



Locally Produced Microbial Inoculants and Plant Bioactive Extracts Reduced Soybean Pest Damage and Improved Biomass Productivity

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ABSTRACT

Pest infestation can severely affect soybean productivity, and synthetic pesticides are frequently used to control crop damage, which may cause deleterious effects on the environment and humans, while their misuse can lead to pest resistance. This study evaluated the potential of locally produced bio-inoculants of microbial and plant origins to control bean leaf beetle and pod borer pests, and the impact on soybean growth and biomass production as a sustainable alternative to synthetic pesticides in Buea – Cameroon. Experiments were conducted in August 2021 and repeated on the same plots in April 2022 to compare performance across seasons, while a new site was used in April 2023 to confirm the effectiveness of treatments in similar seasonal conditions. Experiments were established as Randomized Complete Block Design, with thirteen treatments and four replications. Treatments include control–no input, chemical (NPK fertilizer+Lamida gold insecticide), plant growth-promoting bacteria (PGPB1)+endophytic fungi (EF), PGPB2+EF, PGPB1+*Piper* extract (P), PGPB2+P, *Rhizobium* (R)+P, R+EF, *Mucuna* extract (M)+P, M+EF, PGPB1+M+EF+P, PGPB2+M+EF+P, and M+R+EF+P. Results show similar performance of bio-inoculants from microbial or plant origin and synthetic chemicals in reducing beetle infestation, severity and damage by 15–75%, leading to significantly ($P<0.05$) higher soybean growth and biomass at 15–100% when compared to the untreated control for all experiments. Bean leaf beetle infestation correlated negatively with plant height, number of leaves, leaf area index and soybean biomass ($P<0.05$). These findings highlight the potential of locally formulated bio-inoculants as a sustainable alternative to synthetic pesticides, which opens up avenues for further research on their mechanism of action that can be explored for biological pest control to enhance soybean performance.

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1. Introduction

Soybean (*Glycine max* (L.) Merrill) provides half of global edible oil and vegetable protein, and its cultivation provides income for many farmers (Tong et al., 2021; Halerimana et al., 2022). The bean leaf beetle – BLB (*Cerotoma trifurcate* or *Oothea* spp., Coleoptera: Chrysomelidae) and bean pod borer – BPB (*Maruca*

vitrata, Lepidoptera: Crambidae) are common pests that directly damage soybean leaves and pods (Tong et al., 2021; Halerimana et al., 2022). The invasive and polyphagous leaf beetle is a voracious soybean pest that causes lodging, reduces photosynthetic ability, leading to growth retardation and poor productivity (Halerimana et al., 2022; Schutze et al., 2022). In cases of severe infestation, the BLB and BPB pests can cause up to 100%

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crop losses (Tong et al., 2021; Halerimana et al., 2022). Synthetic pesticides are widely used to control pests, but their continuous use has negative effects on the environment and humans (Hassaan and Nemr, 2020; Pathak et al., 2022). Moreover, excessive use of synthetic pesticides can lead to pesticide resistance by pests, soil water contamination, and harm to beneficial insects and human health challenges such as neurological effects and cancer (Pathak et al., 2022). In recent years, there has been growing interest in using plant growth-promoting microorganisms (PGPMs) and plant extracts (PEs) as sustainable alternatives to synthetic pesticides (Fenibo et al., 2021; Kumar et al., 2021). These PGPMs and PEs have proven to be effective in controlling some economically important insect pests (Tanyi et al., 2020; Becke et al., 2024).

PGPMs are abundant in the rhizosphere of plants and play a variety of vital roles (Cui et al., 2019; Katsenios et al., 2022; Olougou et al., 2024). However, unsustainable agricultural practices continue to harm their diversity, abundance, and functions (Lehman et al., 2015; Bertola et al., 2021). By fostering a hostile environment to phytopathogens and inducing systemic pest resistance through their interactions with plant signaling mechanisms, PGPMs have the potential to enhance plant defense (Tchakounté et al., 2018; Kumar et al., 2021). Some typical fungal and bacterial genera, namely *Beauveria*, *Trichoderma*, *Kosakonia*, and *Bacillus* are often employed against insect pests (Srinivasan et al., 2015, 2019; Drobek et al., 2019).

Some plant bio-active extracts have been used to effectively control economically important pests of various crop types (Tanyi et al., 2020; Achiri et al., 2025). Plant bio-active compounds such as piperine derivatives from the African black pepper (*Piper guineense*) can be explored for biological control of insect pests (Celis et al., 2014; Gupta et al., 2014; Tepa-Yotto et al., 2022). The pungent, smelly piperine is a secondary metabolite that is neurotoxic with a strong deterrent effect to insects (Celis et al., 2014; Pimentel et al., 2021). Plant extracts can also improve soil physico-chemical and biological properties that contribute to crop nutrition and growth (Okur et al., 2010; Godlewska et al., 2021). Studies on *Mucuna cochinchinensis*, which is an abundant plant in Africa, demonstrated the benefits of improving plant health, restoring degraded soils and fertility due to its biological nitrogen fixing ability, enhancing soil biological and chemical properties, and ultimately improving soil productivity (Hu et al., 2016; Ngosong et al., 2018). However, despite their availability and potential, these dual-property plant bio-active materials are not currently being explored in organic crop production systems, probably due to limited information on their importance and lack of readily available products.

This study aimed to formulate and produce local bio-inoculants of microbial and plant origins, and assess their potential to serve as bio-pesticides for the control of bean leaf beetle and pod borer pest infestation and damage on soybean plants, within the integrated pest management nexus to improve soybean growth and productivity. Therefore, it was hypothesized that the individual and combined actions of locally produced bio-inoculants of microbial and plant origins will reduce bean leaf beetle and pod borer pest infestation and damage on soybean plants, leading to enhanced growth and biomass production.

2. Materials and methods

2.1. Experimental site and design

This multi-year field study was conducted at the teaching and research farm of the University of Buea–Cameroon, situated between longitudes 9°27'88" to 9°27'96" east of the Greenwich Meridian and latitudes 4°14'83" and 4°14'89" north of the equator. The site is located in the humid forest agro-ecology of Cameroon with a monomodal rainfall pattern, with the rainy season from March to October. Average annual rainfall is between 3000 and 5000 mm, and the relative humidity of 85–90%. Average annual temperature is between 20–28 °C, with 900–1200 h sunshine (Sounders et al., 2017). The soil is loamy with sand (59%), silt (27%), and clay (14%) (Fomenky et al., 2017). The first planting season is between March and July, with heavy rains from April to July, and the second season is between August and December, with heavy rains from August to October (Sounders et al., 2017). The experiments were conducted in August 2021 and repeated on the same plots in April 2022 to compare performance across seasons. A new site was used for experiments in April 2023 to confirm treatment effectiveness in similar seasonal conditions.

