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Quality and resistance optimization of banana (*Musa acuminata* L.) vegetative seeds through the addition of indole butyric acid with biological agents induction against fusarium wilt disease intensity

Abstract. Banana plants are a vital agricultural commodity in Indonesia, but Fusarium wilt disease (Fusarium oxysporum) significantly hampers production. This research aims to improve the quality and resilience of vegetative banana seedlings by selecting superior seedlings and vegetative propagation techniques such as budding, shoot cutting, and tissue culture management. The research used a randomized complete block design (RCBD) factorial with a combination of two factors, namely the addition of IBA and biological agents, with nine treatment combinations and three replications. IBA treatment significantly affected the initial emergence of shoots and leaf area, with the best result from treatment Z2 (IBA concentration of 1.5 g/l). The biological agent factor treatment significantly affected plant height, lower stem diameter, number of leaves, and hypothetical vigor index, with the best value shown by treatment B1. The use of IBA and biological agents can optimize the quality and resilience of vegetative banana seedlings, aiding farmers in enhancing the productivity and quality of agricultural products sustainably.

Keywords: Banana · Biological agent Fusarium wilt Disease · Vegetative seeds

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Introduction

Banana plants (*Musa acuminata* L.) are one of the important agricultural commodities in Indonesia due to their high economic value and widespread cultivation by farmers in various regions. Indonesia has a diverse collection of banana germplasm. According to (Iskandar et al., 2018)the 66 banana species in the world, 12 species have been recorded in Indonesia. Fourteen cultivars were found in Senduro and Pasrujambe Districts, with some of the leading cultivars in Lumajang including Agung Semeru, Mas Kirana, Cavendish, and others. However, one of the main problems in banana production is Fusarium wilt disease caused by the fungus *Fusarium oxysporum* f. sp. Cubense (Niwas et al., 2021). This disease can reduce the quality and quantity of banana fruit production and quickly spread and extensively damage plants (Mostert et al., 2017; Zakaria et al., 2024).

Fusarium is a plant pathogenic fungus that can attack various types of plants, including banana plants (Dita et al., 2018; Zakaria et al., 2024). This disease is caused by the fungus *Fusarium oxysporum* species that attacks the plants' roots, impeding water and nutrient flow, causing plants to wilt and eventually die (Pérez Vicente et al., 2014). The initial symptoms that appear are usually wilting and yellowing of the plant's lower leaves, followed by the drying up of these leaves and eventual death of the plant (Dita et al., 2021). This disease can spread through soil (Islam et al., 2021; Ivayani et al., 2018), infected seedling (Ploetz, 2015), or contaminated tools (Jamil et al., 2019). Control of fusarium disease can be done by using healthy seedlings, good sanitation, fungicide use, and biological agents that can control the growth of pathogenic fungi.

The percentage of damage to banana plants caused by Fusarium can vary depending on many factors, including the banana variety planted (Ploetz, 2015), environmental conditions, and the disease control methods applied. However, Fusarium disease can cause significant damage to banana plants, even leading to destruction(Islam et al., 2021). In some cases, Fusarium infection in banana plants can cause wilting symptoms and plant death relatively quickly. In other cases, milder symptoms may appear, such as leaf discolourations or brown spots on the banana stem (González et al., 2024)

Generally, the percentage of damage to banana plants caused by Fusarium can vary from a few percent to more than 50%, depending on the environmental conditions and severity of the disease in a particular area (Rampersad, 2020). Therefore, it is important to control Fusarium infection correctly to prevent further damage to banana plants. To address this problem, one effort can be made to improve the quality and resistance of vegetative banana seedlings. Quality and disease-resistant seeds can help farmers increase agricultural productivity and quality sustainably(Edel-Hermann & Lecomte, 2019). Therefore, research is needed to optimize the quality and resistance of vegetative banana seedlings.

Indole-3-butyric acid (IBA) is a synthetic auxin crucial in enhancing plant growth, particularly by promoting root induction and elongation. It stimulates cell division and differentiation in the root meristem, leading to a more robust root system that improves the plant's ability to absorb water and nutrients, essential for healthy development. Additionally, IBA helps maintain hormonal balance within the plant, influencing other growth hormones such as cytokinins and gibberellins, and aids in the plant's response to environmental stresses by enhancing root development and overall resilience (Elyazid et al., 2021).

Biological agents like endophytic bacteria and *Lysinibacillus fusiformis* contribute signficantly to plant resistance against pathogens such as *Fusarium oxysporum*. Endophytic bacteria reside within plant tissues without causing harm and produce antimicrobial compounds that directly inhibit pathogen growth. The induce systemic resistance (ISR) within the plant, priming its immune system to respond more effectively to attacks and compete with pathogens for essential nutrients, limiting the pathogen's ability to thrive.

