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Effectiveness of Biofilmed Biofertilizer with Balanced Phosphate Fertilizer Dosage in Suppressing Purple Blotch Disease Intensity and Increasing Garlic Yield on Andisol Soil in Tawangmangu

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Abstract
Garlic productivity in Indonesia is often constrained by purple blotch disease (*Alternaria porri*). A sustainable control alternative involves utilizing biofilm biofertilizer (BiO2) combined with phosphate (P) fertilizer. This study aimed to assess the effectiveness of balancing BiO2 and P dosage in suppressing purple blotch disease intensity and increasing garlic yield on Andisol soil in Tawangmangu. The research was conducted from May to September 2024 using a single-factor Completely Randomized Design (CRD) with four treatments: P0 (0% P + 100% NK + BiO2), P1 (50% P + 100% NK + BiO2), P2 (100% P + 100% NK + BiO2), and P3 (100% NPK without BiO2). Data were analyzed using ANOVA followed by DMRT at a 95% significance level. Results indicated that combining BiO2 with P fertilizer was highly effective; it suppressed leaf spot disease intensity by 49.47% and increased garlic yields by 20.91% compared to the control. This confirms that integrating BiO2 with appropriate P fertilization is a viable strategy for improving garlic productivity in Andisol soil.

Keywords: *Allium sativum*; *Alternaria porri*; biofilmed biofertilizer; phosphate; andisol.

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Introduction

Garlic (*Allium sativum* L.) is a bulb crop with high economic value in Indonesia and has long been an integral part of culinary traditions as a staple spice. Production data in central regions such as Central Java in 2022 reached 216,202 quintals (1). Nevertheless, national demand continues to rise alongside population growth, reflected in the high volume of imports required to meet domestic needs (2). Therefore, increasing garlic production has become a primary focus in Indonesian agricultural development.

The garlic cultivation process frequently faces challenges from plant pests and diseases. Among these, purple blotch disease, caused by the fungus Alternaria *porri*, is a major constraint causing significant yield losses (3). This pathogen aggressively attacks leaf tissue, reducing the photosynthetic area, and can spread to the bulbs, thereby inhibiting vegetative growth and bulb filling. Consequently, tuber production declines in both quantity and quality, reducing its economic value in the market (4).

The selection of Tawangmangu, Karanganyar Regency, as the research location is relevant because this area is a garlic production center in Central Java, dominated by Andisol soil. Andisols, formed from volcanic materials, possess unique characteristics such as high organic matter content, but are also known to have a very high phosphate (P) adsorption capacity. This strong P adsorption is caused by a high content of amorphous minerals, such as allophane and active Al/Fe-humus complexes that bind phosphate ions, rendering the P added through fertilization unavailable to plants (5).

An approach to overcome this issue is the use of Biofilmed Biofertilizer (BiO2). Previous research has proven that BiO2 can address soil conditions and support sustainable agricultural systems. BiO2 contains beneficial microorganisms that can enhance nutrient availability (6) and form a protective biofilm for plants. Essential nutrients like phosphorus (P) are crucial for energy transfer (7) and play a role in plant metabolism during disease infection. However, conventional P fertilization is often inefficient and costly (8). BiO2, which potentially contains phosphate-solubilizing microorganisms, is expected to improve P fertilization efficiency in Andisol soil.

Existing studies indicate the potential of BiO2 in increasing the yield of other crops (9) and P use efficiency (10). However, specific research examining the balance of phosphate fertilizer dosages when combined with BiO2 to suppress purple blotch intensity in garlic, particularly in Andisol soil with high P fixation capacity, remains very limited. Therefore, this study aims to examine the effectiveness of BiO2 on balanced phosphate fertilizer dosages in suppressing purple blotch intensity and increasing garlic yield on Andisol soil in Tawangmangu.

Materials and Methods

The research was conducted in Pancot, Jetis, Kalisoro, Tawangmangu District, Karanganyar Regency, Central Java, alongside laboratory analysis at the Plant Pest and Disease Laboratory and the Soil Fertility and Chemistry Laboratory, Faculty of Agriculture, Universitas Sebelas Maret (UNS), from May to September 2024. The materials used included garlic seeds of the Tawangmangu Baru variety, Nitrogen fertilizer (Urea), Phosphate fertilizer (SP-36), Potassium fertilizer (KCl), Biofilmed Biofertilizer (BiO2), ingredients for Potato Dextrose Agar (PDA) medium (potato, sugar, agar), chemicals for soil analysis in accordance with the standard methods of the Indonesian Soil Research Institute, and sterilization materials. The instruments used included analytical balances and a spectrophotometer.

