

Yield responses of multi-crops to interaction of manure and microbial bio-fertiliser in a Surjan raised-bed intercropping system on rainfed drylands

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ABSTRACT

The intercropping cultivation system on rainfed land during the dry season often faces water availability constraints and declining soil fertility, which negatively affect crop productivity. The use of manure and liquid bio-fertilisers is one approach to increase the soil's organic matter content and microbiological activity, thereby improving nutrient availability for plants. This study aimed to analyse the effects of manure and liquid bio-fertiliser dosages, determine the optimum doses of these two inputs, and evaluate their interaction on the yield of five crop commodities in an intercropping system on rainfed land during the dry season. The results showed that crop response to the treatments was commodity-specific. The highest *Cucumis sativus* L. yield (23.1 t ha⁻¹) was obtained with a combination of 25 t ha⁻¹ manure and 3 L ha⁻¹ liquid bio-fertiliser. In comparison, butternut squash achieved a maximum yield of 15.0 t ha⁻¹ with a combination of 15 t ha⁻¹ manure and 3 L ha⁻¹ liquid bio-fertiliser. For *Cap-sicum frutescens* L., the highest yield (0.146 t ha⁻¹) was achieved with a combination of 25 t ha⁻¹ manure and 6 L ha⁻¹ liquid bio-fertiliser. In comparison, *Vigna unguiculata* L. produced its highest yield (0.70 t ha⁻¹) with the same combination. For *Ipomoea aquatica* F., the highest biomass yield (6.3 t ha⁻¹) was obtained with a combination of 25 t ha⁻¹ manure and 3 L ha⁻¹ liquid bio-fertiliser. Optimisation analysis indicated that *Cucumis sativus* L. and butternut squash yields followed a quadratic pattern, with optimum liquid bio-fertiliser dosages of approximately 2.88 L ha⁻¹ and 3.14 L ha⁻¹, respectively. In comparison, the optimum manure dosage for butternut squash was estimated to be approximately 15.19 t ha⁻¹. At the system scale, the intercrop was advantageous under every treatment (LER 1.75–2.95, mean 2.07; ATER 1.30–2.20), with cucumber acting as the dominant component. These results indicate that integrating manure and liquid bio-fertilizers increases the productivity of intercropping systems by improving soil organic matter content and microbial activity under rainfed conditions during the dry season.

Keywords: intercropping, manure, liquid bio-fertilizers, rainfed land, crop productivity.

INTRODUCTION

Rainfed agriculture during the dry season faces increasing pressure due to rising aridity, unpredictable rainfall, and the need to sustainably boost production. Field studies in Mediterranean dry areas show that drylands now cover about 41% of Earth's surface, drought frequency may increase by 5–10 times, and irrigation demand could rise

by around 18% (Matsi et al., 2025). In this context, intercropping has become an essential agricultural approach as it optimizes the use of water, light, and nutrients. Zhou et al. (2024) found that intercropping maize with soybeans resulted in a 19.8% increase in platform yield and a 37.8% decrease in phosphorus needs at the optimal turning point compared to growing them separately. These findings theoretically confirm that dryland

productivity is affected not only by water availability but also by the coordinated management of nutrients and the biological support from the soil.

In rainfed areas, the primary issue during the dry season extends beyond just a lack of water; it also involves low efficiency in input use due to diminished soil biological activity and restricted nutrient availability. Rice fields in these regions, characterized by Vertisol-type heavy clay with low porosity, poor drainage, and significant cracking in dry periods, face a high risk of water stress and erratic crop development. To address these challenges, manure is essential for enhancing the soil's physical and chemical attributes, while liquid biofertilisers are necessary to boost nutrient absorption and stress resilience. Nevertheless, incorrect application rates can lead to inefficiencies, including unsynchronized mineralization, inadequate microbial colonization, wasted resources, and inconsistent yields in multi-crop intercropping systems.

Various empirical studies have attempted to address this issue, but the efforts have remained partial. Moradi & Siosemardeh (2025) demonstrated that integrating fertilizers and biofertilizers in wheat cultivation on rainfed land can reduce chemical inputs while maintaining productive performance. Zhang et al. (2025) reported that *Bacillus* and *Trichoderma*-based biofertilizers increased maize yields by 16.07% and 12.98%, as well as soybean yields by 17.48% and 15.32%, compared to chemical fertilizers. In intercropping systems, Michalitsis et al., (2024) obtained LER values of 1.30 and 1.19 in faba bean–wheat intercropping, while Matsi et al., (2025) reported that carrot yield efficiency increased by more than 27% in a rainfed thyme-based intercropping system. Nevertheless, previous studies generally assessed only one or two commodities, have not tested manure and liquid bio-fertilizers simultaneously, have not determined the optimum dosage of both inputs for five commodities at once, and have not explained the interaction effects of both on intercropping under rainfed conditions during the dry season. As a result, there is still no truly evidence-based foundation for recommending whether the response of each commodity is linear, quadratic, or is greatly influenced by the combination of both inputs. This is the research gap underlying the present study.

Based on this gap, the objectives of this research are to analyze the effect of manure dosage and liquid bio-fertilizer dosage on the yields

of five crop commodities in intercropping cultivation on rainfed land during the dry season; to optimize both dosages for the production of the five commodities; and to evaluate the interaction effects of the two on the yield of each crop. This research is important because it establishes a scientific basis for more precise, commodity-specific fertilizer recommendations suitable for rainfed land. Practically, its findings have the potential to increase the efficiency of organic and biological inputs, enhance the stability of multi-commodity production during the dry season, and support more sustainable agricultural intensification.

MATERIALS AND METHODS

Study site and climatic conditions

the experiment was conducted in Klitih Hamlet, Wajik Village, Lamongan District, Lamongan Regency, East Java, Indonesia (7°08'27.10"S, 112°23'46.79"E), at an elevation of approximately 7 m above sea level. The site is classified as a lowland rainfed paddy field with a Vertisol soil type, characterized by a heavy clay texture, low porosity, and poor drainage, which typically cause waterlogging during the rainy season and develop deep cracks during the dry season (Bronick & Lal, 2005). The mean daily temperature ranges from 27 °C to 29 °C, and the long-term average annual rainfall is 1,482 mm. The study was conducted over two consecutive growing seasons in a rainfed paddy field using a surjan (raised-bed–sunken-bed) system. During the dry season from August to October 2022. The *surjan* system is an indigenous land management practice in lowland Java that creates elevated planting beds separated by lower furrows, enabling diversified cropping on rainfed land with seasonally constrained water availability. The research was conducted on raised beds in a surjan system. The average size of the beds is 3 meters wide by the length (along the length of the rice field bed). The initial chemical properties of the topsoil (0–20 cm), determined before treatment application, are presented in Table 1.

Prior to treatment, the topsoil was characterized by very low organic carbon (0.78%; equivalent to $\approx 1.34\%$ soil organic matter) and very low total nitrogen (0.10%), indicating a limited native pool of organic matter and nitrogen typical of intensively cropped Vertisols during the dry season.

Table 1. Initial chemical properties of the topsoil (0–20 cm) at the experimental site before treatment application

Parameter	Method	Value	Unit	Category*
pH (H ₂ O)	Electrometry	6.50	–	Slightly acidic
pH (KCl)	Electrometry	5.60	–	–
C-organic	Walkley & Black	0.78	%	Very low
N-total	Kjeldahl	0.10	%	Very low
C/N ratio	–	8.00	–	Low
P-available	Bray I	85.09	mg kg ⁻¹	Very high
K-exchangeable	NH ₄ OAc 1 N pH 7	0.45	cmol(+) kg ⁻¹	Medium
Na-exchangeable	NH ₄ OAc 1 N pH 7	1.52	cmol(+) kg ⁻¹	High
Ca-exchangeable	NH ₄ OAc 1 N pH 7	23.25	cmol(+) kg ⁻¹	High
Mg-exchangeable	NH ₄ OAc 1 N pH 7	0.32	cmol(+) kg ⁻¹	Low
Cation exchange capacity (CEC)	NH ₄ OAc 1 N pH 7	50.75	cmol(+) kg ⁻¹	Very high
Sum of bases	NH ₄ OAc 1 N pH 7	25.55	cmol(+) kg ⁻¹	–
Base saturation	NH ₄ OAc 1 N pH 7	50.00	%	Medium
Soil structure	Field description	Angular blocky	–	–

Note: * Category based on the soil chemical-property rating criteria of the Indonesian Soil Research Institute (Balai Penelitian Tanah).