This study conducted a combination of treatments of adding IBA and biological agents to determine their effect on the acclimatization and initial growth conditions of vegetative banana seedlings and the effectiveness of biological agents in controlling Fusarium wilt disease. This research is expected to effectively improve the quality and resistance of banana vegetative seedlings and the problem of Fusarium wilt disease.

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Materials and Methods

Preparation of Isolate Endophytic Bacteria. The Isolate Endophytic Bacteria suspension solution was prepared by adding 10 ml of sterile distilled water into a petri dish containing the endophytic bacterial isolate. Then, 1 ml of the endophytic isolate was taken and mixed with 50 ml of Potato Dextrose Broth (PDB) solution (Zakia et al., 2017). The preparation of the growing media referred to the research by (Nurhayati et al., 2022) which involved a mixture of cocopeat, charcoal, and soil in a ratio of 1:1:2 (v/v/v). The homogenous media were placed in plastic pots and bags and rested for 2-3 days before planting. The acclimatization of the banana plants was carried out in the evening.

Seed preparation. Seed preparation involved cleaning the seeds from the remaining media, washing them thoroughly, and drying them at room temperature before planting in the prepared media (Safitri et al., 2023; Setyowati et al., 2024). The seeds were selected based on the complete plant organs (roots, stem, and leaves), with a green non-transparent apex stem, robust growth, roots filling the media, and 3-4 cm height, with an age of 3 months after multiplication (Mekaunint et al., 2024). Then, the plantlets were ready to be planted in the acclimatization media. The planted media were covered with a UV plastic lid and placed in a room with a 40-watt lamp and a temperature of ±22-27°C and air humidity ±43% (Krishna et al., 2022; Setyowati et al., 2024)., or in a room exposed to sunlight. After 3 weeks, the plants were transplanted into plastic bags containing a mixture of cocopeat, charcoal, and Malang sand in a ratio of 1:1:1 (v/v/v) (Chaidir et al., 2021). The treatments were applied in the 4th week after transplanting into the polybag media by adding each treatment in liquid form after planting the banana seeds into the polybag.

The Endophytic Inoculation. The endophytic inoculation meth(Septia & Parlindo, 2019)ch by (Septia & Parlindo, 2019) with modification. 10-20 ml of the endophytic isolate bacterial suspension solution with a concentration of 108-109 cfu/ml was sprinkled around the roots at a 25 ml/plant. Plant growth regulator (PGR) IBA (Yusnita et al., 2018) was applied according to the treatment concentration dose, 1 g/l and 1.5 g/l, by mixing the IBA powder with technical solvent solution and then adding water. The dose of IBA given to the plant was 25 ml/plant. The application of liquid organic fertilizer was carried out according to the concentration, which was 20 ml/l, and the dose given to the plant was 25 ml/plant.

Plant Maintenance. Plant maintenance included watering, weeding, pest control, and fertilization. Pest control was carried out using Dupont brand pesticide with 40% active ingredient methomyl, applied by spraying every 2 weeks (Tama & Frimansyah, 2023). The first subsequent fertilization was carried out one month after planting using the Urea fertilizer with an injection into soil method of 15 g/l, with a dose of 250 ml per plant (de Barros et al., 2023).

The Resistance Test of The Banana Plant. The resistance test of the vegetative banana seedlings was obtained from farmers. The tested seedling was aged 2-3 months. The planted seedlings in the polybag were arranged according to the treatment plan on the Edu Park field of Muhammadiyah Malang University. Planting was carried out using a soil medium of 2 kg consisting of a mixture of garden soil and kendang fertilizer in a ratio of 2:1, which was then sterilized using an autoclave at a pressure of 1.4 psi at 121°C for 20 minutes(Rocha et al., 2021).

Inoculate Pathogenic Fungi and Biological Agents. The initial stage is to inoculate pathogenic fungi and biological agents into polybag banana seedlings by watering. The concentration of the biological agent suspension was calculated with a hemocytometer until it reached a density according to the treatment (Milan et al., 2024). Calculation of conidia density can be done with a hemocytometer using the formula of Sudarjat et al., (2024):

$$K = \frac{(t \times d)}{(n \times 0{,}25)} \times 10^6$$

Note:
K= Number of fungal conidia
t = Total conidia in all boxes counted
d = dilution factor
n = The sum of all squares counted

The initial stage of watering is to sprinkle 25 ml/plant of the Fusarium pathogenic fungus in the root area of the banana seedlings and leave it for one week. After that, the banana seeds are watered with biological agents according to each treatment, and the amount is as much as 25 ml/per plant (Soesanto et al., 2011). Then, the Leaf Symptom Index (LSI), the

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symptoms of wilting on the leaves, is observed seven times weekly, starting one week after inoculation. Rhizome Discoloration Index (RDI), or observation of rotting symptoms on rhizomes, was carried out during the last observation using the method Mak *et al*. (2004).

