chromosome-scale reference genome of black pepper provides insight into piperine biosynthesis. *Nature communications*, **10**(1), 4702.

Kweho, M. V., & Sharma, A. Department of Agricultural Economics, Nagaland University School of Agricultural Sciences, Medziphema Campus District: Chumoukedima, Nagaland.

Nimisha. (2014). Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper *(Piper nigrum L.)*. Kerala Agricultural University]. Vellayani.

Patidar, M., & Mali, A. (2004). Effect of farmyard manure, fertility levels and bio-fertilizers on growth, yield and quality of sorghum (*Sorghum bicolor*). *Indian Journal of Agronomy*, **49**(2), 117–120.

Prongjunthuek, K., Meunchang, P., & Panichsakpatana, S. (2019). Effects of Azospirillum on germination and seedling growth of commercial sweet corn varieties Insee 2 and Hi-Brix 3. *Seed*, **10**, 100.

Ramakrishnan, K., & Selvakumar, G. (2012). Effect of biofertilizers on enhancement of growth and yield on Tomato *(Lycopersicum esculentum* Mill.). *Int J Res Bot*, **2**(4), 20–23.

Reshma, P., Neethu, R., & Sreekala, G. (2022). Genetic diversity of black pepper (*Piper nigrum* L.) in India: A review. *J. Pharm. Innov*, **11**(10), 832–839.

Sarma, Y., Manohara, D., Premkumar, T., & Nair, R. (2013). Orthotropic shoots as source of planting material in black pepper: An approach to boost up production levels. *Spice India*, **26**(8), 4–9.

Selvakumar, G., & Thamizhiniyan, P. (2011). The effect of the arbuscular mycorrhizal (AM) fungus Glomus intraradices on the growth and yield of chilli (*Capsicum annuum* L.) under salinity stress.

Subramanian, S., Rajeswari, E., Chezhiyan, N., & Shiva, K. (2003). Effect of Azospirillum and graded levels of nitrogenous fertilizers on growth and yield of turmeric (*Curcuma longa* L.). National Seminar on New Perspectives in Spices, Medicinal and Aromatic Plants,

Thanuja, T., Hegde, R. V., & Sreenivasa, M. (2002). Induction of rooting and root growth in black pepper cuttings (*Piper nigrum* L.) with the inoculation of arbuscular mycorrhizae. *Scientia horticulturae,* **92**(3-4), 339–346.