Biostimulant Activity of Fermented Plant Juice Derived from Indigenous Tropical Species on the Vegetative Growth and Biomass Accumulation of Pechay (*Brassica rapa* L.)

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ABSTRACT

Intensification of synthetic fertilizer use in smallholder vegetable production has raised persistent concerns over soil degradation and input costs, prompting a renewed interest in biologically active organic alternatives. This study evaluated the growth-stimulating potential of fermented plant juice (FPJ), prepared from kangkong (*Ipomoea aquatica* Forssk.), kulitis (*Amaranthus spinosus* L.), alugbati (*Basella alba* L.), and their combination, as organic biostimulants for pechay (*Brassica rapa* L.) under a Randomized Complete Block Design with six treatments and five replications. Growth performance was assessed at 35 days after transplantation across ten morphological parameters. Multivariate analysis confirmed a significant overall treatment effect (Wilks' λ = 0.097, $p < 0.001$, partial $\eta^2 = 0.374$), with all individual parameters significant at $\alpha = 0.05$. Kangkong FPJ produced the highest leaf count, stem length, and biomass yield among organic treatments, while Alugbati FPJ recorded superior plant height and growth rate. The combined Kangkong–Kulitis–Alugbati FPJ surpassed all other treatments, including the Triple 14 commercial fertilizer, in root length and stem circumference, and was statistically equivalent to the commercial control in leaf width. All FPJ treatments significantly outperformed the unfertilized control. Growth promotion of FPJ is attributed to fermentation-associated microorganisms (*Lactobacillus, Bacillus, Aspergillus* spp.) that facilitate phosphorus solubilization, nitrogen fixation, and biosynthesis of auxins, cytokinins, and gibberellic acids, alongside improved soil biological health. These findings demonstrate locally sourced FPJ formulations as cost-effective and ecologically viable for *Brassica* production systems, with kangkong and alugbati based preparations offering the strongest agronomic performance, with multi-season open-field trials recommended to support large-scale adoption.

Keywords: *Brassica rapa*; biostimulant; fermented plant juice; organic fertilizer; pechay; plant growth regulators

INTRODUCTION

Global food security is increasingly challenged by the dual pressures of population growth and increased agricultural activity. The widespread dependence on synthetic fertilizers, particularly nitrogen (N), phosphorus (P), and potassium (K) formulations, has enabled unprecedented crop yields but has simultaneously imposed long-term costs on soil health, ecosystem integrity, and smallholder farm economics (Herren et al., 2020). Prolonged application of inorganic fertilizers degrades the natural composition of agricultural soils, disrupts microbial communities, and reduces the bioavailability of essential micro- and macronutrients, thereby compromising the productive capacity of soils for successive cropping cycles (Gonzales et al., 2015; Herren et

al., 2020). These consequences are particularly acute in tropical smallholder systems, where narrow profit margins make input cost a critical determinant of agricultural viability (Wortmann et al., 2019).

In response to these challenges, there is growing scientific and policy interest in biologically active organic alternatives that can maintain or enhance crop productivity while reducing dependence on synthetic inputs. Biostimulants defined under EU Regulation 2019/1009 as substances or microorganisms applied to plants or the rhizosphere to improve nutrient use efficiency, tolerance to abiotic stress, and crop quality represent one of the most rapidly expanding categories of sustainable agricultural inputs globally (du Jardin, 2015). Among the diverse forms of biostimulants, liquid organic fertilizers derived from fermented plant materials have attracted attention for their multi-functional properties, including the delivery of plant-available nutrients, beneficial microorganisms, and endogenous plant growth regulators such as auxins, cytokinins, and gibberellic acids (Glick, 2012; Sulok et al., 2021). In the Philippines, this approach is formally supported by Republic Act 10068, the Organic Agriculture Act of 2010, which mandates the identification and promotion of cost-effective, indigenous, and ecologically sound inputs for sustainable crop production (The LAWPhil Project, 2010).

Fermented plant juice (FPJ) is a liquid organic biostimulant produced through anaerobic fermentation of young, vigorously growing plant shoots with brown sugar as an osmotic extractant (Miller et al., 2013). The fermentation process mobilizes plant-associated microorganisms, predominantly *Lactobacillus*, *Bacillus*, and *Aspergillus* species, that contribute to nutrient solubilization, nitrogen fixation, and the biosynthesis of bioactive compounds beneficial to plant growth (Sulok et al., 2021). Despite its longstanding use in natural and organic farming traditions across Southeast Asia, controlled experimental evidence for the agronomic efficacy of FPJ on vegetable crops remains limited. Existing studies have demonstrated growth-promoting effects of FPJ in pechay (*Brassica rapa* L.) (Tagotong & Corpuz, 2015; Bercero et al., 2014) and tomato (Sakimin et al., 2017), but these investigations have largely examined single-source FPJ formulations and have not systematically compared multiple plant-derived FPJ types under standardized experimental conditions, nor have they explored whether combining plant sources yields additive biostimulant effects.