Table 1. Leaf Symptom Index (LSI) based on (Djohan et al., 2020)

Score	Information
1	There are no yellow leaves. Plants look healthy
2	There is a yellow color on the lower leaves
3	The bottom three leaves turn yellow
4	The yellow color begins to spread or is found in all leaves
5	Plants die

Table 2. Rhizome Discoloration Index (RDI) based on (Ribeiro et al., 2011)

Score	Information
1	There was not discoloration of the Pseudostem and root area or around the tissue
2	There does not discoloration on the Pseudostem, the change is at the junction of the roots and the root area
3	Discoloration of up to 5% on the Pseudostem
4	6−20% discoloration on pseudostem
5	21−50% discoloration on pseudostem
6	More than 50% discoloration on pseudostem
7	Discoloration on all of the root area
8.	Plant die

Resistance criteria are determined based on both DSI and LSI, and symptoms of discoloration in the root area (RDI)(Ribeiro et al., 2011; Sutanto et al., 2011). DSI for each treatment is calculated using the following formula:

$$\text{DSI} = \frac{\sum(\text{score} \times \text{number of plants in that score})}{\text{treated seeds}}$$

Table 3. Robustness criteria based on DSI based on the method of (Djohan et al., 2020)

DSI Scale for LSI	DSI Scale for RDI	Resistance Criteria
1	1	Resistant
1.1-2	1.1-3	Tolerant
2.1-3	3.1-5	Susceptible
3.1-4	5.1-8	Very Vulnerable

Resistance criteria include resistance, tolerant, susceptible, and very vulnerable, which are determined based on the DSI scale.

Results and Discussion

Shoot Emergence. The analysis of variance in the combination treatment of indole butyric acid and biological agent did not show any interaction that had a significant effect on the shoot emergence variable. The average results of the shoot emergence variables are presented in Table 4.

Table 4. Average shoot emergence of Barangan banana (*Musa acuminata* l.) after application of indole butyric acid treatment and biological agents in the greenhouse

Treatments	Shoots emergence (day after acclimatization)
IBA	
Z0	7.22 a
Z1	6.77 b
Z2	6.83 ab
Tukey 5%	0.55
Biological Agent	
B0	7.05 a
B1	6.88 a
B2	6.88 a
Tukey 5%	0.55

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

The results of the analysis of various variables for the initial observation of shoot emergence showed that there was no interaction between IBA treatment and biological agent factors. However, the IBA treatment factor showed significantly different values between Z0 and Z1 treatments, and neither treatment was significantly different from the Z2 treatment.

The biological agent treatment factor showed values that were not significantly different between B0, B1, and B2 treatments. The biological agents used in B1 and B2 might not have been specifically formulated or effective for promoting shoot emergence. IBA is a type of auxin, which is a hormone that stimulates root growth (Khadr et al., 2020), which is the center of metabolism in the

formation of new plant organs which is influenced by the interaction of endogenous and exogenous hormones so that they can produce shoots (Wu et al., 2024; Iqbal et al., 2022).

Plant Height. The results of the analysis of variance in the combination treatment of IBA and biological agent did not show any interaction that had a significant effect on the plant height variable. The average plant height results are presented in Table 5 and Table 6.

The IBA treatment factor shows values that are not significantly different between Z1, Z2, and Z3 treatments. Stem growth is influenced by auxin and cytokinin which is supported by the availability of food intake in the media and the results of the photosynthesis process in the seed leaves (Sosnowski et al., 2023). The presence of the natural auxin hormone in the plant's young leaves has a significant effect on plant growth (Ogunyale OG et al., 2014). So that, the addition of the auxin hormone does not have a significant(Nair & Padmavathy, 2014)rowth (Nair & Padmavathy, 2014).

Bottom stem diameter. The results of the analysis of variance in the combination treatment of IBA and biological agent did not show any interaction that had a significant effect on the stem diameter at the bottom variable. The results of the average bottom stem diameter are presented in Table 7 and Table 8.

Table 5. Average plant height of barangan banana (*Musa acuminata* L.) after the application of indole butyric acid treatment and biological agents in the greenhouse (cm) from 7-49 DAA

Treatments	Plant height (cm) - day after acclimatization (DAA)						
IBA	7	14	21	28	35	42	49
Z0	18.30 a	19.50 a	22.16 a	27.25 a	33.88 a	37.66 a	41.05 a
Z1	18.94 a	19.88 a	21.88 a	26.63 a	33.00 a	37.44 a	40.38 a
Z2	17.77 a	19.05 a	19.63 b	24.25 a	31.77 a	36.72 a	40.88 a
Biological Agent							
B0	17.77 b	18.27 b	19.11 b	23.97 b	30.44 b	35.22 b	39.61 b
B1	19.80 a	21.69 a	25.52 a	30.61 a	36.61 a	36.61 a	42.38 a
B2	17.44 b	18.47 b	19.05 b	23.55 b	31.61 b	23.55 b	39.88 b
Tukey 5%	1.99	2.16	2.13	3.24	3.21	3.07	2.18

