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Short Communication

Bio-Based Plant Growth Regulator Reduces Inorganic Fertilizer Requirement and Improves Nodulation of Soybean (*Glycine max L.*) on Acid Ultisol of Bengkulu, Indonesia

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Abstract. Soybean (*Glycine max L.*) production on Ultisol soils in Bengkulu, Indonesia, is constrained by low pH, aluminum toxicity, and phosphorus fixation, all of which suppress root nodulation and push farmers toward heavy inorganic fertilizer use. This study evaluated whether a bio-based plant growth regulator (bio-PGR), formulated from seaweed extract and humic acid, could reduce the inorganic NPK requirement of soybean while improving nodulation on acid Ultisol. A field trial used a factorial randomized complete block design with two factors: bio-PGR (absent or applied as a seed treatment plus two foliar sprays) and NPK dose (25, 50, 75, or 100% of the recommended rate), each combination replicated three times. Bio-PGR increased nodule number by 72% and nodule dry weight by 82% relative to the untreated control, averaged across NPK doses, and lowered soil exchangeable aluminum from 2.85 to 2.31 cmol(+) kg^{-1}. The combination of bio-PGR with 50% of the recommended NPK dose produced grain yield (2.38 t ha^{-1}) statistically equivalent to the full NPK dose without bio-PGR (2.41 t ha-1), while nodule number in that treatment was more than double that of the untreated full-dose control. Bio-PGR did not compensate for the lowest fertilizer dose tested (25%), where phosphorus limitation likely capped the response. These results indicate that bio-PGR can substitute for a substantial share of inorganic fertilizer input on acid Ultisol without a yield penalty, offering a practical route to lower fertilizer cost in smallholder soybean systems.

Keywords: Biostimulant, Bradyrhizobium, Nutrient Use Efficiency, Soil Acidity, Soybean Yield.

1. Introduction

Ultisols cover large areas of the humid tropics and are marked by low pH, low base saturation, and aluminum concentrations high enough to injure roots and disrupt legume-rhizobia signaling [1], [2]. In Bengkulu, on the west coast of Sumatra, Ultisol is the dominant soil order used for upland food crops, and its constraints, pH below 5, exchangeable aluminum above 2 cmol (+) kg^{-1}, and low available phosphorus, are usually managed by applying heavy doses of lime and inorganic NPK fertilizer. This practice raises production cost and does little to restore the biological nitrogen fixation capacity that acid stress suppresses in the first place.

Root nodulation in soybean depends on a signaling exchange between Bradyrhizobium and the host root that acidic conditions disrupt at several points, from rhizobial survival in the rhizosphere to the systemic autoregulation of nodulation that the plant itself imposes once soil nitrogen is adequate [2]. Two consequences follow for fertilizer management. Aluminum-tolerant rhizobial strains isolated from Indonesian acid soils nodulate soybean far more reliably than standard inoculants under low pH [1], which argues for amendments that lower aluminum activity rather than for inoculant substitution alone. Because nitrogen fertilizer feeds back on the plant's own regulation of nodule number, a lower mineral nitrogen supply, provided it does not create a phosphorus or micronutrient shortfall, can support more nodulation rather than less [3].

Biostimulants formulated from seaweed extract, humic substances, and related bio-based compounds have been reported to improve legume growth through several of these same pathways: chelation of exchangeable aluminum, stimulation of root

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development, and support for rhizobial survival in the rhizosphere [4], [5]. A systematic review of 72 field and pot studies found that biostimulant application alone raised soybean yield by 4 to 65%, and that pairing biostimulants with reduced mineral fertilizer often matched or exceeded full-dose mineral fertilization [6]. A similar pattern was shown recently in mungbean, where a biochar-biostimulant combination increased nodulation and yield under reduced NPK relative to full-dose NPK alone [7]. Field trials on Indonesian Ultisol have likewise shown that adaptive variety choice and plant growth-promoting rhizobacteria each raise soybean yield under these constraints [8], which is why we paired a nationally released high-yielding variety and a fixed inoculation regime with the amendment tested here rather than varying variety or inoculant strain.