Pechay (*Brassica rapa* L.) is among the most economically significant leafy vegetables in the Philippines, valued for its short production cycle, high market demand, and rich nutritional profile, including vitamins C, B6, folate, beta-carotene, calcium, and phosphorus (Jimenez et al., 2016; Zou et al., 2021). Its rapid growth rate and sensitivity to soil fertility make it a highly suitable model crop for evaluating the short-term agronomic effects of biostimulant inputs (Bandera, 2020; Gonzales et al., 2015). The successful growth and yield of pechay is largely contingent on adequate soil nutrient availability, which underscores the importance of identifying effective and accessible organic fertilization strategies (Barna et al., 2021).

The selection of kangkong (*Ipomoea aquatica* Forssk.), kulitis (*Amaranthus spinosus* L.), and alugbati (*Basella alba* L.) as FPJ source materials in this study was based on their phytochemical and agronomic profiles. Kangkong is a fast-growing aquatic vegetable known to contain substantial concentrations of nitrogen, potassium, and phosphorus, and has been previously associated with elevated auxin content that promotes vegetative growth when used as FPJ (Igwenyi et al., 2011; Himangini et al., 2019). Kulitis (*Amaranthus spinosus*) is a nitrogen-rich weed species with documented macro- and micronutrient content including calcium, zinc, and magnesium (Ganjare & Raut, 2019). Alugbati (*Basella alba*) similarly contains a broad spectrum of minerals and vitamins and has been noted for its succulent, nutrient-dense foliage suitable for liquid extraction (Kumar et al., 2015). The rapid growth habits and high tissue nutrient concentrations of these three species make them strong candidates for FPJ production, yet their comparative and combined biostimulant potential on *Brassica* crops has not been experimentally evaluated.

This study therefore aimed to determine the biostimulant activity of FPJ formulations derived from kangkong, kulitis, alugbati, and their combination on the vegetative growth and biomass accumulation of pechay (*Brassica rapa* L.) under greenhouse conditions. Specifically, the study sought to: (1) characterize the growth responses of pechay across ten morphological parameters at 35 days after transplantation; (2) compare the efficacy of individual and combined FPJ treatments against a commercial inorganic fertilizer (Triple 14) and an unfertilized control; and (3) identify the most effective FPJ formulation for potential adoption as an organic biostimulant in smallholder pechay production.

MATERIALS AND METHODS

Experimental Site and Design

The study was conducted using a Randomized Complete Block Design (RCBD) with six treatments and five replications, each comprising four plants per replicate, for a total of 120 experimental units. The RCBD was selected for its effectiveness in controlling spatial variation through systematic grouping of experimental units into blocks, with each block containing all six treatments exactly once (Grant, 2010; Dafaallah, 2019). The experiment was conducted under a greenhouse structure located at Barangay Tagbac, District of Jaro, Iloilo City, Philippines. A total planting area of 6 m × 5.5 m was subdivided into six plots measuring 5.5 m × 0.5 m with 0.5 m inter-plot spacing. The entire area was enclosed with fine green screens to minimize insect infestation and regulate external moisture. The greenhouse maintained an average daytime temperature of 24-39°C and relative humidity of 74%, with natural photoperiod conditions averaging approximately 12 hours of light per day during the growing period. The transparent plastic roofing allowed sufficient levels of photosynthetically active radiation (PAR) to reach the crop canopy.

Preparation of Fermented Plant Juice (FPJ)

Four FPJ formulations were prepared from three locally available and botanically verified plant species: kangkong (*Ipomoea aquatica* Forssk.), kulitis (*Amaranthus spinosus* L.), and alugbati (*Basella alba* L.). Botanical verification was conducted through visual inspection by plant specialists at the Department of Agriculture–Iloilo Research Outreach Station, Sta. Barbara, Iloilo. These species were selected based on their documented nutrient richness and rapid vegetative growth, which are associated with elevated concentrations of plant growth-promoting compounds including auxins, nitrogen, phosphorus, and potassium (Igwenyi et al., 2011; Ganjare & Raut, 2019; Kumar et al., 2015; Himangini et al., 2019).