In contrast, available phosphorus was very high (85.09 mg kg⁻¹) and the cation exchange capacity was very high (50.75 cmol(+) kg⁻¹), consistent with the 2:1 clay mineralogy of Vertisols. These baseline conditions establish nitrogen and organic matter — rather than phosphorus — as the primary fertility constraints at the site. This directly justifies the application of fermented manure, whose composition (2.64% N-organic and 14.92% C-organic; Table 2) was intended to replenish the depleted soil organic-matter and nitrogen pools, while the liquid bio-fertilizer was expected to enhance the plant-availability of the already-abundant soil phosphorus through microbial solubilization

Plant materials and agricultural inputs

Five crop species were cultivated in an intercropping arrangement on the raised beds: (i) *Cucumis sativus* L F1 hybrid cv. Harmony (Cap Kapal Terbang); (ii) butternut squash (*Cucurbita moschata* Duchesne) F1 hybrid (Cap Panah Merah); (iii) *Capsicum frutescens* L cv. Dewata 42; (iv) *Vigna unguiculata* L cv. KT-5 (red seed, determinate type); and (v) *Ipomoea aquatica* F cv. BISI (Cap Kapal Terbang). All seed materials were certified high-quality commercial varieties. Fermented manure was used as the organic amendment. The chemical characteristics of the manure, analyzed before field application, are presented in Table 2.

The liquid bio-fertilizer (LBF) used in this study was a commercial formulation containing two active microbial agents: *Trichoderma* sp. and *Beauveria* sp. in liquid suspension. *Trichoderma* sp. functions as both a biofertilizer and biocontrol agent through mechanisms including phosphate solubilization, phytohormone production (indole-3-acetic acid, gibberellic acid), and mycoparasitism against soil-borne pathogens (Tyśkiewicz et al., 2022). *Beauveria* sp. is an entomopathogenic fungus used in biological pest management and also aids plant growth by colonizing the

Table 2. Chemical characteristics of manure used in the experiment

Parameter	Value	Unit
pH (H ₂ O)	8.37	–
Moisture content	10.00	%
N-organic	2.64	%
C-organic (Walkley & Black)	14.92	%
Phosphorus (P)	1.45	%
Sulfur (S)	1.55	%
Potassium (K)	0.77	%
Sodium (Na)	0.20	%
Calcium (Ca)	1.10	%
Magnesium (Mg)	0.22	%
Iron (Fe)	1,518.17	ppm
Zinc (Zn)	120.38	ppm
Copper (Cu)	6.68	ppm
Manganese (Mn)	931.37	ppm

rhizosphere (Jaber & Enkerli, 2017). The liquid formulation included a minimum viable spore concentration of $\geq 1 \times 10^6$ colony-forming units (CFU) mL^{-1} for each microbial agent, meeting the quality standards set for commercial liquid bio-fertilizer products (Kumar et al., 2023).

Experimental design

The research employed a Factorial Randomized Complete Block Design (RCBD), incorporating two treatment variables and three replications. The first factor was manure dosage at three levels: 0 t ha^{-1} (control), 15 t ha^{-1} , and 25 t ha^{-1} . The second factor was liquid biofertilizer dosage at three levels: 0 l ha^{-1} (control), 3 l ha^{-1} , and 6 l ha^{-1} . The factorial combination of the two factors produced nine treatment combinations, each replicated three times, resulting in a total of 27 experimental plots. Each plot measured $3 \text{ m} \times 1 \text{ m}$ (3 m^2) on the raised bed of the *surjan* system.

Field implementation

Land preparation involved manual tillage with hoes to establish the *surjan* raised-bed-sunken-bed configuration and to loosen the soil on the planting beds. Fermented manure was incorporated into the topsoil layer (0–20 cm) of each plot one week before transplanting, according to the assigned dosage treatments. The manure was

evenly mixed into the tillage layer to ensure homogeneous distribution of organic matter within the root zone (Adekiya et al., 2020).

Planting was carried out on the raised beds following crop-specific spacing: *Cucumis sativus* L ($40 \times 50 \text{ cm}$, one seed per hole), *Vigna unguiculata* L ($40 \times 20 \text{ cm}$, one to two seeds per hole), *Capsicum frutescens* L ($50 \times 70 \text{ cm}$, seedlings transplanted after emergence of two flower buds), *Ipomoea aquatica* F ($20 \times 15 \text{ cm}$, one to two seeds per hole), and butternut squash ($100 \times 50 \text{ cm}$, one seed per hole). Prior to sowing, seeds were primed by immersion in a solution of plant growth-promoting rhizobacteria (PGPR) to enhance germination rates and reduce the risk of pathogen attack at the seedling stage (Kumar et al., 2023). The following is the intercropping design of *Cucumis sativus* L-butternut squash-*Capsicum frutescens* L-*Vigna unguiculata* L-*Ipomoea aquatica* F (Figure 1).

The liquid biofertilizer containing *Trichoderma* sp. and *Beauveria* sp. was applied as foliar and basal sprays according to the assigned dosage treatments at 7-day intervals over a 60-day application period, commencing at the early vegetative stage. This weekly application regime was designed to sustain *rhizosphere* and *phyllosphere* colonization by the bioagents throughout the critical growth phases, as the establishment and persistence of *Trichoderma* in the root zone requires repeated inoculation under tropical field



Figure 1. Intercropping model of *Cucumis sativus* L, butternut squash, *Capsicum frutescens* L, *Vigna unguiculata* L, *Ipomoea aquatica* F, on raised beds in the *Surjan* system.

Note: ▲ – *Capsicum frutescens* L, ● – *Ipomoea aquatica* F, ■ – *Vigna unguiculata* L, ○ – butternut squash, *Cucumis sativus* L, + – *Cucumis sativus* L.

conditions (Kumar et al., 2023). Plant maintenance included irrigation as required, staking *Cucumis sativus* L. plants 1 week post-planting, and implementing biological pest management through scheduled LBF applications. Harvesting was conducted at physiological maturity for each crop species, as determined by standard agronomic indicators.

Yield variables and measurement

Yield variables were measured for all five crop species in the intercropping system. For *Cucumis sativus* L., butternut squash, *Capsicum frutescens* L., and *Vigna unguiculata* L., the variables observed included: fruit or pod weight per plant ($\text{g}\cdot\text{plant}^{-1}$), fruit or pod weight per plot ($\text{g}\cdot\text{plot}^{-1}$), fruit or pod weight per hectare ($\text{t}\cdot\text{ha}^{-1}$ or $\text{ku}\cdot\text{ha}^{-1}$), number of fruits or pods per plant, number of fruits or pods per plot, fruit or pod length (cm), and fruit diameter (cm). For *Vigna unguiculata* L., additional measurements were taken on seed weight per plant ($\text{g}\cdot\text{plant}^{-1}$), seed weight per plot ($\text{g}\cdot\text{plot}^{-1}$), seed weight per hectare ($\text{t}\cdot\text{ha}^{-1}$), and number of seeds per plant and per plot. For *Ipomoea aquatica* F., yield variables comprised plant fresh weight per plant ($\text{g}\cdot\text{plant}^{-1}$), plant fresh weight per plot ($\text{g}\cdot\text{plot}^{-1}$), and plant fresh weight per hectare ($\text{t}\cdot\text{ha}^{-1}$).

Conversion of plot-level yield to per-hectare productivity was calculated using the yield per hectare (YPH) formula with correction for effective land proportion, as follows:

$$YPH = \frac{10\,000\text{ m}^2}{\text{plot area}} \cdot \text{yield per harvest plot} \cdot \% \text{ effective land} \quad (1)$$

$$\% \text{ effective land} = \frac{\text{bed width}}{(\text{bed width} + \text{inter-bed spacing})} \times 100\% \quad (2)$$

This correction accounts for the proportion of the total field area occupied by the productive raised beds relative to the non-productive sunken furrows within the surjan system, thereby ensuring accurate extrapolation of plot-scale results to a per-hectare basis.

The agronomic performance of the intercropping system was quantified using four standard intercropping indices, computed against sole-crop yields obtained from monoculture plots established at the same site and in the same season. The land equivalent ratio (LER) was calculated as $\text{LER} = \sum (Y_i/S_i)$, where Y_i and S_i are the intercrop and sole-crop yields of species i . The area-time equivalent ratio (ATER) adjusted the

partial LERs for the growth duration of each species: $\text{ATER} = \sum [(Y_i/S_i) \times d_i]/D$, where d_i is the duration of species i (cucumber 70, chili 95, water spinach 21, butternut squash 90, cowpea 90 days) and D is the total duration of the intercrop system (95 days) (Hiebsch & McCollum, 1987). Aggressivity (A) and the competition ratio (CR) were computed for each species relative to the remaining components of the mixture to describe the competitive hierarchy, where $A > 0$ and $CR > 1$ identify a dominant species and $A < 0$ and $CR < 1$ a subordinate one (Willey & Rao, 1980).