Experiments were set up as Randomized Complete Block Design (RCBD) with thirteen treatments replicated four times (Table 1). The 2500 m² (70×25 m) was manually cleared with a cutlass and raked to remove debris. The field was partitioned into 52 experimental plots, 4×4 m, which represent the thirteen treatments and four replicates each. About 30 cm raised beds were made for each plot by manually tilling with a hoe. The surrounding experimental sites were separated from the adjacent experimental units by a 2 m buffer, while a 1.5 m buffer separated plots within each replicate, and a 2 m buffer separated the replicates.

Table 1. Treatments comprising plant growth-promoting bacteria (PGPB), endophytic fungi (EF), plant extracts (*Mucuna cochinchinensis* and *Piper guineense*) and chemical inputs.

Code	Treatments Description
Control	No Input
Chemical	NPK fertilizer (Urea, Triple Superphosphate and Muriate of Potash)+Lamida Gold Insecticide
PGPB1+EF	Plant growth promoting bacteria 1 (<i>Bacillus</i> sp., <i>Arthrobacter</i> sp., <i>Paenibacillus</i> sp. and <i>Kosakonia radicincitans</i>)+Endophytic fungi (<i>Beauveria</i> sp. and <i>Trichoderma</i> sp.)
PGPB2+EF	Plant growth promoting bacteria 2 (<i>Bacillus</i> sp., <i>Arthrobacter</i> sp., <i>Paenibacillus</i> sp., <i>Kosakonia radicincitans</i> and <i>Bradyrhizobium japonicum</i>)+Endophytic fungi
PGPB1+P	Plant growth promoting bacteria 1+ <i>Piper guineense</i>
PGPB2+P	Plant growth promoting bacteria 2+ <i>Piper</i>
R+P	<i>Bradyrhizobium japonicum</i> + <i>Piper</i>
R+EF	<i>Bradyrhizobium japonicum</i> +Endophytic fungi
M+P	<i>Mucuna cochinchinensis</i> + <i>Piper</i>
M+EF	<i>Mucuna</i> +Endophytic fungi
PGPB1+M+	Plant growth-promoting bacteria
EF+P	1+ <i>Mucuna</i> +Endophytic fungi+ <i>Piper</i>
PGPB2+M+	Plant growth-promoting bacteria
EF+P	2+ <i>Mucuna</i> +Endophytic fungi+ <i>Piper</i>
M+R+ EF+P	<i>Mucuna</i> + <i>Bradyrhizobium</i> +Endophytic fungi+ <i>Piper</i>

2.2. Production of microbial inoculants

Consortia of symbiotic and non-symbiotic PGPMs were used in this study (Table 2). Except for *Kosakonia radicincitans*, which was isolated from the phyllosphere of winter wheat in Germany and deposited in NCBI as DSM 16656^T and Genbank as CP018016.1, CP018017.1, and CP018018.1 (Becker et al., 2018; Brock et al., 2018), four strains of non-symbiotic plant growth-promoting bacteria (PGPB) were isolated from the rhizosphere of maize plants in Cameroon (Tchakounté et al., 2018). The International Institute of Tropical Agriculture's Soil Microbiology Laboratory in Yaoundé, Cameroon, provided the fungal endophytes (*Beauveria spp.* and *Trichoderma spp.*) isolated from the rhizosphere of banana. The symbiotic biological nitrogen-fixing *Bradyrhizobium japonicum* isolated from soybean root nodules was obtained from the Soil Microbiology Laboratory, Biotechnology Center, University of Yaoundé I, Cameroon. Before being used, all microbial stock cultures were stored at the Rhizobiology Laboratory of the Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon. Microbes were selected for inclusion in the inoculant formulations based on their ability to produce or stimulate production of secondary metabolites that can induce systemic pest resistance in plants, produce phytohormones and siderophores, and other plant growth-promoting properties (Brock et al., 2018; Tchakounté et al., 2018). To obtain viable and pure discrete colonies, each bacterial strain from each stock culture of PGPB was sub-cultured individually on sterilized petri dish agar media (Standard nutrient agar I, Carl Roth, Germany) for 48 hours at 28°C. Using an inoculating loop, a pure discrete colony was selected from each of the newly streaked plates and inoculated into separate 100 mL of sterilized nutrient broth (Standard nutrient agar I, Carl Roth, Germany) in 500 mL Pyrex Erlenmeyer flasks. The flask was incubated at 28 °C for 48 hours in a horizontal shaker at 200 rpm. The various broth cultures were centrifuged at 5000 rpm for 10 min to create a pellet, which was then resuspended in sterile water and diluted to 10⁷ mL⁻¹ colony-forming unit (CFU).

Before application, microbial consortia were formulated by assembling independently cultured symbiotic and non-symbiotic PGPB in a 1000 mL Pyrex Erlenmeyer flask and mixed thoroughly. Each fungal isolate was cultured on Sabouraud dextrose agar (Sabouraud 2% glucose agar, Carl Roth, Germany) with 50 g mL⁻¹ chloramphenicol and 50 g mL⁻¹ streptomycin and kept at 28 °C in complete darkness for 10 days until sporulation (Meyling and Eilenberg, 2007). Conidia from each isolate were collected under sterile flow conditions using a sterile steel spatula by scraping them into 200 mL glass tubes containing 100 mL 0.3% (v/v) Tween® 80 solution (Amatuzzi et al., 2018). Conidial suspension from each isolate was vortexed at 120 rpm for 15 min, filtered through sterile gauze (10×15 cm) and put into new 30 mL glass tubes (Amatuzzi et al., 2018). Concentrations of suspensions were modified by serial dilution to produce an equivalent number of conidia per millilitre in aqueous 0.3% Tween® 80 solution. 0.01% sterile aqueous Triton X-100 was used to adjust suspensions to 5×10⁷ spores mL⁻¹ (Gurulingappa et al., 2010).