Plant materials were harvested before sunrise to maximize the concentration of endogenous growth hormones and microorganisms present on leaf surfaces during the early morning period (Miller et al., 2013; Barna et al., 2021). Only healthy, morphologically intact specimens free from pest damage or disease symptoms were selected to prevent the introduction of potentially pathogenic organisms into the fermentation substrate (Barna et al., 2021; Sulok et al., 2021). Materials were deliberately left unwashed after collection to preserve the naturally occurring epiphytic microorganisms essential for fermentation (Miller et al., 2013; Barna et al., 2021; Sulok et al., 2021). Collected materials were chopped into approximately 2–3 cm segments on the day of collection to increase surface area and accelerate osmotic extraction of plant juices (Miller et al., 2013; Barna et al., 2021; Sulok et al., 2021).

Chopped plant materials were weighed, combined with an equal mass of brown sugar (1:1 w/w ratio), and thoroughly mixed until all plant surfaces were uniformly coated (Miller et al., 2013; Barna et al., 2021; Sulok et al., 2021). The plant-sugar mixture was packed into labeled food-grade plastic containers, which were covered with cheesecloth secured with string to permit gas exchange during fermentation while preventing contamination by insects and airborne debris (Miller et al., 2013). Containers were stored under ambient conditions in a well-ventilated area, protected from direct sunlight and temperature extremes to maintain stable fermentation conditions (Miller et al., 2013; Sulok et al., 2021). Fermentation was allowed to proceed for seven days; completion was confirmed by the presence of small gas bubbles, flotation of plant material above a clear liquid layer, and the development of a mild alcoholic odor characteristic of successful lactic acid and yeast fermentation (Miller et al., 2013). The fermented liquid was strained through a fine mesh into sterilized, labeled glass bottles, with residual solids lightly pressed to maximize yield recovery. Bottles were loosely capped to permit continued off-gassing, and the solid residues were composted. Each formulation yielded approximately 2 L of FPJ per batch. Nutrient characterization of the prepared FPJ formulations, including proximate analysis of N, P, and K content, is recommended for future studies to enable more precise attribution of growth responses to specific nutrient fractions (Chontal et al., 2019; Sulok et al., 2021).

Treatments

Six treatments were employed: Treatment A- kangkong FPJ (1 kg plant material : 1 kg brown sugar); Treatment

B- kulitis FPJ (1 kg : 1 kg); Treatment C- alugbati FPJ (1 kg : 1 kg); Treatment D- combined FPJ (1 kg kangkong + 1 kg kulitis + 1 kg alugbati : 3 kg brown sugar); Treatment E- Triple 14 (14-14-14) complete fertilizer as a positive control, sourced from a licensed commercial agricultural supplier; and Treatment F- well water only, serving as the negative control to reflect growth under soil nutrient conditions alone.

Seedling Production and Transplantation

Brassica rapa seeds were sown in bamboo-framed seed boxes filled with a 1:1 (v/v) mixture of garden soil and well-decomposed animal manure (Racoma & Beato, 2017; Gonzales et al., 2015). Seedlings were irrigated twice daily (Bercero et al., 2014), protected from insect damage using fine mesh screens, and progressively hardened by incremental exposure to natural sunlight over the nursery period to promote stem structural rigidity prior to transplantation (Gonzales et al., 2015). At fourteen days after sowing, uniform seedlings of comparable height (range 6.9–7.6 cm) were selected for transplantation into the experimental plots at 30 cm inter-plant spacing (Bercero et al., 2014; Tagotong & Corpuz, 2015). Transplanting was conducted in the late afternoon to minimize transplant shock and water stress (Gonzales et al., 2015). Each seedling was carefully lifted with its surrounding root ball intact to minimize root disturbance during transfer. Two seedlings were initially established per planting station and thinned to one plant per station upon emergence of the first true leaves to ensure uniform stand density (Bandera, 2020).

Fertilizer Application and Crop Management

The first FPJ application commenced ten days after transplantation (DAT), once seedlings had visibly established. FPJ was applied at a dilution of 4 tablespoons per liter of water (approximately 1:16 v/v), a rate consistent with established protocols for vegetable production (Tagotong & Corpuz, 2015), delivering approximately 150 mL per plant through a combined soil drench and foliar spray method. The combined soil and foliar application approach was employed as it has been demonstrated to maximize nutrient uptake and yield parameters relative to either method alone (Bandera, 2020; Rahman et al., 2015). FPJ applications were repeated at three-day intervals until harvest, consistent with the practice of applying FPJ according to visible plant vigor and in the absence of a documented phytotoxic threshold for FPJ at this concentration (Racoma & Beato, 2017; Agricultural Training Institute, 2011). The three-day interval was adopted based on prior FPJ studies on *Brassica* crops (Tagotong & Corpuz, 2015) and reflects the approximate time required for foliar-applied organic amendments to be assimilated before the next application cycle. The positive control (Treatment E) received 150 mL per plant of a solution prepared by dissolving 150 g of Triple 14 in 16 L of water, applied weekly. The negative control (Treatment F) received 200 mL of local well water per irrigation event without any fertilizer supplementation. All treatments received regular supplemental irrigation of approximately 250 mL per plant per session via hand-held sprinkler. Plants were pre-watered prior to each FPJ application to prevent potential osmotic stress or root scorching from direct application to dry soil. Weed management was performed by manual hand-pulling, and insect pests were controlled through manual handpicking throughout the growing period to avoid confounding effects from pesticide application.