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Table 6. Average plant height of barangan banana (*Musa acuminata* L.) after the application of indole butyric acid treatment and biological agents in the greenhouse (cm) from 56-91 DAA

Treatments	Plant height (cm) - day after acclimatization (DAA)					
IBA	56	63	70	77	84	91
Z0	40.27 a	40.83 a	43.33 a	61.44 a	67.94 a	69.66 a
Z1	40.88 a	41.11 a	43.88 a	59.05 a	67.11 a	68.72 a
Z2	40.72 a	41.05 a	43.66 a	61.16 a	68.00 a	70.16 a
Biological Agent						
B0	39.61 b	40.11 b	42.77 a	61.11 a	67.05 a	69.00 a
B1	42.38 a	42.72 a	45.05 a	60.61 a	69.27 a	71.05 a
B2	39.88 b	40.16 b	43.05 a	59.94 a	66.72 a	68.50 a
Tukey 5%	1.91	2.33	2.69	3.74	4.28	4.49

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

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The IBA treatment factor showed that there are significantly different values between Z2 and Z0 treatments only in the 6th week of observation; both treatments were not significantly different from Z1 treatment in the same week. The highest value was in Z2 treatment until the 13th week of observation. The biological agent treatment factor showed that B1 treatment showed significantly different values from B0 and B2 treatments at 2, 3, 4, 5, 6, 7, and 8 weeks of observation. The B0 treatment showed significantly different values from B1 treatment at 1 and 9 weeks of observation. The B2 treatment showed values that were not significantly different from B0 and B1 treatments in the first week of observation with the highest value in B1 treatment until the 13th week of observation. The treatment effect was expressed on plant diameter in the early growth phase. Both auxin and the biological agent given can stimulate an increase in cell number and size (Khan, 2023), so that it is directly proportional to the increase in plant diameter which is faster than the control.

Number of Leaves. The effect of Indole butyric acid and biological agent did not show any significant interaction on the variable number of plant leaves. The average number of leaves results are presented in Table 9 and Table 10.

The biological agent treatment factor shows that there are significantly different values in B0 and B1 treatment in 1, 3, and 4 weeks of observation, but not significantly different from B2 treatment in the same week of observation. The highest value was in treatment B1 until the 13th week of observation. Micronutrients are essential nutrients that plants need in small amounts, however, a lack of micronutrients can also limit plant development.

Table 7. Average bottom stem diameter of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse (mm) from 7-49 DAA

Treatments	Bottom stem diameters (mm) - day after acclimatization (DAA)						
IBA	7	14	21	28	35	42	49
Z0	5.45 a	6.15 a	7.32 a	9.12 a	11.18 a	13.05 a	13.05 a
Z1	5.48 a	6.07 a	7.15 a	9.28 a	10.82 ab	12.45 ab	12.45 ab
Z2	5.20 a	5.80 a	6.90 a	8.76 a	10.05 b	10.05 b	12.08 a
Biological Agent							
B0	5.17 b	5.64 b	6.50 b	8.65 b	9.81 b	11.78 b	14.21 b
B1	5.76 a	6.73 a	8.10 a	10.15 a	12.16 a	13.93 a	15.57 a
B2	5.20 ab	5.65 a	6.77b	8.37 b	10.09 b	11.86 b	14.36 b
Tukey 5%	0.56	0.57	0.66	0.76	1.01	0.85	0.94

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Table 8. Average bottom stem diameter of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse (mm) from 56-91 DAA

Treatments	Bottom stem diameters (mm) - day after acclimatization (DAA)					
IBA	56	63	70	77	84	91
Z0	15.53 a	16.31 a	19.02 a	22.12 a	24.56 a	25.42 a
Z1	15.43 a	16.25 a	19.22 a	22.40 a	24.45 a	25.45 a
Z2	15.09 a	15.83 a	18.69 a	21.90 a	24.76 a	25.52 a
Biological Agent						
B0	39.61 b	40.11 b	42.77 a	61.11 a	67.05 a	69.00 a
B1	42.38 a	42.72 a	45.05 a	60.61 a	69.27 a	71.05 a
B2	39.88 b	40.16 b	43.05 a	59.94 a	66.72 a	68.50 a
Tukey 5%	1.05	1.16	1.24	1.29	1.49	1.48

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Table 9. Average number of leaves of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse (DAA)