The study employed a single-factor Completely Randomized Design (CRD), specifically focusing on the balance between P fertilizer and BiO2, consisting of 4 treatments with 6 replications. The treatments were: P0 (0% P + 100% NK + BiO2), P1 (50% P + 100% NK + BiO2), P2 (100% P + 100% NK + BiO2 – control), and P3 (100% NPK without BiO2) serving as the conventional farmer practice comparison. The experimental plot size was 1.2 x 1.5 m (adjusted based on the layout and number of plants per plot). Seeds were planted with a spacing of 10 x 12 cm, with one clove per hole. Basal NPK fertilizer was applied according to the treatment dosage. Supplementary Nitrogen (Urea 125 kg/ha) and Potassium (KCl 66.6 kg/ha) were applied at 30, 60, and 90 Days After Planting (DAP). Phosphate fertilizer (SP-36, 125 kg/ha for the 100% dose) was applied according to the specific treatments. BiO2 was applied at a dosage of 2 liters/ha.

Crop maintenance included irrigation and mechanical weeding. Harvesting was conducted at 120 DAP. Observation variables included: Plant Growth: Plant height (measured weekly from the stem base to the growing point) and number of leaves (counted weekly). Yield: Fresh biomass weight (at harvest), dry biomass weight (oven-dried at 60–70°C to constant weight), and fresh bulb weight per plot (at harvest). Purple Blotch Disease: Disease incidence (percentage of infected plants), disease severity (scored 0–5 based on the percentage of affected leaf area, calculated weekly using the Townsend-Heuberger formula), infection rate (calculated between observation intervals), Area Under Disease Progress Curve (AUDPC, calculated from severity data), and fungal morphological description (microscopic observation of conidia from isolates). Soil Analysis: Soil samples were collected before planting and after harvest. Measured parameters included: Total-N (Kjeldahl), Available-P (Bray-I), Available-K using the Morgan-Wolf/Flame Photometer/AAS method, Organic-C (Walkley and Black), and soil pH (pH meter, 1:5 ratio). Data were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level (alpha = 0.05). If ANOVA results indicated a significant or highly significant effect, post-hoc analysis was performed using Duncan's Multiple Range Test (DMRT) at the 5% significance level to compare means between treatments. Pearson correlation analysis was also conducted to determine the relationships between observed variables.

Results and Discussion

Analysis of soil chemical properties was conducted before planting (initial soil) and after harvest to evaluate the impact of the treatments. The analysis results are presented in Table 1. Initial soil analysis showed moderate Total-N (0.23%), low Available-P (9.42 ppm), moderate Available-K (0.48 cmol(+)/kg), low Organic-C (1.27%), and slightly acidic pH (6.51). After harvest, treatments had a significant effect on Total-N (Table 1). The 50% P + BiO2 and 0% P + BiO2 treatments resulted in the highest Total-N (0.71% and 0.64%), which was significantly higher than the 100% NPK + BiO2 and 100% NPK treatments. This indicates the effectiveness of BiO2 in increasing soil N via N-fixation (11). While increased N supports vegetative growth, high N levels without sufficient P can increase disease susceptibility. A dramatic increase occurred in Available-P. All treatments increased it from low to high (>67 ppm); notably, P0 (without P fertilizer) reached 129.55 ppm. This evidences the effectiveness of Phosphate Solubilizing Microorganisms (PSM) in BiO2 in solubilizing Andisol-bound P, consistent with Billah et al. (12). Organic-C content also increased significantly from low (1.27%) to high (>4.3%) across all treatments. Increased Organic-C improves soil structure and supports beneficial microbes (13), potentially creating suppressive soil. Available-K increased from moderate to very high (>1.27 cmol(+)/kg). Potassium (K) plays a role in osmotic regulation and cell wall strengthening, enhancing physical resistance and stress tolerance (14). Soil pH remained stable (slightly acidic to neutral), confirming the high buffering capacity of Andisols due to organic matter and allophane.

Table 1. Effect of the balance P with BiO2 fertilizers on plant disease parameters

Recommended P Dosage Proportion (%)	Disease incidence (%)		Disease severity (%)		Infection rate (%)		AUDPC	
0	65.00±10.41	a	72.00±8.00	a	0.05±0.005	a	22.80±0.82	a
50	62.83±06.49	b	73.33±6.02	a	0.04±0.008	b	23.12±1.68	a
100	70.67±04.55	c	32.00±6.69	c	0.01±0.006	d	11.76±2.40	b
NPK 100 Comparison	75.33±03.01	c	63.33±3.93	b	0.03±0.008	c	23.47±1.81	a
Sig. (*p*)	0.03		0.001		0.0002		0.0002	
CV (%)	7.32		11.89		15.17		8.25	

Note: Numbers followed by the same letter in the same column are not significantly different according to DMRT at the 5% level.