Statistical analysis

All yield data were subjected to a two-way analysis of variance (ANOVA) using a factorial RCBD to test the main effects of manure dosage and liquid biofertilizer (LBF) dosage, as well as their interaction (manure $\times$ LBF). When the ANOVA indicated statistically significant effects ($P \leq 0.05$), means were separated using Tukey's honestly significant difference (HSD) test at the $\alpha = 0.05$ significance level. For the interaction analysis, simple effects were examined by comparing levels of one factor within each fixed level of the other factor, with the critical value for pairwise comparisons derived from the pooled experimental error (Gomez & Gomez, 2004). Optimum dosage combinations were determined using quadratic regression of productivity data on dosage levels, with optimum points identified from the first derivative of the fitted polynomial. All statistical computations were performed using SmartstatXL software version 3.6.5.4 (Setiawan, 2023).

RESULTS AND DISCUSSION

The interaction between manure and liquid biofertiliser is an important factor determining the effectiveness of integrated nutrient management in intercropping systems, especially on rain-fed land during the dry season, which faces limited water availability and reduced soil biological activity (Nyawade et al., 2019). The synergistic effect between organic matter, as a decomposition substrate, and liquid biofertiliser, as a rhizosphere biostimulator, has the potential to multiplicatively increase productivity, surpassing the additive effects of each factor. However, this interaction pattern is highly specific to the commodity and the dose levels applied (Backer et al., 2018). It has been proposed in other systems that intercropping

may enhance rhizosphere metabolite chemodiversity, which could in turn drive microbial community complexity and promote nutrient uptake, provided that microbial inputs remain within the carrying capacity of the rhizosphere environment (Jiang et al., 2024).

The intercropping cultivation system on rainfed land during the dry season encounters the dual challenge of limited water availability and declining soil fertility, necessitating the optimization of agronomic inputs to sustain multi-commodity productivity (Brooker et al., 2015). Manure, as a source of organic matter, contributes to enhancing soil structure, increasing cation exchange capacity, and gradually supplying macro- and micronutrients. Meanwhile, rhizosphere microorganism-based liquid bio-fertilizers function as biostimulators, enhancing nutrient uptake efficiency and plant tolerance to drought stress (Adekiya et al., 2020; Backer et al., 2018). Recent reviews have confirmed that *Trichoderma*-based biofertilizers are particularly effective in this regard, as they not only solubilize phosphates and produce phytohormones but also remodel rhizosphere microbial communities to favor plant growth (Asghar et al., 2024). The following sections present, for each crop group, the main effects of manure and LBF dosage, their interaction, and the dose-optimization analysis (Figures 2 and 3; Tables 3–10).

Cucurbitaceae – cucumber and butternut squash

Cucumber (*Cucumis sativus* L.)

In *Cucumis sativus* L. plants, the manure factor only had a significant effect on fruit weight per plant (Table 3). A dose of 25 t ha⁻¹ produced the highest average fruit weight per plant (1,717 ± 323 g), which was not significantly different from the control (1,571 ± 263 g), but both were significantly higher than the 15 t ha⁻¹ dose (1,237 ± 463 g). The decrease at the 15 t ha⁻¹ dose may be due to the nitrogen mineralization rate from the manure not being optimal at this intermediate dose during the dry season (Adekiya et al., 2020). This temporary depression at intermediate manure doses is consistent with nitrogen immobilization dynamics reported in organic-amended soils, where microbial demand for nitrogen during the early decomposition phase can temporarily reduce plant-available N (Tang et al., 2025). There were no significant differences in fruit weight per plot, productivity, fruit number, fruit length, or fruit diameter across manure treatments. In contrast, the bio-agent factor significantly influenced fruit weight per plot and productivity per hectare (Table 3). A dose of 3 L ha⁻¹ yielded the highest productivity (18.9 ± 4.0 t ha⁻¹), an increase of 42.1% compared to the control (13.3 ± 2.9 t ha⁻¹) and 52.4% compared to the 6 L ha⁻¹ dose (12.4 ±

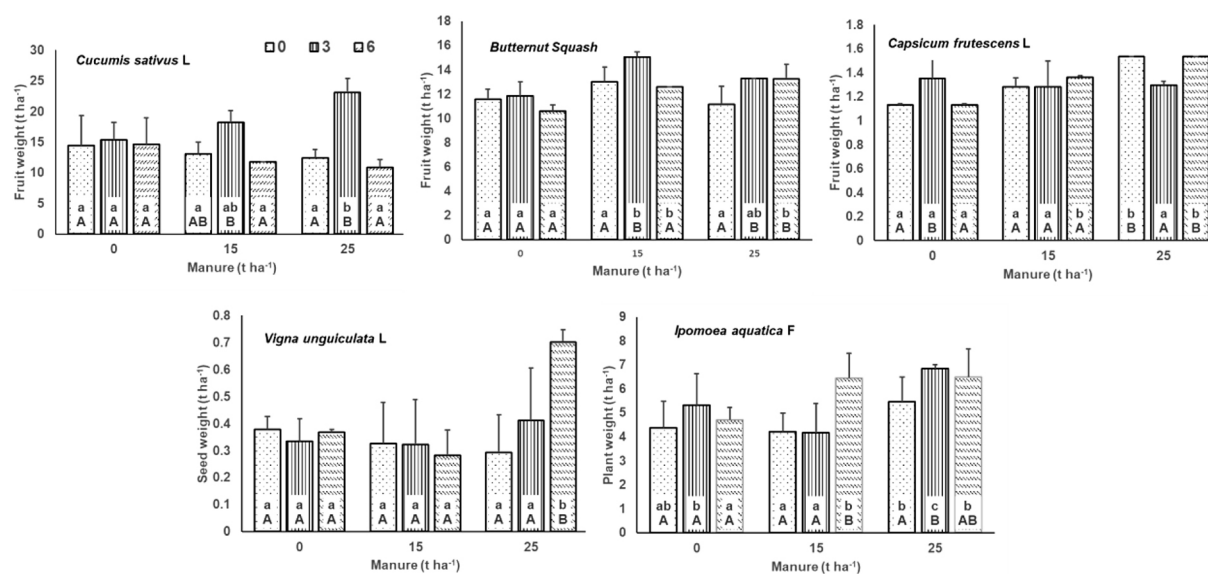


Figure 2. Average interaction between manure dosage and liquid bio-fertilizer dosage on five crops in rainfed fields during the dry season. Mean values followed by the same letter are not significantly different according to Tukey's post hoc test (BNJ) at the 0.05 significance level. Lowercase letters are read vertically, comparing between two manure levels at the same liquid bio-fertilizer dose; uppercase letters are read horizontally, comparing between two liquid bio-fertilizer doses at the same manure level.

Table 3. Average *Cucumis sativus* L. yield at different doses of manure and liquid bio-fertilizers during the dry season

Dosage	Yield components						
	Fruit weight (g plant ⁻¹)	Fruit weight (g plot ⁻¹)	Fruit weight (t ha ⁻¹)	Number of fruits (fruit plant ⁻¹)	Number of fruits (fruit plot ⁻¹)	Fruit length (cm)	Fruit diameter (cm)
Manure (t ha ⁻¹)							
0	1,571 ± 263 b	4,444 ± 1,081	14.8 ± 3.6	6.8 ± 0.8	11.9 ± 1.3	19.4 ± 2.1	5.1 ± 0.2
15	1,237 ± 463 a	3,960 ± 1,577	14.4 ± 3.2	6.7 ± 1.0	11.4 ± 1.7	18.7 ± 1.2	5.1 ± 0.1
25	1,717 ± 323 b	4,586 ± 2,105	15.5 ± 6.0	7.0 ± 1.1	11.5 ± 1.3	19.3 ± 1.1	5.1 ± 0.2
Liquid bio-fertilizer (l ha ⁻¹)							
0	1,583 ± 274	4,028 ± 1,064 a	13.3 ± 2.9 a	6.7 ± 0.9	11.7 ± 1.9	18.7 ± 1.8	5.1 ± 0.1
3	1,610 ± 295	5,549 ± 1,756 b	18.9 ± 4.0 b	7.1 ± 0.9	11.9 ± 1.3	19.2 ± 1.4	5.1 ± 0.2
6	1,331 ± 556	3,413 ± 1,181 a	12.4 ± 2.8 a	6.6 ± 1.1	11.2 ± 0.8	19.4 ± 1.3	5.0 ± 0.2
Tukey manure 0.05	310.5	ns	ns	ns	ns	ns	ns
Tukey liquid bio-fertilizer 0.05	310.5	1316.7	3.3	ns	ns	ns	ns
CV (%)	16.9	25.0	18.0	10.4	10.3	7.0	2.7

Note: Values are mean ± SD. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$). CV: coefficient of variation; ns: not significant.