Whether a bio-PGR substitution of this kind holds for soybean on the specific acid Ultisol conditions found in Bengkulu had not, to our knowledge, been tested directly. This study asked two questions. Does a bio-based plant growth regulator, applied as a seed treatment and foliar spray, increase nodule number and nodule biomass on acid Ultisol? And, more relevant to farm-level decisions, can bio-PGR allow a reduction in inorganic NPK dose without a corresponding loss of grain yield? We tested these questions in a factorial field trial crossing bio-PGR presence with four NPK dose levels, and report nodulation, growth, yield, and post-harvest soil chemical outcomes.

2. Method

2.1 Study site and soil characteristics

The trial ran from May to August 2025 at the experimental field of the Faculty of Agriculture, Universitas Muhammadiyah Bengkulu, Kandang Limun, Bengkulu City (approximately 3°46' S, 102°16' E; 12 m above sea level). The soil is a Typic Kanhapludult (Ultisol) with the following initial properties in the 0-20 cm layer: pH H_2O 4.6, organic C 1.4%, available P (Bray-1) 6.8 mg kg^{-1}, exchangeable Al 2.9 cmol (+) kg^{-1}, and cation exchange capacity 11.2 cmol(+) kg^{-1}. Dolomite lime was broadcast at 1.5 Mg ha-1 across the entire trial area two weeks before planting to bring the soil to a workable pH for soybean establishment; this amendment was applied uniformly and is not a treatment factor.

2.2 Experimental design and treatments

The trial used a randomized complete block design arranged as a 2 x 4 factorial. Factor A was bio-PGR (P0, without bio-PGR; P1, with bio-PGR), and Factor B was inorganic NPK dose (25, 50, 75, and 100% of the recommended rate of 50 kg N, 75 kg P2O5, and 50 kg K2O ha-1, supplied as urea, SP-36, and KCl). The eight treatment combinations were replicated three times across 24 plots of 3 x 4 m, separated by 0.5 m guard paths. Fertilizer was split-applied at planting and at 30 days after planting (DAP).

2.3 Plant material and cultivation

Soybean variety Anjasmoro was sown at 40 x 15 cm spacing, two seeds per hill thinned to one plant after emergence. Seed was inoculated with a commercial carrier-based Bradyrhizobium japonicum inoculant at planting in every plot, so inoculum availability was held constant and any nodulation difference reflects the bio-PGR and fertilizer treatments rather than inoculation itself. Pest and weed management followed standard local extension recommendations uniformly across plots.

2.4 Bio-PGR formulation and application

The bio-PGR combined Ascophyllum nodosum seaweed extract and humic acid (1:1 v/v, diluted to 3 mL L^{-1}), chosen for their documented capacity to chelate exchangeable aluminum, supply low-molecular-weight organic acids, and support root and rhizosphere development in legumes [4], [5]. Bio-PGR plots received the working solution as a 30-minute seed soak before sowing, followed by foliar application at 15 and 30 DAP with a knapsack sprayer calibrated to 500 L ha^{-1}. Control plots (P0) received an equal volume of water at the same timings.

2.5 Data collection

Nodulation was assessed destructively at 35 DAP (early flowering) from three plants per plot; roots were washed free of soil, and nodules were counted and oven-dried at 70 degrees C for 48 hours for dry weight. Plant height was measured on five tagged plants per plot at harvest (about 85 DAP). At harvest, filled pods per plant, 100-seed weight, and grain yield (adjusted to 14% moisture, expressed per hectare) were recorded from a 6 m2 net harvest area per plot, excluding border rows. Post-harvest soil samples from the 0-20 cm layer were analyzed for pH (1:2.5 soil-water suspension) and exchangeable Al (1 M KCl extraction, titration).

2.6 Statistical analysis

Variance homogeneity was checked with Levene's test before analysis. Effects of bio-PGR, NPK dose, and their interaction were tested by two-way analysis of variance (ANOVA) at alpha = 0.05. Where the F-test was significant, means were separated with Duncan's Multiple Range Test (DMRT) at the 5% level, using R version 4.3.

3. Result and Discussion

3.1 Nodulation

Bio-PGR increased nodulation regardless of NPK dose (Table 1). Averaged across the four fertilizer levels, nodule number rose from 14.1 +/- 2.0 per plant without bio-PGR to 24.3 +/- 2.6 per plant with bio-PGR, a 72% increase, and nodule dry weight rose from 0.27 +/- 0.04 to 0.49 +/- 0.06 g per plant, an 82% increase. NPK dose alone also affected nodulation, but not monotonically: nodule number was lowest at the 100% dose (16.2) and highest at the 50% dose (22.9), consistent with suppression of nodulation once mineral nitrogen supply is high.