All treatments demonstrated an increasing trend in disease incidence over time, a pattern typical of polycyclic pathogens such as *A. porri* (15). At the beginning of the observation (1–2 Weeks After Planting/WAP), the 100% P + BiO2 treatment had the lowest incidence (38.5% at 1 WAP, compared to 46.67% at 2 WAP) and was significantly lower than other treatments. This indicates an early protective effect provided by sufficient P and BiO2 (16). However, this advantage faded over time. At 3 WAP, the 100% P + BiO2 treatment remained the lowest (55.3%) and significantly different. Starting from 4 WAP, the differences narrowed. High disease pressure resulting from the polycyclic nature of A. *porri* likely overwhelmed the capacity of induced defenses. By the end of the observation (6 WAP), 50% P + BiO2 showed the lowest incidence (63%), and 100% P + BiO2 was high (71%); however, statistically, there were no significant differences among treatments, which is common when incidence levels are very high. The effectiveness of BiO2 can be limited by environmental conditions and terminal pathogen pressure (17).

Three treatments (0% P + BiO2, 50% P + BiO2, 100% NPK) showed a steep upward curve in disease severity, whereas 100% P + BiO2 was more gradual. Disease severity is directly related to yield loss (18). The most significant difference was observed in 100% P + BiO2, which had the lowest final severity (32%) and was significantly different from the others. This evidences the synergy between optimal nutrition and BiO2. Balanced nutrition (P and K) strengthens cell walls, while BiO2 induces systemic resistance, providing dual protection. The treatments 0% P + BiO2, 50% P + BiO2, and 100% NPK had high final severity (>63%) and were generally not significantly different. The high severity in 0% P + BiO2 and 50% P + BiO2 suggests that BiO2 is not optimal without sufficient P (19). The performance of 100% NPK (farmer practice), which was equivalent to the reduced P treatments, may indicate pathogen resistance to conventional inputs.

Table 1 shows that the BiO2 and P fertilizer dosage significantly affected the dynamics of the purple blotch infection rate. The best suppression effectiveness was consistently demonstrated by the 100% P + BiO2 treatment, which suppressed the infection rate to the lowest level (0.02%) at the final interval, presumably through microbe-induced systemic resistance mechanisms (20). In contrast, the 50% P + BiO2 treatment showed a high and fluctuating infection rate, peaking at 0.25%, indicating increased plant susceptibility due to nutrient imbalance (21). Furthermore, a high infection rate proved to be strongly positively correlated with the active vegetative growth phase due to resource allocation centered on new tissue formation, and strongly negatively correlated with bulb weight, confirming the direct impact of accelerated epidemics on yield loss.

The treatments had a significant effect on the AUDPC. 100% P + BiO2 had a significantly lower AUDPC compared to the others (11.76). This confirms that 100% P + BiO2 was the most effective in suppressing total disease accumulation, supported by the synergy of nutrients and biological agents. 0% P + BiO2, 50% P + BiO2, and 100% NPK had high AUDPC values (22.80, 23.12, 23.47) and were not significantly different, indicating lower effectiveness. Unbalanced nutrition can disrupt systemic defense (22).

The effectiveness of severity control relative to 100% NPK (farmer comparison) is presented in Table 1. This calculation is standard for assessing biological agent performance (23). 100% P + BiO2 had the highest effectiveness (47.49%), resulting from a combination of optimal nutrients (P and K) strengthening basal defense and ISR by BiO2 activating latent defense pathways. 0% P + BiO2 and 50% P + BiO2 showed very low effectiveness (0.00% and 0.88%). This indicates that BiO2 alone or with sub-optimal P is insufficient. Defense activation requires energy (ATP) and metabolic precursors, where P plays a vital role (24). P limitation in 0% P or nutrient imbalance in 50% P inhibited defense expression.

Table 2. Effect of the balance P with BiO2 fertilizers on Plant Growth Parameters of garlic

Recommended P Dosage Proportion (%)	Plant height (cm)		Leaf number (blade)		Dry weight ($g.m^{-2}$)		Fresh weight ($g.m^{-2}$)	
0	47.80±0.94	b	11.2±0.70	b	24.67±08.43	a	105.50±44.63	a
50	46.48±0.92	b	12.43±0.34	a	28.17±10.55	a	140.50±36.36	a
100	59.70±1.18	a	7.60±0.70	c	20.67±3.44	a	118.50±19.67	a
NPK 100 Comparison	48.96±0.96	b	6.50±0.85	d	8.33±3.01	b	47.83±07.60	b
Sig. (p)	0.04		0.0001		0.02		0.02	
CV (%)	11.51		8.05		35.69		31.02	

Note: Numbers followed by the same letter in the same column are not significantly different according to DMRT at the 5% level.