2.8 t ha⁻¹). The quadratic response pattern may indicate that high levels of liquid biofertiliser could promote microbial competition in the rhizosphere and reduce the effectiveness of root colonization, although these processes were not directly measured here (Backer et al., 2018). In line with this, moderate applications of a *Trichoderma*-enriched biofertilizer enhanced cucumber yield by promoting beneficial *Aspergillus* populations in the soil, whereas excessive inoculant doses interfered with these beneficial microbial interactions (Hang et al., 2022). Furthermore, continuous fertilization studies on cucumber rhizosphere microbiomes have shown that excessive inputs can deteriorate microbial network stability through accumulation of inhibitory ions (Tang et al., 2025).

A simple analysis of the interaction effect in *Cucumis sativus* L. revealed a highly specific synergistic pattern in the combination of 25 t ha⁻¹ manure with 3 L ha⁻¹ bio-agent. This combination yielded the highest productivity (23.1 ± 2.3 t ha⁻¹), which was significantly different vertically from the 0 and 15 t ha⁻¹ manure treatments at the same liquid biofertiliser level (15.4 ± 2.8 and 18.2 ± 1.9 t ha⁻¹, respectively), and significantly different horizontally from the 0 and 6 L ha⁻¹ liquid biofertiliser treatments at 25 t ha⁻¹ manure (12.4 ± 1.4 and 10.8 ± 1.3 t ha⁻¹, respectively). The 86.3% increase in productivity from the lowest combination (25 t ha⁻¹ × 6 L ha⁻¹ = 10.8 t ha⁻¹) to the highest combination (25 t ha⁻¹ × 3 L ha⁻¹ = 23.1 t ha⁻¹) indicates that the synergistic effect

of high manure and moderate liquid biofertiliser surpasses the additive effects of the two factors. This phenomenon may be related to the increased organic substrate, which stimulates optimal proliferation of liquid biofertiliser colonies at the 3 L ha⁻¹ dose, whereas at 6 L ha⁻¹, inter-colony competition may suppress effectiveness (Backer et al., 2018). Without manure (0 dose), there were no significant differences among liquid biofertiliser levels, suggesting that the effect of the liquid biofertiliser on *Cucumis sativus* L. may depend on the availability of organic matter as a carbon source for rhizosphere microbes.

The quadratic regression model for the productivity of *Cucumis sativus* L. in relation to manure dosage is represented by $y = 0.0055x^2 - 0.1087x + 14.8$ ($R^2 = 0.671$). This model identifies an optimal dosage of 9.88 t ha⁻¹, predicting a productivity of 14.26 t ha⁻¹ (Figure 3a). The positive x^2 coefficient yields a convex curve, indicating that productivity initially decreases at moderate doses before rising again at higher doses, likely due to nitrogen immobilization during the early stages of manure decomposition under drought conditions (Wu et al., 2023). In contrast, the response to liquid bio-fertilizers is described by $y = -0.6722x^2 + 3.8833x + 13.3$ ($R^2 = 0.743$), with an optimal dosage of 2.88 L ha⁻¹ and a maximum predicted productivity of 18.91 t ha⁻¹ (Figure 3f). The negative x^2 coefficient creates a concave curve, and the higher R^2 value suggests that liquid bio-fertilizers have a more significant

impact on *Cucumis sativus* L. productivity than manure. The decrease in productivity at dosages above 2.88 L ha⁻¹ is consistent with, but does not by itself demonstrate, intraspecific competition among microbial colonies that could reduce root colonization efficiency at high inoculant concentrations (Backer et al., 2018). This phenomenon of microbial competition has been further explained, highlighting poor colonization ability and unstable field performance of biofertilizers as major challenges, especially when inoculant doses surpass the host rhizosphere's carrying capacity (Fadiji et al., 2024).

Butternut squash

In butternut squash, manure has a significant effect on productivity and the number of fruits per plot (Table 4). A dose of 15 t ha⁻¹ resulted in the highest productivity (13.5 ± 1.3 t ha⁻¹), followed by 25 t ha⁻¹ (12.6 ± 1.4 t ha⁻¹), both of which were significantly higher than the control (11.3 ± 0.9 t ha⁻¹). The progressive increase in the number of fruits per plot from 2.3 (control) to 3.2 at the 25 t ha⁻¹ dose indicates that a continuous supply of organic nutrients supports fruit formation in *Cucurbita* (Capo & Blandino, 2021). The biotic agent factor also had a significant effect on the same two parameters. A dose of 3 L ha⁻¹ produced the highest productivity (13.4 ± 1.5 t ha⁻¹) and the highest number of fruits per plot (3.2 ± 1.1), while the 6 L ha⁻¹ dose (12.1 ± 1.4 t ha⁻¹) was not significantly different from the control. The quadratic pattern shared with *Cucumis sativus* L.

supports the hypothesis of a saturation point for the use of biotic agents within the Cucurbitaceae family. This saturation threshold is consistent with research showing that biofertilizers amended with *Trichoderma* significantly boosted plant biomass in field trials; however, this positive effect was achieved through the selective enrichment of soil fungal communities rather than a straightforward dose-dependent reaction (Capo & Blandino, 2021). Fruit weight per plot, number of fruits per plant, fruit length, and fruit diameter were not significantly affected by either treatment factor.

The interactions observed in butternut squash cultivation exhibit a more intricate pattern. The application of 15 t ha⁻¹ manure with 3 L ha⁻¹ liquid biofertiliser resulted in the highest yield (15.0 ± 0.4 t ha⁻¹), significantly greater than the control (11.6 ± 0.8 t ha⁻¹). At a manure dose of 25 t ha⁻¹, both 3 L ha⁻¹ (13.3 t ha⁻¹) and 6 L ha⁻¹ (13.2 ± 1.2 t ha⁻¹) of liquid biofertiliser differed significantly (horizontally) from the liquid biofertiliser control (11.2 ± 1.4 t ha⁻¹). An important finding is the shift in the optimal liquid biofertiliser dose: at 15 t ha⁻¹ manure, 3 L ha⁻¹ is optimal, whereas at 25 t ha⁻¹ manure, 6 L ha⁻¹ is equally effective. This may indicate that increasing organic substrate expands the rhizosphere's carrying capacity for liquid biofertiliser populations in Cucurbitaceae, although rhizosphere populations were not measured in this study (Yadav et al., 2020). Without manure, there was no meaningful interaction.

Butternut squash exhibits a concave reaction to manure application, described by $y =$

Table 4. Average butternut squash yield at different doses of manure and liquid bio-fertilizers during the dry season

Dosage	Yield components					
	Fruit weight (g plot ⁻¹)	Fruit weight (t.h ⁻¹)	Number of fruits (fruit plant ⁻¹)	Number of fruits (fruit plot ⁻¹)	Fruit length (cm)	Fruit diameter (cm)
Manure (t ha ⁻¹)						
0	3,314 ± 522	11.3 ± 0.9 a	1.8 ± 0.6	2.3 ± 1.2 a	19.1 ± 3.3	7.5 ± 0.4
15	3,580 ± 1,082	13.5 ± 1.3 b	1.8 ± 0.6	2.6 ± 1.0 ab	19.0 ± 2.7	7.7 ± 0.8
25	3,176 ± 1,065	12.6 ± 1.4 b	2.1 ± 0.5	3.2 ± 1.0 b	19.9 ± 3.1	7.2 ± 0.9
Liquid bio-fertilizer (l ha ⁻¹)						
0	3,395 ± 779	11.9 ± 1.3 a	1.8 ± 0.6	2.3 ± 1.0 a	18.8 ± 3.6	7.3 ± 1.1
3	3,429 ± 1,147	13.4 ± 1.5 b	2.0 ± 0.5	3.2 ± 1.1 b	19.5 ± 3.1	7.6 ± 0.7
6	3,246 ± 854	12.1 ± 1.4 a	1.9 ± 0.7	2.7 ± 1.1 ab	19.7 ± 2.2	7.6 ± 0.4
Tukey manure 0.05	ns	1.1	ns	0.8	ns	ns
Tukey liquid bio-fertilizer 0.05	ns	1.1	ns	0.8	ns	ns
CV	21.4	7.3	13.6	23.9	8.4	7.8

Note: Values are mean ± SD. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$). CV: coefficient of variation; ns: not significant.