Treatments	Number of leaves - day after acclimatization (DAA)						
IBA	7	14	21	28	35	42	49
Z0	4.33 a	4.38 a	4.88 a	5.83 a	6.33 a	6.83 a	6.77 a
Z1	4.38 a	4.33 a	4.88 a	5.66 a	6.22 a	6.66 a	6.61 a
Z2	4.00 a	4.16 a	4.50 a	5.66 a	5.83 a	6.05 a	6.38 a
Biological Agent							
B0	4.00 b	4.22 ab	4.33 b	5.38 b	5.77 a	6.05 a	6.44 a
B1	4.55 a	4.72 a	5.44 a	6.27 a	6.44 a	6.72 a	6.61 a
B2	4.16 ab	3.94 b	4.50 b	5.50 b	6.16 b	6.77 a	6.72 a
Tukey 5%	0.76	0.75	0.89	0.81	1.08	1.08	0.77

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Table 10. Average number of leaves of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse (DAA)

Treatments	Number of leaves - day after acclimatization (DAA)					
IBA	56	63	70	77	84	91
Z0	6.77 a	6.77 a	6.94 a	7.83 a	8.50 a	8.55 a
Z1	6.61 a	7.00 a	6.83 a	7.83 a	8.33 a	8.61 a
Z2	6.50 a	7.00 a	6.61 a	7.55 a	8.05 a	8.05 a
Biological Agent						
B0	6.38 a	6.77 a	6.83 a	7.77 a	8.27 a	8.22 a
B1	6.55 a	7.00 a	6.83 a	7.66 a	8.27 a	8.66 a
B2	6.83 a	7.00 b	6.72 a	7.77 a	8.33 a	8.33 a
Tukey 5%	0.67	0.42	0.85	0.83	0.85	0.82

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Leaf Area. The analysis of variance results in the combination treatment of indole butyric acid and biological agent did not show any significant interaction on the leaf area variable. The results of the average leaf area are presented in Table 11. The IBA treatment showed significantly different values because IBA is an auxin hormone that regulates cell growth and elongation, so it has a significant influence on plant leaf area variables. The intensity of the light factor received also influences the plant leaf area (Dikayani et al., 2017).

Biological agents, such as beneficial bacteria and fungi, play a vital role in enhancing the availability and uptake of essential micronutrients in plants, which are crucial for various physiological processes, including leaf development. These microorganisms can solubilize minerals in the soil, making micronutrients like zinc, iron, and manganese more accessible to plant roots. They also produce chelating agents that bind to these nutrients, preventing them from becoming insoluble and enhancing their absorption.

Table 11. Average leaf area of barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse.

Treatments	Leaf aera (cm^2)
IBA	
Z0	275.10 b
Z1	273.38 b
Z2	309.03 a
Tukey 5%	5.03
Biological Agent	
B0	280.39 b
B1	300.18 a
B2	276.95 b
Tukey 5%	5.03

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

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By promoting root growth and forming symbiotic relationships with plants, such as mycorrhizal associations, these biological agents expand the root system's reach, allowing plants to access more nutrients. An adequate supply of micronutrients supports better chlorophyll production, efficient photosynthesis, and a balanced hormonal environment, all of which contribute to the initiation and growth of leaves. As a result, plants with enhanced micronutrient uptake tend to have a higher leaf count, better overall health, and increased resilience to environmental stresses.

Root Fresh Weight. The analysis of variance results in the combination treatment of Indole butyric acid and Biological Agent did not show any significant interaction on the root fresh weight variable. The results of the average root fresh weight are presented in Table 12. The IBA treatment factor shows values that are not significantly different between Z0, Z1, and Z2 treatments, with the highest value being Z0 treatment. The biological agent treatment factor shows values that are not significantly different between B0, B1, and B2 treatments with the highest value being in B1 treatment. The banana plant is a type of monocotyledonous plant whose body is mostly water. The phases of cell growth and death experienced by fleshy and dry species alike are extensively regulated by transient concentrations of phytohormones (Fenn & Giovannoni, 2021).

Table 12. Average fresh root weight of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse.

Treatments	Root fresh weight (gram)
IBA	
Z0	52.00 a
Z1	48.70 a
Z2	49.29 a
Tukey 5%	9.72
Biological Agent	
B0	49.93 a
B1	50.59 a
B2	49.46 a
Tukey 5%	9.72

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Root Dry Weight. The analysis of variance in the combination treatment of indole butyric acid and biological agent did not show any interaction that had a significant effect on the root dry weight variable. The results of the average dry weight of the roots are presented in Table 13. The IBA treatment factor shows values that are not significantly different between Z0, Z1, and Z2 treatments, with the highest value being in Z0 treatment. The biological agent treatment factor shows values that are not significantly different between B0, B1, and B2 treatments with the highest value being in B2 treatment.

Dry weight of the plant, indicates the actual weight of the plant. The dry weight shows net photosynthesis results of the plants and is an actual measure of the biomass (Amalia & Widiyanto, 2019; Dikayani et al., 2017) The higher the dry weight value of the plant indicates that the assimilation process in the plant is going well.