2.3. Extraction of plant extracts

Harvested fresh *Mucuna cochinchinensis* leaves and soft stems were thoroughly washed, chopped into 2 cm pieces and transferred into a water-filled 50 L plastic container at 1:1 (v/v), covered in shade for 28 days, filtered through 0.5 mm mesh, and stored (Tatseguouck et al., 2020). *Piper guineense* extract was produced from West African black pepper (*Piper guineense*) according to Tanyi et al. (2020). Briefly, 1.5 kg mature sun-dried *Piper* seeds were crushed with a kitchen blender. The fine powder was mixed with 6 L of vegetable oil (OILIO, Douala, Cameroon) and 60 g detergent (SABA, Douala, Cameroon), and thoroughly stirred for 3 min. The setup was kept for 24 h, and sieved through a 0.5 mm mesh to obtain oil-soluble extract, which was stored at room temperature before use.

2.4. Treatment application and crop maintenance

Soybean seeds (Mak soy 3N variety) were surface-sterilized by immersion in 70% ethanol for 1 min and thoroughly stirred, immersed in 2% sodium hypochlorite (NaOCl) solution for 15 min, and washed thrice in distilled water. For chemical treatment, single fertilizers (urea, triple superphosphate, and muriate of potash) were applied in 2 split doses at 15 kg ha⁻¹, 24 kg ha⁻¹, and 18 kg ha⁻¹, respectively, according to the soybean fertilizer recommendation by Chabi et al. (2021). The first fertilizer dose was applied at sowing and the second at 4 weeks after sowing (WAS) by ringing at a 5 cm radius of soybean plants. Insecticide Lamida Gold (LG) 90EC (30 g L⁻¹ Imidaclopride + 60 g L⁻¹ Lambda cyhalothrine) was sprayed using a knapsack at two WAS by mixing 25 mL in 15 L of water. This procedure was carried out twice a month until soybean plants started podding. For microbial treatments, soybean seeds were inoculated with PGPB and EF at 100 mL inoculant kg⁻¹ seed and gently mixed for 5 min for all seeds to be coated with inoculants. Inoculation of the seed was conducted under shade to maintain the viability of microbial cells, and seeds were allowed to air-dry for 15 min before sowing.

In order to prevent contamination, plots containing non-inoculated seeds were sown first. Three soybean seeds were sown per hole at approximately 4 cm depth, with 25 cm intra-row and 50 cm inter-row spacing. After emergence, seedlings were thinned to 2 vigorous plants per stand, giving 256 plants per plot, which is equivalent to 160,000 plants ha⁻¹. For treatments with plant extracts, *Mucuna cochinchinensis* extract was applied at 400 L ha⁻¹ by adding 5 mL extract into the rhizosphere of each soybean stand at 5 cm radius using a slip tip syringe (Keeler, USA) at 2 and 4 WAS. A knapsack sprayer was used to apply *Piper* extract at 2 WAS by mixing 100 mL in 15 L of water and spraying weekly until the soybean plants started podding. *Mucuna* extract was applied at the rhizosphere to target root interactions and enhance soil biota, whereas *Piper* extract was sprayed for direct pest control efficacy. The field site was regularly monitored for weed emergence and manually weeded every 4 weeks. Soil moisture during the entire experimental period depended on the local rainfall regime.

Table 2. Plant growth-promoting bacteria and endophytic fungi used in the consortium of microbial inoculants, their functional traits and potential roles as biofertilizers and biopesticides

Bacterial isolates	Accession number	Genus	Family	Phylum	nifH gene – atmospheric nitrogen fixation	Siderophores	Phosphorus solubilization	Biocontrol activity	Reason for selection and inclusion in the consortium
NKa11	AY599102	<i>Bradyrhizobium</i>	Rhizobiaceae	Proteobacteria	Yes	No	No	No	Nitrogen fixation
V64	MN128891	<i>Arthrobacter</i>	Micrococcaceae	Actinobacteria	No	Yes	Yes	No	Siderophore production and phosphorus solubilization
V65	OP209869	<i>Bacillus</i>	Bacillaceae	Firmicutes	No	Yes	Yes	yes	Siderophore production and phosphorus solubilization
V12	OP209823	<i>Paenibacillus</i>	Paenibacillaceae	Firmicutes	No	No	Yes	No	Nitrogen fixation
D5/23	DSM 16656 ^T	<i>Kosakonia</i>	Enterobacteriaceae	Proteobacteria	Yes	Yes	Yes	Yes	Biocontrol, Nitrogen fixation, siderophore production and phosphorus solubilization
Bb-SV-CG24	Bb11	<i>Beauveria</i>	Cordycipitaceae	Ascomycota	No	No	No	Yes	Biocontrol activity
Tri-Sv-CG41	NGT158	<i>Trichoderma</i>	Hypocreaceae	Ascomycota	No	No	No	Yes	Biocontrol activity

2.5. Data collection

2.5.1. Infestation and severity of bean leaf beetle and pod borer

Data were collected on pest infestation and severity on 10 randomly selected soybean plants from the central row of each plot. Soybean plants were assessed for BLB infestation at 6 weeks after sowing, while BPB infestation was assessed at 14 weeks after sowing (Achiri et al., 2025). BLB and BPB infestation was assessed as the presence or absence of the BLB and BPB, respectively. Severity of BLB and BPB was recorded by visually counting the number of BLBs and BPBs, respectively, on infested plants and pods. Damage of soybean plants was assessed by presence or absence of leaves with irregular holes on the 10 randomly selected plants, while the severity of damaged plants by BLB was evaluated and scored from 0 to 4, based on the degree of leaf damage (0–20% = 1, 21–40% = 2, 41–60% = 3, 61–80% = 4, 81–100% = 5) (Achiri et al., 2025). The degree of damage was visually observed and assessed based on the portion of soybean plants (%) damaged by BLB. The equations below were used to assess pest infestation and damage (Achiri et al., 2025):

$$\text{BLB infestation (\%)} = \frac{\text{Number of sampled plants infested by BLB (n)}}{\text{Number of sampled plants (N)}} \times 100 \text{ --- 1}$$

$$\text{BPB infestation (\%)} = \frac{\text{Number of sampled pods infested by BPB (n)}}{\text{Number of sampled pods (N)}} \times 100 \text{ --- 2}$$

$$\text{Damage incidence on plants (\%)} = \frac{\text{Number of sampled plants damaged (n)}}{\text{Number of sampled plants (N)}} \times 100 \text{ --- 3}$$