$-0.0095x^2 + 0.2887x + 11.3$ ($R^2 = 0.728$), reaching an optimal point at 15.19 t ha^{-1} with an anticipated yield of 12.49 t ha^{-1} (Figure 3b). The negative x^2 coefficient signifies a true maximum, where increasing the dosage beyond 15.19 t ha^{-1} reduces productivity, probably due to an imbalance in the C/N ratio at higher doses that hampers nutrient mineralization (Xin Zhang et al., 2023). For the liquid biofertiliser factor, $y = -0.1556x^2 + 0.9667x + 11.9$ ($R^2 = 0.697$) shows an optimum at 3.14 L ha^{-1} with maximum productivity of 13.44 t ha^{-1} (Figure 3g). The consistent concave pattern in both Cucurbitaceae members (*Cucumis sativus* L. and butternut squash) confirms that this group has a saturation threshold for liquid biofertiliser around 3 L ha^{-1} ; exceeding this dose leads to counterproductive microbial competition (Yadav et al., 2020). Overall, both Cucurbitaceae members share a saturation threshold for liquid biofertiliser near 3 L ha^{-1} combined with moderate-to-high manure, distinguishing them from the other three commodities.

Solanaceae – chili pepper (*Capsicum frutescens* L.)

Capsicum frutescens L. is the commodity most responsive to both treatment factors. Manure has a significant effect on four of seven measured parameters: fruit weight per plant, fruit weight per plot, productivity, and number of fruits per plot (Table 5). A dose of 15 t ha^{-1} significantly

increases fruit weight per plant ($17.6 \pm 3.0 \text{ g}$) compared to the control ($15.0 \pm 3.5 \text{ g}$), while productivity shows a linear increase, with the 25 t ha^{-1} dose reaching the highest value ($0.146 \pm 0.012 \text{ t ha}^{-1}$). The biotic agent factor significantly affects weight per plant, weight per plot, and the number of fruits per plot. A dose of 6 L ha^{-1} results in the highest fruit weight per plant ($18.6 \pm 3.1 \text{ g}$) and the highest weight per plot ($42.8 \pm 5.7 \text{ g}$), significantly different from doses of 0 and 3 L ha^{-1} . Unlike the Cucurbitaceae group, *Capsicum frutescens* L. shows a positive linear response to biotic agents up to the highest dose, possibly related to the capacity of Solanaceae rhizospheres to accommodate a larger microbial population, a mechanism not directly assessed in this study (Adesemoye & Kloepper, 2009). This family-specific tolerance to higher biofertilizer doses is consistent with evidence that Solanaceae crops recruit diverse and functionally resilient rhizosphere microbial communities, enabling them to benefit from greater microbial inputs without encountering saturation effects (Asghar et al., 2024).

Interactions in *Capsicum frutescens* L. yielded a fundamentally different pattern from Cucurbitaceae. Two combinations produced the highest, equivalent yields: 25 t ha^{-1} manure $\times$ 0 L ha^{-1} liquid biofertiliser (0.154 t ha^{-1}) and 25 t ha^{-1} manure $\times$ 6 L ha^{-1} liquid biofertiliser (0.154 t ha^{-1}). Both combinations were significantly different from $25 \text{ t ha}^{-1} \times 3 \text{ L ha}^{-1}$ ($0.130 \pm 0.003 \text{ t ha}^{-1}$), which represented the lowest value at the 25 t ha^{-1}

Table 5. Average *Capsicum frutescens* L. yield at different doses of manure and liquid bio-fertilizers during the dry season

Dosage	Yield components						
	Fruit weight (g plant ⁻¹)	Fruit weight (g plot ⁻¹)	Fruit weight (ku h ⁻¹)	Number of fruits (fruit plant ⁻¹)	Number of fruits (fruit plot ⁻¹)	Fruit length (cm)	Fruit diameter (cm)
Manure (t ha ⁻¹)							
0	15.0 $\pm$ 3.5 a	32.1 $\pm$ 3.2 a	1.20 $\pm$ 0.14 a	25.7 $\pm$ 7.8	17.8 $\pm$ 8.1 a	3.6 $\pm$ 0.2	0.53 $\pm$ 0.06
15	17.6 $\pm$ 3.0 b	39.8 $\pm$ 5.1 b	1.31 $\pm$ 0.12 a	27.1 $\pm$ 11.0	29.6 $\pm$ 1.8 b	3.6 $\pm$ 0.1	0.53 $\pm$ 0.04
25	17.0 $\pm$ 2.0 ab	38.6 $\pm$ 7.1 b	1.46 $\pm$ 0.12 b	24.8 $\pm$ 8.3	33.5 $\pm$ 8.8 b	3.6 $\pm$ 0.1	0.51 $\pm$ 0.05
Liquid bio-fertilizer (l ha ⁻¹)							
0	16.5 $\pm$ 2.1 ab	34.6 $\pm$ 4.9 a	1.31 $\pm$ 0.18	24.2 $\pm$ 8.0	21.9 $\pm$ 9.0 a	3.6 $\pm$ 0.1	0.52 $\pm$ 0.03
3	14.5 $\pm$ 2.3 a	33.0 $\pm$ 2.5 a	1.31 $\pm$ 0.14	27.4 $\pm$ 10.4	28.7 $\pm$ 7.2 b	3.5 $\pm$ 0.1	0.52 $\pm$ 0.07
6	18.6 $\pm$ 3.1 b	42.8 $\pm$ 5.7 b	1.34 $\pm$ 0.18	25.9 $\pm$ 8.6	30.4 $\pm$ 10.9 b	3.7 $\pm$ 0.1	0.54 $\pm$ 0.04
Tukey manure 0.05	2.34	2.87	0.12	ns	6.42	ns	ns
Tukey liquid bio-fertilizer 0.05	2.34	2.87	0.12	ns	6.42	ns	ns
CV	11.64	6.41	7.43	15.83	19.55	3.74	7.75

Note: Values are mean $\pm$ SD. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$). CV: coefficient of variation; ns: not significant.

manure dose. This pattern contrasts sharply with Cucurbitaceae, where a dose of 3 L ha⁻¹ was superior. In *Capsicum frutescens* L., the highest productivity is achieved when high manure doses are combined with liquid biofertiliser doses at either extreme (0 or 6 L ha⁻¹), whereas the intermediate dose (3 L ha⁻¹) is suboptimal. This phenomenon may be related to the specificity of Solanaceae–microbe rhizosphere interactions, in which a moderate dose of a liquid biofertiliser could disrupt the balance of the endogenous community without being dominant enough to elicit a biostimulatory effect, although this was not directly assessed here (Kumar et al., 2023).

Capsicum frutescens L. shows a fundamentally different regression pattern from that of the Cucurbitaceae. For manure application, $y = 0.00003x^2 + 0.00027x + 0.12$ ($R^2 = 0.728$) yields a curve with a very small positive x^2 coefficient, indicating an almost linear response. The optimum point at 4.5 t ha⁻¹ with a productivity of 0.119 t ha⁻¹ (Figure 3c) represents the minimum point of a convex curve, not a maximum. Productivity continues to increase beyond this point up to the highest tested dose (25 t ha⁻¹ = 0.146 t ha⁻¹), indicating that the response capacity of *Capsicum frutescens* L. to organic matter has not reached saturation within the tested dose range (Kumar et al., 2023). For the liquid biofertiliser factor, $y = 0.00017x^2 - 0.0005x + 0.131$ ($R^2 = 0.672$) is also convex with a minimum point at 1.47 L ha⁻¹ (Figure 3h), where productivity marginally increases

from 0.131 to 0.134 t ha⁻¹ at a dose of 6 L ha⁻¹. The minimal impact of the bio-agent suggests that for *Capsicum frutescens* L., the availability of organic nutrients is a more significant limiting factor than the stimulation of the rhizosphere (Adesemoye & Kloepper, 2009). This is consistent with recent findings indicating that the effectiveness of biofertilizers varies greatly depending on crop type and soil quality, with crops in nutrient-deficient soils responding more to organic matter additions than to microbial biostimulants alone (Agustina, Rahmawati, et al., 2025).

Grain legume–cowpea (*Vigna unguiculata* L.)