Table 13. Average root dry weight of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse.

Treatments	Root dry weight (gram)
IBA	
Z0	4.13 a
Z1	3.76 b
Z2	3.97 b
Tukey 5%	0.26
Biological Agent	
B0	3.86 b
B1	3.78 b
B2	4.21 a
Tukey 5%	0.26

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Root Length. The results of the analysis of variance in the combination treatment of Indole butyric acid and Biological Agent did not show any interaction that had a significant effect on the root length variable. The results of the average root length are presented in Table 14.

Table 14. Average root length of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse

Treatments	Root length (cm)
IBA	
Z0	72.22 a
Z1	73.00 a
Z2	63.38 a
Tukey 5%	17.19
Biological Agent	
B0	71.11 a
B1	69.55 a
B2	67.94 a
Tukey 5%	17.19

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Table 15. Average crown width of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse

Treatments	Crown width (cm)
IBA	
Z0	82.00 a
Z1	81.78 a
Z2	83.11 a
Tukey 5%	8.01
Biological Agent	
B0	83.61 a
B1	81.67 a
B2	81.61 a
Tukey 5%	8.01

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

The IBA treatment factor shows values that are not significantly different between Z0, Z1, and Z2 treatments, with the highest value being in Z1 treatment. The biological agent treatment factor shows values that are not significantly different between B0, B1, and B2 treatments, with the highest value being in B0 treatment.

Crown Width. The analysis of variance results in the combination treatment of Indole butyric acid and Biological Agent did not show any significant interaction on the crown width variable. The average header width is presented in Table 15.

Light intensity becomes a factor affecting the width of the plant header. The effect of sunlight on header width is inverse. If the higher the intensity of light hits the plant, the size of the plant crown decreases, on the other hand, if the intensity of light hitting the plant is low, the width of the plant crown is wider (Mahfudza & Linda, 2018).

Hypothetical Vigour Index. The results of the analysis of variance in the combination treatment of Indole butyric acid and Biological Agent did not show any interaction that had a significant effect on the hypothetical vigour index variable. The average results of the hypothetical vigour index are presented in Table 16 and Table 17

The B0 treatment differed significantly from B1 in the 5th and 6th weeks of observation, but neither differed significantly from treatment B2 in the same week of observation. The hypothetical vigour index is one of the variables used to determine the ability to grow in the field in the early growth phase. A higher hypothetical vigour index value indicates the potential of seeds to undergo initiation to become young plants (Rocha et al., 2021)

The Resistance Test of Banana Vegetative Seeds. The resistance test of banana vegetative seeds to pathogen application was carried out by inoculating 10^6 conidia/ml of *Fusarium* sp. into the root area of banana seedlings as much as 25 ml. Then induce biological agents B1, B2 conidia/ml as much as 25 ml, so that results are obtained as shown in Table 18.

The results of the DSI (Disease Severity Index) scale calculation as in table 14 show differences in Barangan banana plants' value and resistance status. In further testing, namely the ANOVA test, the results showed no significant difference between the effect of treatment on the parameters for observing Fusarium disease attacks in terms of the DSI calculation scale. Further testing using ANOVA showed that the results were not significantly different, which was thought to be caused by several factors, including allegedly because there were no sick roots of the Barangan banana plants due to injury or injury, so the soil fungi, especially the *Fusarium* sp. cannot infect plant tissue. According to (Ishak & Jumjunidang, 2024)*Fusarium* sp. carries infection into plant tissue through wounds on the roots. A similar opinion was expressed by (Islam et al., 2021; Hartati et al.,

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2016; Srivastava et al., 2018; Sukorini et al., 2021)stated that pathogens use injured roots or rhizomes as a way to enter plant tissue. Furthermore, the research results of (Hastuti & Rahmawati, 2016) showed that with the inoculation treatment of the fungus *Fusarium* sp. without injury, the percentage of root damage to pepper plants is 0%.

Injury to plant tissue as a medium for soil pathogen infection can occur naturally depending on soil conditions and the population of microorganisms in the soil. Soil conditions on conventional land are generally a habitat for various microorganisms ranging from fungi, bacteria, and nematodes. Nematodes are microorganisms in the soil that are important for plants because they can cause natural injury to plant tissue and act as disease-causing vectors, opening the way for other pathogens to enter. According to (Meena et al., 2016) the nematode type *Melodogyne incognita*. capable of producing wounds mechanically and chemically. Mechanically, the nematode can penetrate the roots using the stylet organ, causing the root tissue to open. Meanwhile, chemically, *Melodogyne incognita* releases enzyme activity so that plant cells are damaged and will act as chemotrophy for *Fusarium* sp.