The interaction between bio-PGR and NPK dose was significant for both nodulation variables (Table 2). Nodule number in the bio-PGR + 50% NPK treatment (28.9 +/- 3.4 per plant) was more than double that of the untreated full-dose control (12.2 +/- 1.7 per plant) and did not differ statistically from the bio-PGR + 75% NPK treatment (24.6 +/- 2.8). The lowest NPK dose (25%) did not sustain the same nodulation benefit from bio-PGR, which likely reflects phosphorus limitation at that dose, since phosphorus availability constrains nodule initiation independently of nitrogen suppression.

3.2 Growth and soil chemical properties

Plant height and root dry weight followed the same pattern as nodulation (Table 1). Bio-PGR-treated plants were on average 18% taller (61.6 vs. 52.2 cm) and had 42% greater root dry weight (4.4 vs. 3.1 g $plant^{-1}$) than untreated plants. Post-harvest soil analysis showed that bio-PGR plots had higher pH (5.0 vs. 4.7) and lower exchangeable Al (2.31 vs. 2.85 cmol(+) kg^{-1}) than untreated plots (Table 3), consistent with the aluminum-chelating capacity attributed to humic and seaweed-derived organic acids.

3.3 Yield components and grain yield

Grain yield responded to both factors and their interaction (Table 2). Without bio-PGR, yield rose with NPK dose in the expected pattern, from 1.61 t ha-1 at the 25% dose to 2.41 t ha^{-1} at the 100% dose. With bio-PGR, yield at the 50% NPK dose (2.38 t ha-1) was statistically indistinguishable from the untreated 100% NPK control (2.41 t ha-1), and yield at the bio-PGR + 75% and + 100% doses (2.47 and 2.53 t ha-1) was numerically higher, though not statistically different from that control. The bio-PGR + 25% NPK combination did not reach parity with the full-dose control, mirroring the nodulation shortfall at that dose. Hundred-seed weight showed a similar pattern of smaller magnitude (Table 2).

Table 1. Main effects of bio-PGR and NPK dose on nodulation and vegetative growth of soybean.

Factor / Level	Nodule number (plant^{-1})	Nodule dry weight (g plant^{-1})	Plant height (cm)	Root dry weight (g plant^{-1})
		Bio-PGR		
Without (P0)	14.1 b	0.27 b	52.2 b	3.1 b
With (P1)	24.3 a	0.49 a	61.6 a	4.4 a
		NPK dose		
25%	18.5 ab	0.37 ab	51.0 c	3.1 c
50%	22.9 a	0.46 a	56.8 bc	3.7 bc
75%	19.3 ab	0.39 ab	60.4 ab	4.0 ab
100%	16.2 b	0.30 b	63.8 a	4.2 a

Note: values are treatment means (n = 12 for bio-PGR levels, n = 6 for NPK dose levels). Means within a factor and column followed by the same letter are not significantly different by DMRT ($p < 0.05$).

Table 2. Interaction effect of bio-PGR and NPK dose on nodulation, grain yield, and 100-seed weight.

Treatment	Nodule number (plant^{-1})	Nodule dry weight (g plant^{-1})	Grain yield (t ha^{-1})	100-seed weight (g)
P0 x 100% NPK	12.2 c	0.21 d	2.41 a	13.8 a
P0 x 75% NPK	14.0 bc	0.27 cd	2.28 ab	13.5 a
P0 x 50% NPK	16.9 bc	0.34 c	2.02 bc	12.9 ab
P0 x 25% NPK	13.3 c	0.25 cd	1.61 d	11.6 c
P1 x 100% NPK	20.1 b	0.39 bc	2.53 a	14.2 a
P1 x 75% NPK	24.6 a	0.51 ab	2.47 a	14.0 a
P1 x 50% NPK	28.9 a	0.58 a	2.38 a	13.7 a
P1 x 25% NPK	23.6 ab	0.48 ab	1.98 bc	12.6 b

Note: P0 = without bio-PGR, P1 = with bio-PGR. Values are treatment means (n = 3). Means within a column followed by the same letter are not significantly different by DMRT ($p < 0.05$).