Vigna unguiculata L. exhibits a complex and differential response pattern between pod components (Table 6) and seed components (Table 7). In the pod component, application of 15 t ha⁻¹ manure resulted in the highest pod weight per plant (79.9 ± 9.7 g) and the highest number of pods per plot (169 ± 18), whereas the 25 t ha⁻¹ dosage yielded the lowest pod weight per plant (67.5 ± 3.8 g). Conversely, in the seed component, the 25 t ha⁻¹ dosage produced the highest seed weight per plot (140.6 ± 65.8 g) and the highest seed yield (0.47 ± 0.22 t ha⁻¹). This opposing trend indicates that increased manure levels redirect photosynthate from pod development to seed filling (Fageria et al., 2005; Fadiji et al., 2024). Similar changes in assimilate distribution in cowpea under different organic and

Table 6. Average *Vigna unguiculata* L. pod yield at different doses of manure and liquid bio-fertilizers during the dry season

Dosage	Yield components					
	Pod weight (g plant ⁻¹)	Pod weight (g plot ⁻¹)	Pod weight (t h ⁻¹)	Number of pod (pod plant ⁻¹)	Number of pod (pod plot ⁻¹)	Pod length (cm)
Manure (t ha ⁻¹)						
0	73.5 ± 11.4 ab	103 ± 48	0.35 ± 0.13	54.9 ± 6.6	156 ± 24 ab	17.1 ± 0.5
15	79.9 ± 9.7 b	95 ± 62	0.34 ± 0.19	56.0 ± 4.9	169 ± 18 b	17.0 ± 0.5
25	67.5 ± 3.8 a	95 ± 55	0.30 ± 0.12	55.7 ± 5.8	148 ± 12 a	17.0 ± 0.5
Liquid bio-fertilizer (l ha ⁻¹)						
0	70.1 ± 6.6 a	76 ± 47 a	0.26 ± 0.14 a	52.9 ± 5.8	152 ± 7 a	17.0 ± 0.6
3	70.7 ± 7.2 a	127 ± 58 b	0.42 ± 0.11 b	56.2 ± 4.7	170 ± 24 b	17.1 ± 0.3
6	80.1 ± 12.5 b	90 ± 45 ab	0.32 ± 0.15 ab	57.5 ± 5.8	151 ± 21 a	17.1 ± 0.6
Tukey manure 0.05	7.9	ns	ns	ns	17.7	ns
Tukey liquid bio-fertilizer 0.05	7.9	41.3	0.1	ns	17.7	ns
CV	8.8	34.7	28.2	9.9	9.3	2.5

Note: Values are mean ± SD. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$). CV: coefficient of variation; ns: not significant.

Table 7. Average *Vigna unguiculata* L. seed yield at different doses of manure and liquid bio-fertilizers during the dry season

Dosage	Yield components				
	Seed weight (g plant ⁻¹)	Seed weight (g plot ⁻¹)	Seed weight (t ha ⁻¹)	Number of seed (seed plant ⁻¹)	Number of seed (seed plot ⁻¹)
Manure (t ha ⁻¹)					
0	16.3 ± 1.7	108.2 ± 15.7 a	0.36 ± 0.05 a	574 ± 63	973 ± 481 b
15	16.8 ± 2.5	93.3 ± 37.1 a	0.31 ± 0.12 a	574 ± 78	1,059 ± 171 b
25	18.8 ± 5.1	140.6 ± 65.8 b	0.47 ± 0.22 b	570 ± 58	571 ± 190 a
Liquid bio-fertilizer (l ha ⁻¹)					
0	18.0 ± 0.2	99.8 ± 33.7 a	0.33 ± 0.11 a	568 ± 34	727 ± 322 a
3	16.4 ± 3.1	106.9 ± 42.3 ab	0.36 ± 0.14 ab	574 ± 90	1,152 ± 290 b
6	17.4 ± 5.2	135.3 ± 59.6 b	0.45 ± 0.20 b	576 ± 63	724 ± 356 a
Tukey manure 0.05	ns	30.7	0.1	ns	225.8
Tukey liquid bio-fertilizer 0.05	ns	30.7	0.1	ns	225.8
CV	14.9	22.1	22.1	11.7	21.4

Note: Values are mean ± SD. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$). CV: coefficient of variation; ns: not significant.

biological fertilization conditions have been observed, where the use of integrated biofertilizers with PGPR notably improved pod dry weight and total grain carbohydrates, suggesting that microbially facilitated nutrient availability channels carbon towards seed reserves (Omer et al., 2023). The bio-agent factor significantly influenced pod weight per plant, pod weight per plot, pod yield, number of pods per plot, seed weight per plot, and seed yield. A dosage of 3 L ha⁻¹ was ideal for pod production (plot weight 127 ± 58 g; yield 0.42 ± 0.11 t ha⁻¹), whereas 6 L ha⁻¹ was optimal for pod weight per plant (80.1 ± 12.5 g) and seed yield (0.45 ± 0.20 t ha⁻¹). This dose-dependent partitioning response is consistent with recent findings that the combined application of inorganic fertilizers, organic manure, and consortium biofertilizers significantly increased soil enzymatic activity and nutrient availability, ultimately boosting cowpea seed yield and soil quality (Kaur et al., 2024).

Interactions among seed components in *Vigna unguiculata* L. exhibit a synergistic effect localized exclusively to a single combination. The combination of 25 t ha⁻¹ manure × 6 L ha⁻¹ liquid biofertiliser produced a seed yield of 0.70 ± 0.05 t ha⁻¹, an increase of 150% compared to the average of the other eight combinations (0.28–0.41 t ha⁻¹). No other combination showed significant differences in either direction. This distinct reaction suggests a threshold effect: the combined impact of manure and liquid biofertiliser on *Vigna*

unguiculata L. becomes evident only when both are applied at their highest levels simultaneously. Mechanistically, the combination of a high manure dose providing abundant carbon and nitrogen substrates with a high dose of liquid biofertiliser is thought to create favorable nutritional conditions for seed filling in legumes (Nyawade et al., 2019). Cowpea yield responded positively to combined organic manures and biofertilizers under water-stressed conditions, where the synergistic effect of organic carbon supply and microbial-enhanced nitrogen fixation was essential for overcoming soil fertility constraints in sandy and degraded soils (Omer et al., 2023).

The regression analysis of *Vigna unguiculata* L. focused on the seed component as the primary indicator of productivity. For manure application, $y = 0.0008x^2 - 0.0149x + 0.36$ ($R^2 = 0.710$) forms a convex curve, reaching its minimum at 9.31 t ha⁻¹ with a productivity of 0.29 t ha⁻¹ (Figure 3d). This trend mirrors that of *Capsicum frutescens* L., where productivity decreases at moderate doses and then rises significantly at 25 t ha⁻¹ (0.47 t ha⁻¹), indicating that higher manure doses may redirect photosynthate towards seed filling. Recent studies have verified that moderate nitrogen supplementation boosts cowpea pod numbers and biological nitrogen fixation, while excessive amounts hinder nodulation through feedback mechanisms, highlighting the necessity of optimizing input levels for legume crops (Danso et al., 2025).

Leafy vegetable – water spinach (*Ipomoea aquatica* F.)

Ipomoea aquatica F. showed the most consistent response to both treatment factors (Table 8). Manure had a significant effect on all measured parameters: individual plant weight, plot weight, and productivity. A dose of 25 t ha⁻¹ resulted in the highest values for all three — plant weight of 58.6 ± 6.5 g, plot weight of 2,357 ± 479 g, and productivity of 6.3 ± 1.0 t ha⁻¹, a 31.3% increase compared to the control (4.8 ± 1.0 t ha⁻¹). Liquid bio-fertilizers had a significant effect on plot weight and productivity, but not on individual plant weight. Doses of 3 L ha⁻¹ (5.4 ± 1.5 t ha⁻¹) and 6 L ha⁻¹ (5.9 ± 1.2 t ha⁻¹) were consistently higher than the control (4.7 ± 1.0 t ha⁻¹). The positive linear response of *Ipomoea aquatica* F. to both factors indicates that leafy vegetable crops harvested vegetatively utilize organic nutrients and benefit from biostimulation more efficiently (Gharagozloo et al., 2019). This finding is corroborated by research showing that cow manure significantly increased chlorophyll content, shoot number, and fresh weight of water spinach, with the optimal dose depending on the balance between nutrient mineralization rate and plant demand; combined organic and inorganic fertilizer strategies have likewise been shown to synergistically improve biomass accumulation and nutrient uptake in leafy vegetables through enhanced soil microbial activity (Danso et al., 2025).

Interactions in *Ipomoea aquatica* F. present the most diverse pattern among the five

commodities. The highest yields were achieved by two combinations: 25 t ha⁻¹ manure × 3 L ha⁻¹ liquid biofertiliser (6.8 ± 0.2 t ha⁻¹) and 25 t ha⁻¹ manure × 6 L ha⁻¹ liquid biofertiliser (6.5 ± 1.2 t ha⁻¹). With 15 t ha⁻¹ manure, only 6 L ha⁻¹ liquid biofertiliser resulted in a significant increase (6.4 ± 1.0 t ha⁻¹) compared to the control at the same dose (4.2 ± 0.8 t ha⁻¹). Without manure, there was no meaningful interaction. This pattern shows that, in *Ipomoea aquatica* F., increasing the manure dose progressively expands the plant's response to liquid biofertiliser: from unresponsive (0 t ha⁻¹), to responsive only at high liquid biofertiliser doses (15 t ha⁻¹), to responsive at all liquid biofertiliser levels (25 t ha⁻¹). This gradation is consistent with the hypothesis that organic matter serves as a prerequisite for the effectiveness of biostimulation in vegetative crops (Li et al., 2020) a systematic understanding of the responses of soil microbial biomass and diversity to biochar addition is lacking. Here, a meta-analysis of 999 paired data points from 194 studies shows that biochar increases microbial biomass but has variable effects on microbial diversity. Generally, the effects of biochar on microbial biomass are dependent on biochar properties, while that on microbial diversity is dependent on soil properties. The application of biochar, particularly that produced under low temperature and from nutrient-rich feedstocks, could better increase soil microbial biomass (based on phospholipid fatty acid analysis (MBCPLFA).