Table 16. Average hypothetical vigour index of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse (DAA)

Treatments	Hypothetical vigour index - day after acclimatization (DAA)						
IBA	7	14	21	28	35	42	49
Z0	4.87 a	4.91 a	5.00 a	5.18 a	5.31 a	5.39 a	5.43 a
Z1	4.86 a	4.87 a	4.96 a	5.11 a	5.25 a	5.37 a	5.34 a
Z2	4.85 a	4.90 a	4.95 a	5.14 a	5.27 a	5.42 a	5.35 a
Biological Agent							
B0	4.81 a	4.85 b	4.88 b	5.07 b	5.20 a	5.29 a	5.36 a
B1	4.93 a	4.99 a	5.12 a	5.26 a	5.35 a	5.41 a	5.43 a
B2	4.85 a	4.85 b	5.10 b	5.10 b	5.28 ab	5.38 ab	5.42 a
Tukey 5%	0.18	0.15	0.12	0.15	0.67	0.15	0.12

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Table 17. Average hypothetical vigour index of Barangan banana (*Musa acuminata* L.) after application of indole butyric acid treatment and biological agents in the greenhouse (DAA)

Treatments	Hypothetical vigour index - day after acclimatization (DAA)					
IBA	56	63	70	77	84	91
Z0	5.41 a	5.42 a	5.46 a	5.66 a	5.74 a	5.76 a
Z1	5.38 a	5.40 a	5.42 a	5.61 a	5.61 a	5.72 a
Z2	5.43 a	5.46 a	5.46 a	5.67 a	5.67 a	5.76 a
Biological Agent						
B0	5.36 a	5.39 a	5.43 a	5.64 a	5.70 a	5.71 a
B1	5.42 a	5.46 a	5.47 a	5.65 a	5.74 a	5.77 a
B2	5.43 a	5.44 a	5.45 a	5.66 a	5.73 a	5.75 a
Tukey 5%	0.12	0.13	0.55	0.14	0.15	0.15

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on the Tukey test at the level of 5 %. ($p < 0.05$). Z0 (without IBA) Z1 (IBA concentration 1 g/l), Z2 (IBA concentration 1.5 g/l), B0 (without biological agents), B1 (*Lynicillus fusiformis*), B2 (isolate endophytic bacteria).

Table 18. Results of vegetative seedling resistance tests for banana plants (DSI)

Treatments	DSI			
	LSI	Resistance Criteria	RDI	Resistance Criteria
Control (-) B0-	2.31	Resistant	1.8	Susceptible
Control (+) B0+	1.86	Tolerant	1.1	Tolerant
Fusarium sp + B1	1.97	Tolerant	1	Resistant
Fusarium sp + B2	1.63	Tolerant	1.2	Tolerant

Note: B0-(without biological agent), B0+ (with product biological agent), B1 (*Lynicillus fusiformis*+ Fusarium sp), B2 (isolate endophytic bacteria+ Fusarium sp).

Septia E, Zakia A, Zainudin A, Maftuchah, Gofur A, Abbabiel Y. 2024. Quality and resistance optimization of banana (*Musa acuminata* L.) vegetative seeds through the addition of indole butyric acid with biological agents induction against fusarium wilt disease intensity. Jurnal Kultivasi, 23(2): 133-147.

The research results of (Meena et al., 2016) stated that the interaction between *M. incognita* and *Fusarium* Sp. in pepper it is synergistic and has a high intensity of root damage reaching 62% when compared to treating M. incognita and *Fusarium* sp alone. Research by (Al-Idrus et al., 2017) also states that the high percentage of *Fusarium* wilt disease in banana plants is directly proportional to the density of the *R. similis* nematode population. The large population of these nematodes results in more and more injuries so that the *Fusarium* fungus enters the root tissue and causes a high percentage of banana disease incidence due to this fungus, reaching 64.45%.

The soil used in this test uses soil that has been sterilized at a temperature of 121°C for 20 minutes to minimize the number of microorganisms contained in the soil, and it is hoped that pathogenic and antagonistic fungal infections can infect plants more optimally. It is suspected that the soil does not contain any parasitic nematode populations due to the effect of high-temperature sterilization. (Yaseen et al., 2024) statesthat nematode death in soil media or plant tissue can occur at temperatures above 32°C. The alleged absence of this nematode population resulted in testing the resistance of banana plants to the fungus *Fusarium* sp. did not experience natural injuries. Hence, the treatment is not significantly different.

Another factor that resulted in an insignificant effect in the in vivo test for the resistance test of vegetative banana plant seeds was thought to be the influence of different fungal incubation periods. According to (Ploetz, 2015), differences in the incubation period for fungi can occur due to several factors. One of them is the number of conidia densities. The density of conidia inoculated in the test was in accordance with the standards for using fungi as biological controls. (Niemeyer & De Andrade, 2016) states that the spore density that meets the standards for biocontrol agents or *Trichoderma* sp biological control agents must have a value greater than or equal to 1 x 106 spores/ml.