2.5.2. Soybean growth and biomass production

At 6 weeks after sowing, 10 randomly selected plants from the middle row of each experimental plot were tagged for data collection on plant height (cm), number of leaves, number of branches, stem girth (cm), and leaf area index (leaf length x leaf width x constant). Leaf length and width were measured on the third trifoliate leaf of each tagged plant using a meter rule. The length and width of the leaf were measured from base to apex and from the middle edge of one margin to the other, respectively. Plant height was measured from the base of the soil surface to the end of the terminal bud. At a distance of 10 cm from the soil's surface, the stem girth of each tagged plant was measured with a Vernier caliper. The leaves and visible branches of each tagged plant were counted and recorded. At 13 WAS, the entire above-ground biomass of the 10 randomly selected plants from the central row of each plot was harvested and oven dried at 70 °C to a constant weight to determine the total above-ground dry biomass (Tamiru et al., 2012).

2.6. Statistical analysis

All data sets were analyzed using the statistical software package IBM SPSS Statistics version 23 for Windows. Data were checked for normality and homogeneity using Kolmogorov-Smirnov (Kolmogorov-Smirnov, 2008) and

Levene's (Levene, 1960) tests, respectively. Factorial analysis was performed to test the effects of treatments, seasonal variations, and their interactions as categorical predictors. Where significant effects occurred, univariate analysis of variance (ANOVA, $P < 0.05$) was performed to compare effects on categorical predictors on specific dependent variables. Significantly different means were further separated using *posthoc* Tukey's HSD test ($P < 0.05$). Additionally, Pearson's correlation was performed to check the degree of association between soybean growth and biomass production with bean leaf beetle infestation.

3. Results

3.1. Effect of bio-inoculants on pest infestation, severity and damage

Factorial analysis of all measured parameters revealed significant effects of treatment ($P < 0.001$) and planting season ($P < 0.001$), while interaction between treatment and season was not significant ($P > 0.05$). BLB and BPB infestation, severity and damage on soybean plants differed significantly across treatments in all planting seasons, with microbial inoculants and plant extracts exerting similar effects, which were higher than the untreated control ($P < 0.05$). BLB infestation ranged from 5 to 50% across all planting seasons, with the highest (50%) in untreated control, and the lowest (5%) in M+R+EF+P ($P < 0.05$; Fig. 1). BLB severity ranged from 1 to 5 across all planting seasons, with the highest in untreated control, and the lowest in PGPB1+M+EF+P and M+R+EF+P ($P < 0.05$; Fig. 2). Damage incidence on soybean plants ranged from 5 to 50% across all planting seasons, with the highest in untreated control, and the lowest in M+R+EF+P ($P < 0.01$; Fig. 3). Microbial inoculants, plant extracts, and chemical inputs reduced damage severity on soybean plants by half compared to the untreated control across all planting seasons, although the difference was not statistically significant ($P > 0.05$; Table 3). BPB Infestation differed significantly across treatments in August 2021, ranging from 7.5 to 22.5%, with the highest in the untreated control, and the lowest in PGPB1+M+EF+P ($P < 0.05$; Table 3). No significant difference was observed in BPB infestation across all treatments in April 2022 and 2023 planting seasons ($P > 0.05$; Table 3). Microbial inoculants, plant extracts and chemical inputs reduced the severity of pod borers by half when compared to the untreated control in all planting seasons, although the difference was not statistically significant ($P > 0.05$; Table 3).

3.2. Impact of bio-inoculants on soybean growth and biomass production

All soybean growth parameters and biomass differed significantly across treatments in all planting seasons, with microbial inoculants and plant extracts exerting similar effects that were higher than the untreated control ($P < 0.05$). Plant height ranged from 30.24 to 72.53 cm across all planting seasons, with the highest in R+EF, and the lowest in the untreated control ($P < 0.001$; Table 4). Stem girth ranged from 1.85 to 3.20 cm across all planting seasons, with the highest in PGPB1+EF, and the lowest in the untreated control ($P < 0.05$; Table 4). The number of branches ranged from 3 to 10 across all planting seasons, with the highest in PGPB2+EF, PGPB2+P, M+EF, and PGPB2+M+EF+P, and the lowest in the untreated control

($P < 0.05$; Table 4). Number of leaves ranged from 10 to 32 across all planting seasons, with the highest in PGPB2+M+EF+P, and the lowest in untreated control ($P < 0.01$; Fig. 4).

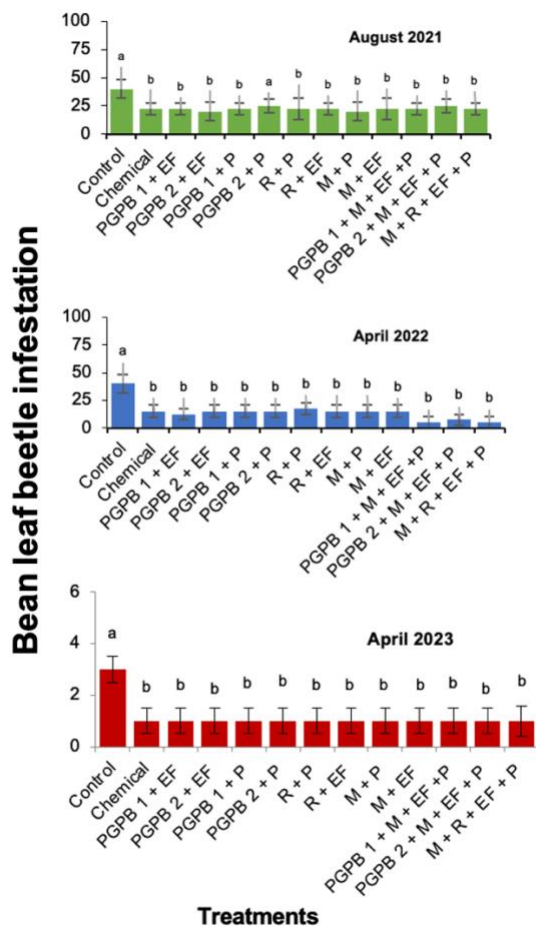


Figure 1. Effect of microbial inoculant and plant extract on bean leaf beetle infestation (Mean \pm SD). Bars with different letters represent significant differences across treatments (Tukey's HSD, $P < 0.05$). Control: No input, Chemical: N, P, K fertilizer + Lamida gold insecticide, PGPB1: Plant growth-promoting bacteria without *Bradyrhizobium japonicum*, EF: Endophytic fungi, PGPB2: Plant growth-promoting bacteria + *Bradyrhizobium japonicum*, R: *Bradyrhizobium japonicum*, P: *Piper guineense* extract, M: *Mucuna cochinchinensis* extract