Harvesting and Growth Measurements

Plants were harvested at 35 days after transplantation (DAT), two days following the final fertilizer application, which corresponds to the commercially accepted harvest stage for pechay at which leaves are immature yet fully expanded (Tagotong & Corpuz, 2015; Bandera, 2020). Harvesting was conducted between 6:00 and 8:00 AM to minimize moisture loss from leaf surfaces and preserve fresh weight integrity (Gonzales et al., 2015). Whole plants were carefully uprooted with root systems intact, and roots were gently agitated in clean water to remove adhering soil before measurement (Bercero et al., 2014).

The following ten growth parameters were recorded for each plant: (1) plant height, measured in centimeters from the base of the stem to the tip of the tallest leaf using a measuring tape (Bandera, 2020); (2) number of leaves, determined by direct counting of all fully expanded leaves; (3) leaf length, measured in centimeters from the point of stem attachment to the leaf tip; (4) leaf width, measured in centimeters at the widest portion of the leaf lamina; (5) stem length, measured in centimeters from the base to the first leaf node; (6) stem circumference, measured in centimeters using a flexible tape at the mid-point of the stem; (7) root length, measured in

centimeters from the stem base to the tip of the longest root; (8) wet biomass, recorded in grams immediately after harvest and root cleaning using a digital analytical balance; (9) dry biomass obtained by sun-drying whole plant samples spread evenly on paper platforms at ambient temperature 28–34 °C until constant weight was achieved over 5–7 days, representing total dry matter content in grams (Managa et al., 2020; Chontal et al., 2019); and (10) relative growth rate calculated as described below.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) at a 95% confidence level. Relative growth rate (GR) was calculated following the height-based index of Hilty et al. (2021) using the formula: $GR = [(H_2 - H_1) / (D_2 - D_1)] \times 100$, where H_2 and H_1 represent plant height (cm) at harvest and at the time of transplantation, respectively, and D_2 (day 35) and D_1 (day 10) denote the corresponding time points in days after transplantation. It is acknowledged that this index represents a height-based growth rate proxy rather than the classical biomass-based relative growth rate (RGR), which requires sequential destructive harvests (Hilty et al., 2021); this approach was adopted given the single-harvest design of the study. Treatment effects on the combined set of growth parameters were assessed using one-way multivariate analysis of variance (MANOVA), followed by tests of between-subjects effects for each individual dependent variable. In parameters where significant treatment effects were detected, pairwise comparisons were conducted using the post-hoc Least Significant Difference (LSD) test to identify specific treatment differences. All statistical analyses were performed using IBM SPSS Statistics v32 at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Vegetative Growth Parameters

Table 1 presents the mean ± SD of all ten growth parameters recorded at 35 days after transplantation (DAT). Initial plant heights were equivalent across all treatment groups, ranging 6.9–7.6 cm, confirming uniform starting conditions prior to treatment application. All FPJ formulations significantly stimulated growth relative to the negative control across all measured parameters, consistent with the documented growth-promoting activity of bio-organic liquid fertilizers applied to *Brassica rapa* (Tagotong & Corpuz, 2015; Bercero et al., 2014).

Table 1. Mean ± SD of growth and development parameters of pechay (*Brassica rapa* L.) at 35 DAT under six treatment groups. Data represent n = 20 observations per treatment (5 blocks × 4 plants). KKA FPJ = Kangkong + Kulitis + Alugbati combined FPJ. GR = height-based growth index calculated as $[(H_2 - H_1)/(D_2 - D_1)] \times 100$.