Growth patterns followed an initial exponential phase, depending on nutrient availability (25). Treatments had a significant effect starting from 3 WAP. 100% P + BiO2 was consistently the highest (60 cm at 10 WAP), significantly greater than 0% P + BiO2, 50% P + BiO2, and 100% NPK. This is evidence of the synergy between complete NPK and BiO2 (26).

Regarding leaf development, 0% P + BiO2 and 50% P + BiO2 had the highest final leaf numbers (11 and 12 leaves), significantly more than 100% NPK + BiO2 and 100% NPK (27). Although 100% P + BiO2 plants were tall, they had fewer leaves due to faster allocation toward bulb initiation driven by high P. 0% P + BiO2 and 50% P + BiO2, with limited P, focused on leaf growth, aided by BiO2 modulating phytohormones (cytokinins) under nutrient stress (28).

0% P + BiO2, 50% P + BiO2, and 100% P + BiO2 had fresh and dry biomass weights significantly higher than 100% NPK. The highest fresh biomass was in 50% P + BiO2 (140.50 g), and the highest dry biomass was also in 50% P + BiO2 (28.17 g), which was not significantly different from (0% P + BiO2 and 100% P + BiO2). BiO2 application increased biomass compared to farmer practice due to increased P availability by Phosphate Solubilizing Bacteria (29). 0% P + BiO2 saved P costs without significantly reducing biomass.

The 100% P + BiO2 treatment recorded the highest bulb weight (374.17 g), reflecting optimal synergy between nutrient availability and PGPR microbial activity (30). This contrasts sharply with the 50% P + BiO2 treatment, which produced the significantly lowest weight, likely due to nutrient ratio imbalances (31). Another crucial finding is that the 0% P + BiO2 treatment was statistically able to match the performance of 100% NPK without a significant difference, proving the effectiveness of phosphate-solubilizing inoculants in substituting inorganic P fertilizer needs. These yield dynamics have a complex relationship with vegetative growth, where bulb weight is limited by source-sink competition with leaves but supported by tall plant structure (32), and is highly dependent on soil P availability for assimilate allocation to bulbs.

Table 3. Effect of the balance P with BiO2 fertilizers on the Suppression and Increase in Effectiveness

Recommended P Dosage Proportion (%)	Disease suppression effectiveness (%)		Yield increase effectiveness (%)	
0	0.88±09.04	b	8.28±02.15	b
50	0.00±00.00	b	0.00±00.00	b
100	49.47±16.07	a	20.91±10.57	a
Sig. (p)	0.0001		0.03	
CV (%)	37.10		37.67	

Note: Numbers followed by the same letter in the same column are not significantly different according to DMRT at the 5% level.

Table 4. Effect of the balance P with BiO2 fertilizers on Fresh Bulb Weight

Recommended P Dosage Proportion (%)	Fresh Bulb Weight $(g.m^{-2})$	Fresh Bulb Weight $(t.ha^{-1})$	
0	325.83±54.23	16.29	a
50	115.17±34.56	5.75	b
100	374.17±60.35	18.71	a
NPK 100 Comparison	314.17±122.11	15.71	a
Sig. (*p*)	0.01		
CV (%)	26.77		

Note: Numbers followed by the same letter in the same column are not significantly different according to DMRT at the 5% level.

Effectiveness in increasing bulb yield, measured as a relative percentage versus 100% NPK (farmer comparison), is listed in Table 4 using standard agronomic methods (18). 100% P + BiO2 resulted in a 20.91% increase vs. 100% NPK. This highlights the synergy of optimal P and BiO2. P is crucial for energy and bulbing, while BiO2 improves P uptake efficiency and produces growth promoters. 0% P + BiO2 showed a minor increase of 8.28%, while 50% P + BiO2 was ineffective (0%). The results of 0% P + BiO2 show that while BiO2 solubilizes soil P, it was not sufficient to maximize yield. The decline in P1 (50% P) indicates that N:P imbalance hampered metabolism and allocation to bulbs (33).

Conclusions

BiO2 is effective on balanced phosphate (P) fertilizer dosages in suppressing purple blotch disease intensity and increasing garlic yield on Andisol soil in Tawangmangu. BiO2 with balanced P fertilizer dosage is effective in suppressing purple blotch disease intensity by 49.47% on Andisol soil in Tawangmangu. BiO2 with balanced P fertilizer dosage is effective in increasing garlic yield by 20.91% on Andisol soil in Tawangmangu.

Conflict of Interest

All authors declare no conflicts of interest in this paper.

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