Ipomoea aquatica F. exhibits a convex regression pattern in response to manure, with y

Table 8. Average *Ipomoea aquatica* F. yield at different doses of manure and liquid bio-fertilizers during the dry season

Dosage	Yield components		
	Plant weight (g plant ⁻¹)	Plant weight (g plot ⁻¹)	Plant weight (t h ⁻¹)
Manure (t.ha ⁻¹)			
0	48.6 ± 9.4 a	1,534 ± 251 a	4.8 ± 1.0 a
15	50.3 ± 13.5 ab	1,658 ± 634 a	4.9 ± 1.4 a
25	58.6 ± 6.5 b	2,357 ± 479 b	6.3 ± 1.0 b
Liquid bio-fertilizer (l.ha ⁻¹)			
0	48.3 ± 9.9	1,586 ± 438 a	4.7 ± 1.0 a
3	55.8 ± 11.3	1,803 ± 574 ab	5.4 ± 1.5 b
6	53.3 ± 11.0	2,160 ± 653 b	5.9 ± 1.2 b
Tukey manure 0.05	9.2	364.1	0.7
Tukey liquid bio-fertilizer 0.05	ns	364.1	0.7
CV	14.4	16.2	10.3

Note: Values are mean ± SD. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$). CV: coefficient of variation; ns: not significant.

$= 0.0053x^2 - 0.0733x + 4.8$ ($R^2 = 0.734$) and a minimum point at 6.91 t ha^{-1} with a productivity of 5.55 t ha^{-1} (Figure 3e). Productivity increases sharply from the minimum point up to a dose of 25 t ha^{-1} (6.3 t ha^{-1}), indicating that the nutrient needs of vegetative crops have not been met within the tested range (Li et al., 2020). A systematic understanding of the responses of soil microbial biomass and diversity to biochar addition is lacking. Here, a meta-analysis of 999 paired data points from 194 studies shows that biochar increases microbial biomass but has variable effects on microbial diversity. Generally, the effects of biochar on microbial biomass are dependent on biochar properties, while that on microbial diversity is dependent on soil properties. The application of biochar, particularly that produced under low temperature and from nutrient-rich feedstocks, could better increase soil microbial biomass (based on phospholipid fatty acid analysis (MBCPLFA). For the liquid biofertiliser factor, a concave response is described by $y = -0.0111x^2 + 0.2667x + 4.7$ ($R^2 = 0.743$), with an optimum point at 12.01 L ha^{-1} and a predicted maximum productivity of 6.3 t ha^{-1} (Figure 3j). The optimum value, well beyond the highest tested dose (6 L ha^{-1}), indicates that *Ipomoea aquatica* F. has a high capacity for biostimulation utilization because all assimilates are allocated to vegetative biomass growth, with no partitioning to reproductive organs (Li et al., 2020). This pattern fundamentally distinguishes *Ipomoea aquatica* F. from the other, generative commodities in the intercropping system.

Integrated synthesis across crops

It should be emphasized that the present study did not measure soil microbial biomass, microbial community composition, enzyme activity, or root colonization. The mechanistic explanations invoked throughout this discussion such as rhizosphere microbial competition, colony proliferation, and shifts in microbial community structure are therefore inferred from the published literature and are presented as *plausible* explanations for the observed yield responses rather than as processes demonstrated by our data. Direct measurement of these microbial and biochemical variables in future studies would be required to confirm the proposed mechanisms.

Optimising the application of manure and liquid biofertiliser is essential for enhancing the yields of the five commodities, because during the

dry season water scarcity is the primary constraint that limits nutrient uptake and plant development (Nyawade et al., 2019; Danso et al., 2025). Quadratic regression was therefore used to model the nonlinear relationship between dosage and productivity and to identify the specific optimum point for each commodity (Yadav et al., 2020). Across the five commodities, the R^2 values of the fitted quadratic models ranged from 0.671 to 0.753, indicating that the models explained 67.1% to 75.3% of the variability in productivity a level considered sufficient for field data collected on rainfed land during the dry season. The concave curves observed for butternut squash with manure and for both Cucurbitaceae members with liquid bio-fertilizer indicate a definitive peak, whereas the convex curves seen for *Capsicum frutescens* L., *Vigna unguiculata* L., and *Ipomoea aquatica* F. with manure suggest the possibility of higher productivity at dosages exceeding the experimental limits.

The interaction analysis revealed four distinct synergistic patterns among the five commodities. First, moderate-to-high synergy in Cucurbitaceae: *Cucumis sativus* L. and butternut squash showed the highest yields with high manure ($15\text{--}25 \text{ t ha}^{-1}$) combined with moderate liquid biofertilizer (3 L ha^{-1}), consistent with a microbial interaction saturation point in this family. Second, bipolar synergy in *Capsicum frutescens* L.: optimal productivity was achieved with 25 t ha^{-1} manure combined with either 0 or 6 L ha^{-1} bio-agent, but not at intermediate doses. Third, in *Vigna unguiculata* L., a threshold synergy occurred, where the interaction effect became apparent only when both factors were applied at their maximum levels simultaneously ($25 \text{ t ha}^{-1} \times 6 \text{ L ha}^{-1}$), leading to a 150% increase in seed yield. Fourth, in *Ipomoea aquatica* F., a gradual synergy was observed as the stepwise addition of manure expanded the effective dose range of liquid biofertilizer. These four patterns collectively highlight the need for a nuanced, plant family-specific approach when combining organic amendments with microbial inoculants. This conclusion is increasingly supported by studies showing that the effectiveness of biofertilizers is influenced by the host plant's rhizosphere ecology and the presence of organic carbon substrates (Alzate Zuluaga et al., 2024). These findings emphasize that recommendations for combination doses in intercropping systems must be tailored to each commodity and cannot be generalized across crops, even when grown on the same land and in the same season.