Observation of the test results, according to Figure 1 does not show a significant difference. The difference was only visible in the B0- treatments, where there were three wilted leaves and slight symptoms of colour change on the banana plant root. Meanwhile, on average, other treatments, namely B1 showed no significant symptoms. Judging from the condition of the weevil, it showed a clean condition without any symptoms. The symptoms shown in the B0- treatment were wilting of the leaves, apart from that, in the B0+ and B2 treatments, there were also symptoms of yellowish wilting on the leaves, but there isn't enough significance.

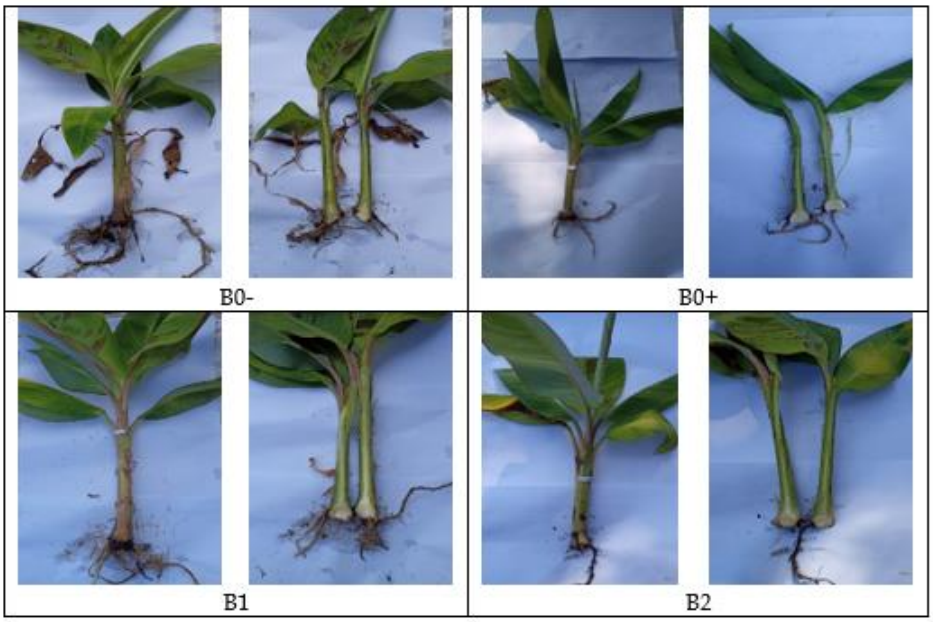

Figure 1. In vivo test results for the resistance of banana vegetative seeds to *Fusarium* wilt. description: B0-(without biological agent), B0+ (with Product Biological Agent), B1 (*Lynicillus fusiformis*+ *Fusarium* sp), B2 (isolate endophytic bacteria+ *Fusarium* sp).

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Symptoms of changes in banana leave colour found in the B0- the activity of the *Fusarium* sp. fungus may cause treatment. As is known, the *Fusarium* sp. fungus. produces many pectinase enzymes. This enzyme causes wilting and causes plant tissue vessels to turn brownish (Sukorini et al., 2021). Furthermore, the mycelium of pathogenic fungi can enter the parenchymal vessels, and the pathogen will form conidia in plant tissue, and the microconidia can be transported through the xylem. As a result, the water transport process was disrupted, and the flow of transpiration was reduced, resulting in wilting of plants (Islam et al., 2021)Symptoms of leaf wilting when observed are shown in treatments B0-, B0+ and B2 in Figure 1. Symptoms of wilting can also cause by phytotoxin activity produced by the fungus *Fusarium* sp. and translocated to the leaves, causing chlorosis symptoms. The toxins produced by *F. solani* include isomarticin and dehydro-fusarubin, which disrupt chloroplast formation and cause vein banding symptoms in oranges. (Nwankiti; & Gwa, 2018; Leslie & Summerell, 2006).

Biocontrol agents are crucial in managing Fusarium by producing antagonistic compounds, competing for resources, inducing systemic resistance in plants, and directly parasitizing the fungus. These mechanisms work together to inhibit Fusarium spread, enhance plant resistance.

Conclusion

This study demonstrated that the combination of Indole-3-butyric acid and biological agents did not exhibit significant interaction effects. Each treatment independently improved vegetative banana seedling quality and resilience. Applying IBA, especially at a concentration of 1.5 g/l, significantly enhanced early shoot emergence and leaf area, showing its potential to promote vigorous early growth. Meanwhile, biological agents notably increased the seedlings' resistance, as reflected in the DSI for leaves and reduced symptoms of root discoloration. These findings suggest that IBA and biological agents are valuable tools for improving banana seedlings' overall quality and resilience.

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