Leaf area index ranged from 20.21 to 119.81 cm² across all planting seasons, with the highest in PGPB2+EF, and the lowest in untreated control ($P < 0.001$; Table 4). Microbial inoculants, plant extracts and chemical inputs resulted in about two-fold increase in soybean biomass production compared to the untreated control across all planting seasons ($P < 0.001$; Fig. 5). Soybean biomass ranged from 8.55 to 29.21 t ha⁻¹ across all planting seasons, with the highest in M+R+EF+P, and the lowest in untreated control ($P < 0.001$; Fig. 5). Correlation analysis indicates strong negative relationship between bean leaf beetle infestation and soybean growth or biomass across all planting seasons ($P < 0.05$; Table 5).

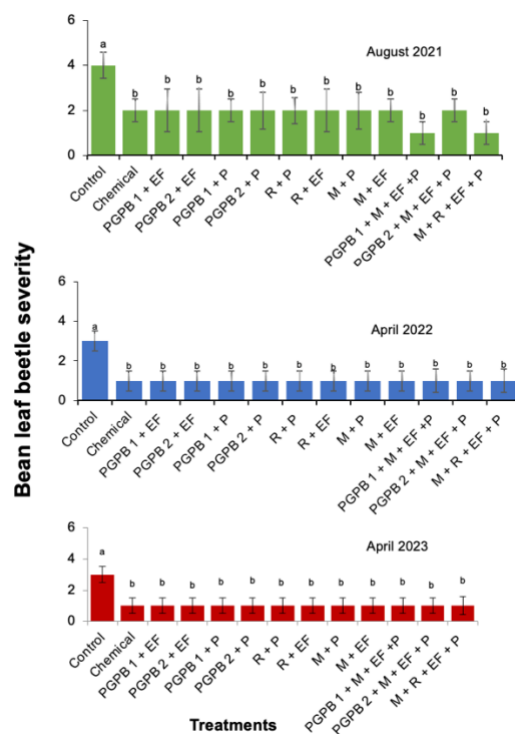


Figure 2. Effect of microbial inoculant and plant extract on bean leaf beetle severity (Mean \pm SD). Bars with different letters represent significant differences across treatments (Tukey's HSD, $P < 0.05$). Control: No input, Chemical: N, P, K fertilizer + Lamida gold insecticide, PGPB1: Plant growth-promoting bacteria without *Bradyrhizobium japonicum*, EF: Endophytic fungi, PGPB2: Plant growth-promoting bacteria + *Bradyrhizobium japonicum*, R: *Bradyrhizobium japonicum*, P: *Piper guineense* extract, M: *Mucuna cochinchinensis* extract.

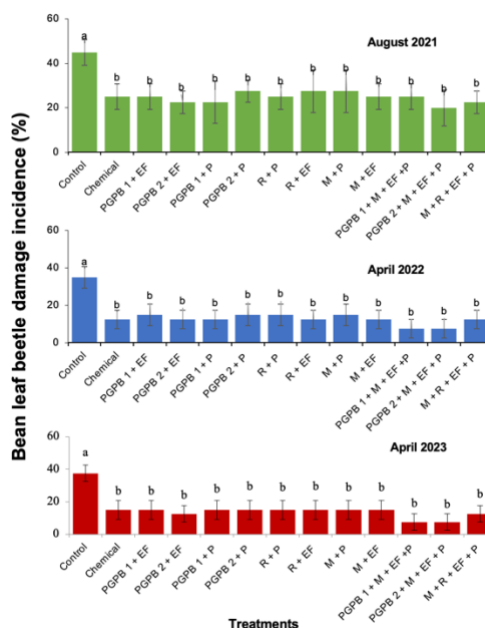


Figure 3. Effect of microbial inoculant and plant extract on bean leaf beetle damage incidence (Mean \pm SD). Bars with different letters represent significant differences across treatments (Tukey's HSD, $P < 0.05$). Control: No input, Chemical: N, P, K fertilizer + Lamida gold insecticide, PGPB1: Plant growth-promoting bacteria without *Bradyrhizobium japonicum*, EF: Endophytic fungi, PGPB2: Plant growth-promoting bacteria + *Bradyrhizobium japonicum*, R: *Bradyrhizobium japonicum*, P: *Piper guineense* extract, M: *Mucuna cochinchinensis* extract.

Bradyrhizobium japonicum, R: *Bradyrhizobium japonicum*, P: *Piper guineense* extract, M: *Mucuna cochinchinensis* extract.

4. Discussion

4.1. Performance of bio-inoculants on soybean pest damage

The use of bio-inoculants of microbial and plant origins as alternatives to synthetic chemicals in agriculture has increased in recent years (Ahmed et al., 2021; Becke et al., 2024; Achiri et al., 2025). The invasion of soybean fields by insect pests and the downside of chemical inputs have necessitated the search for sustainable alternatives (Heinrichs and Muniappan, 2018; Srinivasan et al., 2019). The bean leaf beetle and pod borer were efficiently controlled by *Piper* extract, which has proven to be a successful antidote for a variety of field pests (Sisay et al., 2019; Tanyi et al., 2020; Achiri et al., 2025). This is most likely attributed to the activity of secondary metabolites, isobutyl amides with active components (Piperine and Piperiline) as a neurotoxic and deterrent (Paula, 2000) and phenylpropanoid content that inhibits the functioning of cytochrome in insect tissue, resulting in mortality (Lucena et al., 2017). In the present study, associated microbes in the different inoculant consortia from the genera *Beauveria*, *Trichoderma*, *Bacillus* and *Kosakonia* were likely successful in "priming" soybean seeds upon inoculation that caused systemic resistance (Aime et al., 2013). After exposure to pathogens, herbivorous insects, or abiotic stressors, primed plants may exhibit higher induction of numerous cellular defense mechanisms (Ye et al., 2013; Martinez-Medina et al., 2016; Hokkanen and Menzler-Hokkanen, 2017). The key components of defense mechanisms are repellence and antifeedant or poisonous substances that reduce insect performance (McCormick et al., 2016). Mechanistic actions may also involve volatile emissions that attract natural enemies of some economically important crop pests (Shivaramu et al., 2017). Meanwhile, the ability of *Kosakonia* to repel Lepidopteran pests like *Spodoptera exigua* has been established (Brock et al., 2018).