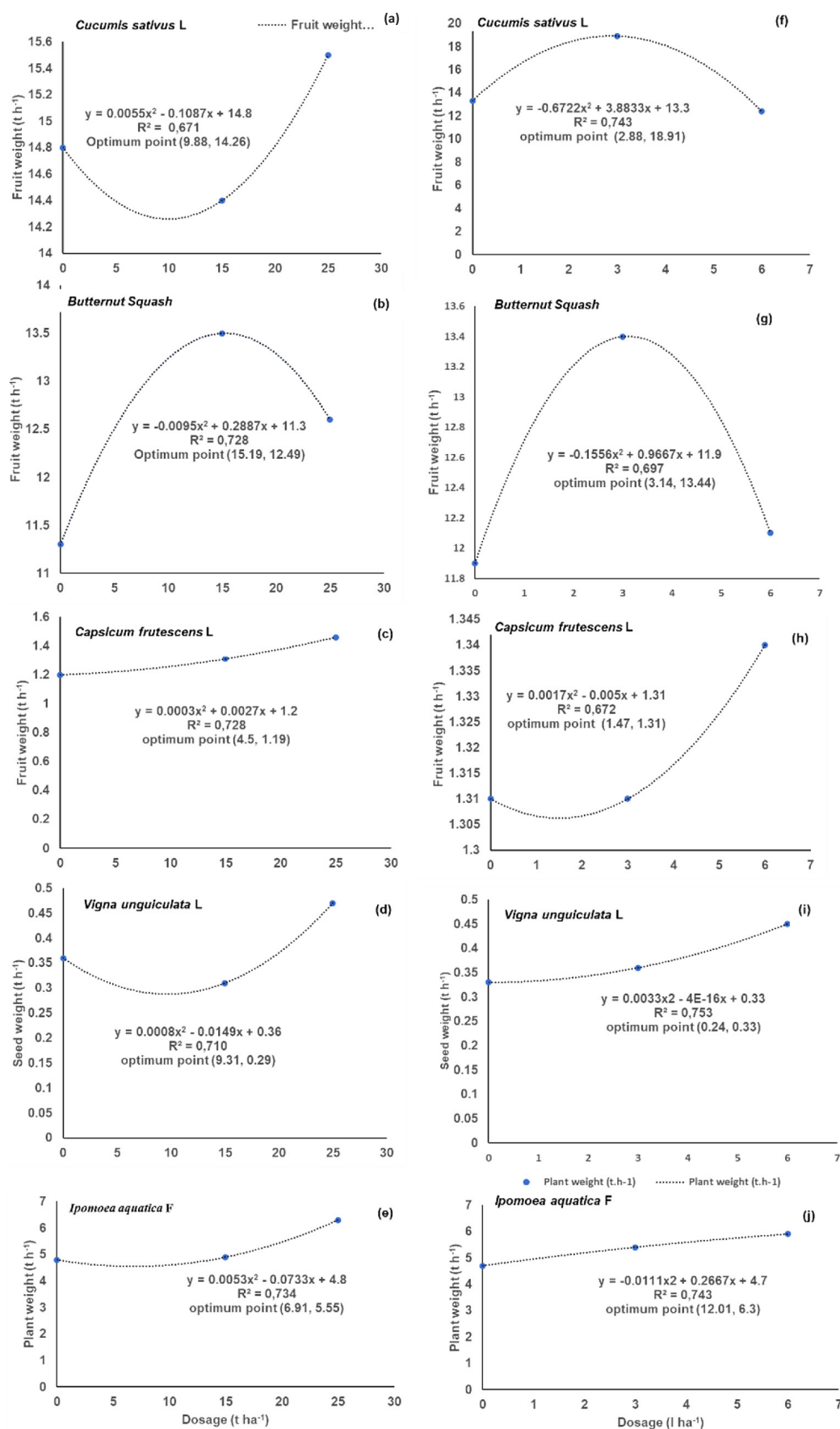


Figure 3. Optimal dose regression of manure (a–e) and liquid bio-fertilizers (f–j) for five crops *Cucumis sativus* L., butternut squash, *Capsicum frutescens* L., *Vigna unguiculata* L., and *Ipomoea aquatica* F. on rainfed land during the dry season

Quadratic regression analysis revealed two fundamental patterns that differentiate the responses of the five commodities to dose optimization. The first is a concave response (negative x^2 coefficient) that produces a true maximum point, found in: (i) liquid biofertiliser for Cucurbitaceae — *Cucumis sativus* L. (optimum 2.88 L ha⁻¹, $R^2 = 0.743$) and butternut squash (optimum 3.14 L ha⁻¹, $R^2 = 0.697$); (ii) manure for butternut squash (optimum 15.19 t ha⁻¹, $R^2 = 0.728$); and (iii) liquid biofertiliser for *Ipomoea aquatica* F. (optimum 12.01 L ha⁻¹, $R^2 = 0.743$). The second is a convex response (positive x^2 coefficient) that results in a minimum point and an accelerated increase at high doses, observed in *Capsicum frutescens* L. and *Vigna unguiculata* L. for both factors, and in *Cucumis sativus* L. and *Ipomoea aquatica* F. for manure. These findings confirm that dose optimization strategies in intercropping systems cannot be universal: Cucurbitaceae require moderate doses of liquid biofertiliser (± 3 L ha⁻¹) to avoid microbial competition, while *Capsicum frutescens* L., *Vigna unguiculata* L., and *Ipomoea aquatica* F. show potential for increased productivity at doses higher than those tested (Khairuddin et al., 2018; Oyebiyi et al., 2026). The practical implication is the need for commodity-specific dose recommendation formulations that integrate regression models, plant family rhizosphere architecture, and harvest organ type in the context of drought stress on rainfed fields.

System-scale performance of the intercrop

Beyond the commodity-level responses, the agronomic advantage of the whole intercrop was evaluated using the land equivalent ratio (LER), area–time equivalent ratio (ATER), aggressivity, and competition ratio (CR), computed against sole-crop yields from monoculture plots established at the same site and in the same season (Mead & Willey, 1980).

Across all fertilizer treatments, the intercropping system was consistently advantageous, with LER ranging from 1.75 to 2.95 (mean 2.07) and ATER from 1.30 to 2.20 (mean 1.56) (Table 9). A mean LER of 2.07 indicates that approximately 2.07 ha of sole crops would be required to match the output of 1 ha of this intercrop a land saving of about 107%. The fact that ATER remained above unity confirms that the advantage was not merely an artefact of differing crop durations but reflected a genuine gain in land- and time-use efficiency (Agustina, Indrazatmiko, et al., 2025). The highest system efficiency was obtained at 25 t ha⁻¹ manure combined with 3 L ha⁻¹ liquid biofertilizer (LER 2.95; ATER 2.20), coinciding with the treatment that produced the highest commodity yields; increasing the bio-fertilizer dose to 6 L ha⁻¹ lowered the LER, mirroring the quadratic yield response reported above.

The competition indices (Table 10) revealed a clear hierarchy: cucumber was the dominant component (partial LER 1.49; aggressivity +1.34; CR 10.31), whereas butternut squash, cowpea, water spinach, and chili were subordinate (partial LER < 1; aggressivity < 0; CR < 1) (Willey & Rao,

Table 9. Land equivalent ratio (LER) and area-time equivalent ratio (ATER) of the five-crop intercrop under different manure and liquid bio-fertilizer treatments during the dry season

Manure (t ha ⁻¹)	LBF (L ha ⁻¹)	LER	ATER
0	0	1.97	1.50
0	3	2.08	1.57
0	6	1.98	1.49
15	0	1.85	1.41
15	3	2.40	1.83
15	6	1.75	1.30
25	0	1.77	1.32
25	3	2.95	2.20
25	6	1.84	1.41
Mean		2.07	1.56

Table 10. Partial land equivalent ratio (pLER), aggressivity, and competition ratio (CR) of the five commodities in the intercrop (values averaged across all treatments)

Commodity	Partial LER	Aggressivity	CR	Status
Cucumber (<i>Cucumis sativus</i> L.)	1.49	+1.34	10.31	Dominant
Butternut squash	0.27	−0.18	0.59	Subordinate
Cowpea (<i>Vigna unguiculata</i> L.)	0.16	−0.32	0.33	Subordinate
Water spinach (<i>Ipomoea aquatica</i> F.)	0.12	−0.37	0.25	Subordinate
Chili (<i>Capsicum frutescens</i> L.)	0.03	−0.48	0.07	Subordinate

1980). The system advantage was therefore driven mainly by cucumber, which exceeded its own sole-crop yield within the mixture, while the four subordinate species contributed complementary yield without strongly competing for the same resources. This complementarity a highly competitive climber combined with lower-canopy and short-duration crops explains how the overall LER exceeded 2 even though four of the five species yielded below their sole-crop potential, and highlights that management should aim to protect the subordinate components from excessive suppression by the dominant cucumber.

CONCLUSIONS

This study shows that manure and liquid biofertiliser doses, as well as their interactions, significantly affect the productivity of five crop commodities in intercropping systems on rainfed land during the dry season, with commodity-specific responses. Quantitatively, the combination of manure at 25 t ha⁻¹ and liquid biofertiliser at 3 L ha⁻¹ resulted in the highest *Cucumis sativus* L. productivity of 23.1 ± 2.3 t ha⁻¹, while for butternut squash, the combination of 15 t ha⁻¹ manure and 3 L ha⁻¹ liquid biofertiliser produced a maximum yield of 15.0 ± 0.4 t ha⁻¹. For *Capsicum frutescens* L., the highest productivity of 0.146 t ha⁻¹ was achieved with the combination of 25 t ha⁻¹ manure and 6 L ha⁻¹ bio-agent. In contrast, for *Vigna unguiculata* L., the same combination yielded the highest seed production of 0.70 ± 0.05 t ha⁻¹. For *Ipomoea aquatica* F., the highest biomass of 6.3 ± 1.0 t ha⁻¹ was obtained from the combination of 25 t ha⁻¹ manure and 3 L ha⁻¹ bio-agent. Regression-based optimization showed that the optimum dose of liquid biofertiliser for *Cucumis sativus* L. was 2.88 L ha⁻¹ (yield 18.91 t ha⁻¹), while for butternut squash it was 3.14 L ha⁻¹ (yield 13.44 t ha⁻¹). The optimum manure dose for butternut squash was estimated at 15.19 t ha⁻¹ (yield 12.49 t ha⁻¹), while for *Cucumis sativus* L. it was 9.88 t ha⁻¹ (yield 14.26 t ha⁻¹). For *Capsicum frutescens* L. and *Ipomoea aquatica* F., the production response still increased up to the highest tested manure dose (25 t ha⁻¹), indicating that the optimum dose may be higher. At the system scale, the intercrop was advantageous under all treatments (LER 1.75–2.95, mean 2.07; ATER 1.30–2.20), with cucumber as the dominant component and the other four crops as complementary, subordinate

species. Overall, integrating high doses of manure (15–25 t ha⁻¹) with moderate to high doses of liquid biofertiliser (3–6 L ha⁻¹) gives a synergistic effect in increasing the productivity of intercropping systems through improved soil organic matter content and soil microbiological activity.

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