Table 3. Post-harvest soil pH and exchangeable aluminum as affected by bio-PGR

Bio-PGR	pH (H_2O)	Exchangeable Al (cmol(+) kg^{-1})
Without (P0)	4.7 b	2.85 a
With (P1)	5.0 a	2.31 b

Note: values are means of four NPK dose levels x three replicates (n = 12). Means within a column followed by the same letter are not significantly different by DMRT ($p < 0.05$).

3.4 Discussion

The central finding, that bio-PGR combined with half the recommended NPK dose produced nodulation and yield equivalent to the full dose without bio-PGR, fits two separate lines of evidence rather than a single mechanism. Acid soil conditions suppress nodulation both through direct aluminum rhizotoxicity and through the plant's systemic autoregulation response, which tightens once fertilizer supplies abundant nitrogen [2]. Reducing the NPK dose while supplying an aluminum-chelating, root-stimulating bio-PGR relieves both constraints together instead of trading one for the other. The magnitude of the yield response we observed at moderate bio-PGR and NPK combinations also matches the pattern reported in a recent systematic review, where biostimulant and reduced mineral fertilizer combinations matched or exceeded full mineral fertilization more often than biostimulant alone did [6].

The failure of bio-PGR to compensate at the lowest NPK level (25%) qualifies the practical recommendation. Phosphorus is required for nodule initiation and for the energy metabolism of nitrogen fixation itself, and on a soil already low in available P, a 25% fertilizer dose may simply not supply enough phosphorus for the biostimulant to act on, regardless of aluminum chelation [9], [10]. Bio-PGR therefore looks like a partial fertilizer substitute rather than a full replacement, and the substitution rate depends on which nutrient becomes limiting once the dose drops.

The reduction in exchangeable aluminum and the modest rise in soil pH under bio-PGR is smaller in absolute terms than lime alone typically achieves, yet it occurred without any lime beyond the uniform base dose applied to all plots. This points to organic acid chelation as a plausible mechanism, in line with reports that humic and seaweed-derived compounds bind exchangeable aluminum and improve root-zone chemistry in acid soils [4]. Aluminum speciation was not measured directly, so this interpretation stays an inference rather than a demonstrated mechanism and needs confirmation with more direct methods.

Compared with microbial approaches to the same problem, such as aluminum-tolerant Bradyrhizobium strains [1] or co-inoculation with phosphate-solubilizing bacteria [9], [11], bio-PGR has the practical advantage of fitting existing inoculation practice rather than requiring a strain switch, since inoculation was held constant across our treatments and bio-PGR still produced a clear nodulation response. Whether pairing an aluminum-tolerant inoculant with bio-PGR gives an additive or redundant effect is a natural next question, since both interventions appear to act partly through the aluminum-toxicity pathway.

The trial ran for a single season at one site, which limits how far the specific recommendation (50% NPK plus bio-PGR) should travel. Ultisol chemistry varies within Bengkulu Province, and a site with more severe aluminum saturation or lower baseline phosphorus might need a higher retained NPK dose to reach the same result. Multi-season, multi-location trials, together with direct measurement of rhizosphere aluminum speciation and nitrogen fixation rate rather than nodule count as a proxy, would strengthen the case for recommending this practice beyond the conditions tested here.

4. Conclusion

Bio-based plant growth regulator formulated from seaweed extract and humic acid increased soybean nodulation and offset most of the yield penalty of reduced inorganic NPK fertilization on acid Ultisol in Bengkulu. Combining bio-PGR with 50% of the recommended NPK dose produced grain yield statistically equal to the full-dose, non-bio-PGR control while more than doubling nodule number, which shows that a substantial share of inorganic fertilizer input can be replaced without yield loss under these conditions. The benefit did not extend to the lowest fertilizer dose tested (25%), where phosphorus limitation likely capped the response. These findings support bio-PGR as one component of reduced-input soybean production on acid soils, pending confirmation across further seasons and locations.

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6. Declaration

Author contributions and responsibilities - The authors made substantial contributions to the conception and design of the study. The authors were responsible for the data analysis, interpretation, and discussion of the results. The authors read and approved the final manuscript.

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7. How to Quote

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