Low infestation and damage in the chemical pesticide Lamida Gold of the present study are consistent with lambda cyhalothrine and imidacloprid active ingredients that are fatal to many insect pests (Djouaka et al., 2018; Becke et al., 2024; Achiri et al., 2025). Since there was no pest management approach applied to the soybean plants in the control treatment, the leaf beetle and pod borer pests were allowed to infest and thrive on those plants, causing maximum damage to the soybean plants and pods. This underscores the difficulties faced by farmers who do not employ pest control methods and the need to develop and promote efficient pesticide solutions that are resource conserving, easily accessible and economically viable (Tanyi et al., 2020).

4.2. Impact of bio-inoculants on soybean growth and biomass production

Besides environmental and genetic variability of crop varieties, farm management strategies have significant impacts on crop production (Cooper et al., 2021; Young et al., 2021). Considering the need to guarantee environmental and human health, the most effective crop protection techniques are those that can control crop pests and/or diseases without negative externalities (Struik and Kuyper, 2017; Achiri et al., 2025).

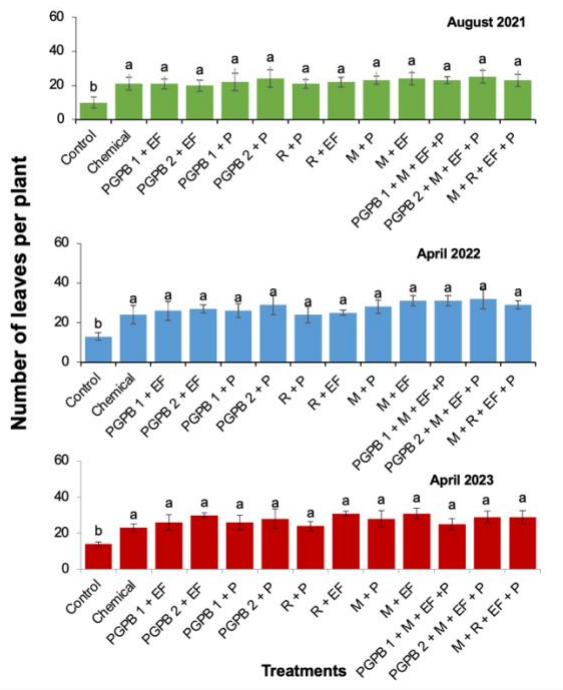


Figure 4. Effect of microbial inoculant and plant extract on number of leaves per plant (Mean \pm SD). Bars with different letters represent significant differences across treatments (Tukey's HSD, $P < 0.05$). Control: No input, Chemical: N, P, K fertilizer + Lamida gold insecticide, PGPB1: Plant growth-promoting bacteria without *Bradyrhizobium japonicum*, EF: Endophytic fungi, PGPB2: Plant growth-promoting bacteria + *Bradyrhizobium japonicum*, R: *Bradyrhizobium japonicum*, P: *Piper guineense* extract, M: *Mucuna cochinchinensis* extract.

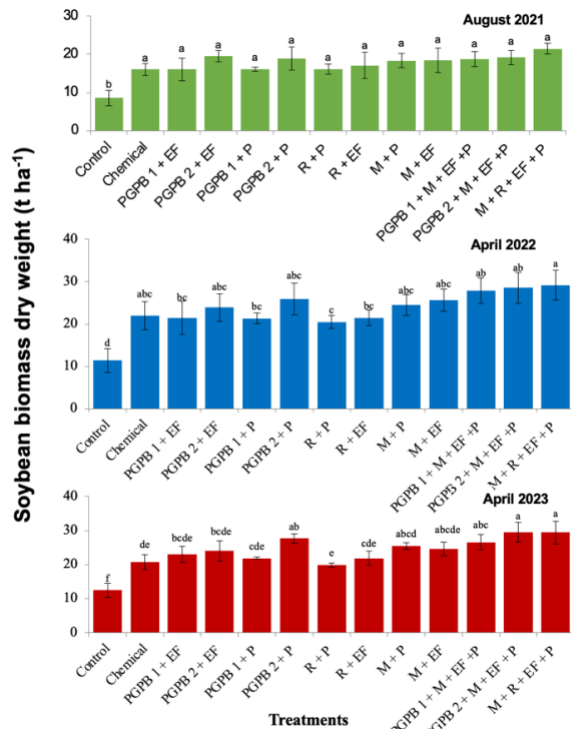


Figure 5. Effect of microbial inoculant and plant extract on soybean biomass dry weight. Bars with different letters represent significant differences across treatments (Tukey's HSD, $P < 0.05$). Control: No input, Chemical: N, P, K fertilizer + Lamida gold insecticide, PGPB1: Plant growth-promoting bacteria without *Bradyrhizobium japonicum*, EF: Endophytic fungi, PGPB2: Plant growth-promoting bacteria +

Table 3. Effect of treatments on damage severity, pod borer infestation and pod borer severity.

Treatments	Damage severity	BPB infestation	BPB Severity	Damage severity	BPB infestation	BPB Severity	Damage severity	BPB infestation	BPB Severity
	August 2021			April 2022			April 2023		
Control	2.00± 0.50a	22.50±5.00a	2.00± 0.58a	2.00± 0.50a	17.50± 5.00a	2.00± 0.50a	2.00± 0.50a	17.50±5.00a	2.00± 0.50a
Chemical	1.00± 0.50a	12.50± 5.00ab	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
PGPB 1 + EF	1.00± 0.50a	7.50±5.00b	1.00± 0.50a	1.00± 0.50a	7.50± 5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
PGPB 2 + EF	1.00± 0.50a	7.50±5.00b	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
PGPB 1 + P	1.00± 0.50a	10.00±0.00b	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
PGPB 2 + P	1.00± 0.50a	12.50± 5.00ab	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
R + P	1.00± 0.50a	7.50±5.00b	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
R + EF	1.00± 0.50a	12.50± 5.00ab	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
M + P	1.00± 0.50a	12.50± 5.00ab	1.00± 0.50a	1.00± 0.50a	12.50± 5.00a	1.00± 0.50a	1.00± 0.50a	12.50±5.00a	1.00± 0.50a
M + EF	1.00± 0.50a	12.50± 5.00ab	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
PGPB 1 + M + EF +P	1.00± 0.50a	7.50±5.00b	1.00± 0.50a	1.00± 0.50a	5.00±5.77a	1.00± 0.57a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
PGPB 2 + M + EF + P	1.00± 0.50a	7.50±5.00b	1.00± 0.50a	1.00± 0.50a	5.00±5.77a	1.00± 0.57a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a
M + R + EF + P	1.00± 0.50a	7.50±5.00b	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a	1.00± 0.50a	7.50±5.00a	1.00± 0.50a

Values (mean ± S.D) within columns with different letters are significantly different (Tukey's HSD, $P < 0.05$). Control: No input, Chemical: Chemical N, P, K fertilizer + Lamida gold insecticide, PGPB1: Plant growth-promoting bacteria without *Bradyrhizobium japonicum*, EF: Endophytic fungi, PGPB2: Plant growth-promoting bacteria + *Bradyrhizobium japonicum*, R: *Bradyrhizobium japonicum*, P: *Piper guineense* extract, M: *Mucuna cochinchinensis* extract.

Table 4. Effect of treatments on soybean growth parameters

Treatments	Plant height (cm)	Stem girth (cm)	Number of branches	leaf area index (cm ²)	Plant height (cm)	Stem girth (cm)	Number of branches	leaf area index (cm ²)	Plant height (cm)	Stem girth (cm)	Number of branches	leaf area index (cm ²)
August 2021 (6 weeks after planting)				April 2022 (6 weeks after planting)				April 2023 (6 weeks after planting)				
Control	30.24 ± 1.14e	1.85 ± 0.12b	3 ± 0.52b	20.21 ± 2.59g	39.27 ± 2.54b	1.93 ± 0.16b	4 ± 0.58b	49.21 ± 8.64b	39.61 ± 1.32b	1.99 ± 0.17b	4 ± 0.58b	51.42 ± 6.58b
Chemical	46.87 ± 5.95abc	2.51 ± 0.26ab	6 ± 0.25a	60.79 ± 5.64c-f	62.17 ± 9.56a	2.84 ± 0.44ab	8 ± 1.89a	99.54 ± 5.74a	64.63 ± 6.86a	2.73 ± 0.35ab	9 ± 2.16a	98.32 ± 3.81a
PGPB 1 + EF	43.58 ± 5.56abcd	2.71 ± 0.21a	7 ± 1.82a	68.48 ± 5.36abc	68.95 ± 8.26a	2.98 ± 0.46a	9 ± 0.96a	106.45 ± 19.46a	68.10 ± 7.55a	3.19 ± 0.30a	9 ± 0.82a	114.86 ± 13.91a
PGPB 2 + EF	45.4 ± 5.16abcd	2.79 ± 0.37a	7 ± 0.71a	64.96 ± 6.02b-e	70.22 ± 3.63a	3.04 ± 0.57a	9 ± 0.82a	118.29 ± 21.32a	68.93 ± 3.10a	2.77 ± 0.26a	10 ± 0.58a	119.81 ± 23.80a
PGPB 1 + P	37.99 ± 5.19cde	2.43 ± 0.33ab	6 ± 0.85a	52.42 ± 2.76f	62.87 ± 8.14a	3.08 ± 0.61a	9 ± 1.29a	98.06 ± 3.53a	65.19 ± 5.58a	2.81 ± 0.26a	9 ± 1.26a	97.93 ± 5.21a
PGPB 2 + P	50.73 ± 4.57a	3.04 ± 0.16a	8 ± 1.69a	76.365 ± 4.95a	64.45 ± 2.88a	2.84 ± 0.36ab	9 ± 1.89a	104.61 ± 3.36a	64.92 ± 4.68a	2.99 ± 0.17a	10 ± 1.73a	105.73 ± 4.67a
R + P	46.72 ± 3.17abc	2.52 ± 0.27ab	6 ± 2.35a	57.71 ± 3.06def	70.03 ± 5.74a	2.66 ± 0.09ab	8 ± 1.41a	93.42 ± 11.72a	70.28 ± 7.29a	2.71 ± 0.11ab	9 ± 1.29a	97.87 ± 9.23a
R + EF	48.78 ± 4.54abc	2.85 ± 0.16a	6 ± 0.62a	63.31 ± 3.77b-e	69.96 ± 8.53a	2.71 ± 0.28ab	9 ± 1.73a	93.97 ± 6.93a	72.53 ± 6.79a	2.75 ± 0.34ab	9 ± 1.41a	96.42 ± 8.41a
M + P	46.23 ± 3.55abcd	2.79 ± 0.23a	7 ± 2.15a	58.52 ± 2.13c-f	67.29 ± 7.68a	2.68 ± 0.21ab	9 ± 2.16a	101.28 ± 14.25a	69.68 ± 6.78a	2.74 ± 0.25ab	9 ± 2.06a	105.62 ± 9.23a
M + EF	38.91 ± 2.35bcde	2.75 ± 0.45a	7 ± 1.69a	56.14 ± 5.34ef	66.71 ± 3.93a	2.89 ± 0.29ab	9 ± 1.89a	97.63 ± 8.31a	66.77 ± 3.67a	2.95 ± 0.41a	10 ± 1.71a	96.26 ± 10.34a
PGPB 1 + M + EF + P	35.66 ± 3.64de	2.55 ± 0.39ab	6 ± 1.99a	67.09 ± 1.01a-d	67.57 ± 6.19a	2.91 ± 0.42a	9 ± 1.41a	100.69 ± 16.79a	69.48 ± 5.37a	3.06 ± 0.39a	9 ± 1.41a	105.96 ± 7.03a
PGPB 2 + M + EF + P	42.92 ± 5.71abcd	2.76 ± 0.31a	6 ± 0.72a	65.54 ± 4.06b-e	69.09 ± 6.57a	2.84 ± 0.22ab	10 ± 1.5a	97.16 ± 15.56a	71.53 ± 4.59a	2.85 ± 0.17a	10 ± 1.41a	96.27 ± 15.84a
M + R + EF + P	48.95 ± 3.55ab	2.79 ± 0.46a	7 ± 1.35a	72.29 ± 3.02ab	63.42 ± 6.58a	2.79 ± 0.49ab	9 ± 1.29a	103.94 ± 13.81a	65.26 ± 6.51a	2.85 ± 0.53a	9 ± 0.82a	110.68 ± 9.60a

Values (mean ± S.D) within columns with different letters are significantly different (Tukey's HSD, $P < 0.05$). Control: No input, Chemical: Chemical N, P, K fertilizer, + Lamida gold insecticide, PGPB1: Plant growth-promoting bacteria without *Bradyrhizobium japonicum*, EF: Endophytic fungi, PGPB2: Plant growth-promoting bacteria + *Bradyrhizobium japonicum*, R: *Bradyrhizobium japonicum*, P: *Piper guineense* extract, M: *Mucuna cochinchinensis* extract.

Table 5. Correlations between soybean growth parameters, biomass dry weight, and bean leaf beetle infestation metrics in August 2021, April 2022, and April 2023 experimental periods.

Soybean growth and biomass	Plant height (cm)	Number of leaves	Leaf area index (cm ²)	Biomass dry weight (tons ha ⁻¹)	Plant height (cm)	Number of leaves	Leaf area index (cm ²)	Biomass dry weight (tons ha ⁻¹)	Plant height (cm)	Number of leaves	Leaf area index (cm ²)	Biomass dry weight (tons ha ⁻¹)
	August 2021				April 2022				April 2023			
Bean leaf beetle infestation	-0.39*	-0.54**	-0.49**	-0.51**	-0.61**	-0.46**	-0.61**	-0.68**	-0.63**	-0.62**	-0.41**	-0.60**
Bean leaf beetle severity	-0.32	-0.41**	-0.56**	-0.55**	-0.54**	-0.38*	-0.62**	-0.70**	-0.57**	-0.52**	-0.49**	-0.69**
Plant damage incidence	-0.52**	-0.53**	-0.52**	-0.60**	-0.65**	0.42**	-0.57**	-0.54**	-0.66**	-0.65**	-0.52**	-0.72**
Plant damage severity	-0.08	-0.40*	-0.37*	-0.54**	-0.49**	-0.17	-0.43**	-0.32*	-0.46**	-0.46**	-0.31*	-0.50**

Significant correlation *r* values are represented by * at $P < 0.05$ and ** at $P < 0.01$ across years.

Moreover, bio-inoculants with the dual potential to improve soil fertility/crop nutrition and protect plants would likely enhance crop productivity. Accordingly, the bio-inoculants of microbial and plant origins probably performed these dual roles, leading to greater productivity in the respective treatments (Luo et al., 2018; Becke et al., 2024; Achiri et al., 2025), as compared to the untreated control with lower soybean growth and biomass production. Microbial inoculants and organic amendments are known to modulate soil biotic interactions that improve nutrient uptake and subsequent increase in plant growth (Ngosong et al., 2022; Olougou et al., 2024; Ngone et al., 2025). Some studies highlighted the role of bio-inoculants in decomposing organic matter (Kallenbach et al., 2016), enhancing biological nitrogen fixation, phosphorus solubilization, production of phytohormone and siderophore (Berger et al., 2015; Becker et al., 2018; Tchakounté et al., 2018; Qi et al., 2021). These processes likely accounted for the increase in soybean growth following application of bio-inoculants. Meanwhile, when pest infestation and damage are effectively controlled, plants incur less damage, which allows for the development of more leaves and photosynthetic tissues, increased water and nutrient uptake, with more energy directed towards growth that eventually leads to higher productivity (Kim et al., 2024; Ansabayeva et al., 2025). Concurrently, while lamida gold insecticide effectively reduced soybean pests, chemical NPK fertilizers directly provided essential nutrients for soybean growth and biomass production. This is demonstrated by the strong negative correlation between soybean growth parameters, biomass production and BLB infestation. This is in line with our hypothesis that locally produced bio-inoculants of microbial and plant origins will reduce pest infestation and damage on soybean plants, leading to enhanced growth and biomass production.

4.3. Exploration of bio-inoculants for sustainable farming systems

The European Union has identified expansion of organic agricultural production methods as a critical factor in the development of sustainable global food systems, with a target share of 25% organic farmland area by 2030 through the Farm-to-Fork strategy (Rees et al., 2023). Organic action plans appear to be a useful agenda-setting tool, but caution is advised in relying on them to produce consistent results, as highlighted by national action plans in some European countries, with some of them being ineffective (Rees et al., 2023). Nonetheless, the use of synthetic fertilizers and pesticides to resolve poor soil fertility status and pest constraints is considered unsustainable due to high cost, product scarcity, and their human or environmental effects (Shahwar et al., 2023). Alternatively, bio-inoculants from microbial and plant origins can be cost-effective and sustainable options to enhance crop productivity without jeopardising human and environmental health (Reed and Glick, 2023; Rios-Ruiz et al., 2023). Recently, bio-inoculant initiatives in the Central African region have focused on producing effective local products to manage soil acidity and drought stress, soil fertility and crop nutrition, pest and disease pressures (Ngosong et al., 2022; Ngone et al., 2023, 2025; Becke et al., 2024; Olougou et al., 2024; Achiri et al., 2025). Meanwhile, the exploration of microbial products for sustainable agricultural production across Eastern, Southern and Western Africa has gained recognition

(Adedeji et al., 2020; Etesami and Maheshwari, 2018; Ohene-Mensah et al., 2024). Therefore, it is important to overcome biotechnological barriers in developing effective bio-inoculants that are adapted to local conditions and the specific needs of farmers (Diaz-Rodriguez et al., 2025).

5. Conclusion

Microbial inoculants and plant extracts significantly reduced bean leaf beetle and pod borer infestation, severity, and damage on soybean plants, which was comparable to synthetic inputs. Soybean growth and biomass production increased with treatment application when compared to the untreated control. This result highlights the potential of locally formulated bio-inoculants of microbial and plant origins as sustainable alternative options to synthetic pesticides for mitigating pest infestation and damage on soybean plants, leading to increased growth and biomass production. These findings open up avenues for further research on their mechanism of action that can be explored for biological pest control and to increase soybean performance.

Data Availability

All data used to support the findings of this study are included in the article.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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