Parameter	Treatment (Mean ± SD)					
	Kangkong FPJ	Kulitis FPJ	Alugbati FPJ	KKA FPJ	Positive Control	Negative Control
Plant Height (cm)	20.3 ± 3.2	20.6 ± 3.9	20.9 ± 4.6	18.4 ± 4.6	23.4 ± 4.8	14.4 ± 4.1
No. of Leaves	9.2 ± 2.1	7.7 ± 1.7	7.8 ± 1.2	8.9 ± 1.3	8.0 ± 1.3	6.3 ± 1.3
Leaf Length (cm)	22.8 ± 2.8	22.0 ± 2.1	23.9 ± 2.1	22.5 ± 2.8	24.9 ± 3.7	18.2 ± 2.6
Leaf Width (cm)	14.7 ± 2.5	15.1 ± 1.6	14.2 ± 1.6	16.0 ± 2.3	16.0 ± 1.7	9.2 ± 1.7
Stem Length (cm)	4.86 ± 1.26	4.01 ± 0.76	4.30 ± 0.42	4.08 ± 0.68	4.78 ± 0.92	3.56 ± 1.44
Stem Circumference (cm)	2.56 ± 0.67	2.41 ± 0.43	2.47 ± 0.54	2.83 ± 0.21	2.79 ± 0.56	1.55 ± 0.43
Root Length (cm)	13.6 ± 3.4	13.7 ± 3.2	14.9 ± 3.1	16.8 ± 3.0	13.6 ± 3.4	8.0 ± 2.2
Wet Weight (g)	105.0 ± 37.7	100.0 ± 35.4	81.8 ± 18.7	94.5 ± 34.9	129.2 ± 45.2	24.0 ± 11.7
Dry Weight (g)	19.8 ± 10.5	17.2 ± 11.0	11.4 ± 5.0	16.2 ± 13.3	27.4 ± 13.3	3.3 ± 2.0
Height-based GR (%)	50.70 ± 14.11	53.3 ± 15.2	55.86 ± 12.6	43.86 ± 18.2	63.28 ± 20.66	49.07 ± 19.53

Plant Height and Height-Based Growth Index

Among FPJ-treated groups, Alugbati FPJ produced the tallest plants at 35 DAT (20.9 ± 4.6 cm), followed closely by Kulitis FPJ (20.6 ± 3.9 cm) and Kangkong FPJ (20.3 ± 3.2 cm), while the combined KKA FPJ yielded the lowest mean height (18.4 ± 4.6 cm) among FPJ groups. The positive control (23.4 ± 4.8 cm) outperformed all FPJ treatments, while the negative control recorded the lowest final height (14.4 ± 4.1 cm). The height-based growth index reflected this pattern, with Alugbati FPJ recording the highest index among FPJ groups (55.86 ± 12.6%), followed by Kulitis FPJ (53.3 ± 15.2%) and Kangkong FPJ (50.70 ± 14.11%). It is acknowledged that this metric represents a height-based proxy rather than the classical biomass-based relative growth rate (RGR), which requires sequential destructive harvests (Hilty et al., 2021); it was adopted here given the single-harvest design employed.

The superior height and growth index of Alugbati FPJ is consistent with the nutrient profile of *Basella alba*, whose succulent foliage is characterized by a broad spectrum of minerals and vitamins that contribute to the nutrient density of the resulting extract (Kumar et al., 2015). Improved plant height in FPJ-treated pechay is broadly attributed to the auxin-like compounds associated with rapidly growing source plants; kangkong (*Ipomoea aquatica*) in particular has been noted for its high endogenous auxin content, which promotes vegetative elongation when incorporated into FPJ (Himangini et al., 2019; Sakimin et al., 2017). The comparatively lower height and growth index recorded under KKA combined FPJ may reflect partial antagonism or dilution effects among the phytochemical constituents of the three source plants at the concentrations applied, an interaction that merits systematic investigation at varying mixing ratios in future studies.

Leaf Parameters: Number, Length, and Width

Kangkong FPJ produced the greatest leaf number among FPJ groups (9.2 ± 2.1), exceeding even the positive control (8.0 ± 1.3), a finding consistent with the nitrogen-rich composition of *Ipomoea aquatica* (Igwenyi et al., 2011). Nitrogen is the dominant macronutrient governing vegetative organ formation, including leaf initiation and expansion (Xu et al., 2005), and its relatively higher availability in kangkong-sourced FPJ likely drove the observed superiority in leaf proliferation. For leaf length, Alugbati FPJ produced the longest leaves (23.9 ± 2.1 cm), while the KKA combined FPJ produced the widest (16.0 ± 2.3 cm), statistically equivalent to the positive control (16.0 ± 1.7 cm; LSD p = 0.987). The base plants for all FPJ formulations, kangkong (Igwenyi et al., 2011), kulitis (Ganjare & Raut, 2019), and alugbati (Kumar et al., 2015), naturally contain nitrogen alongside potassium, phosphorus, calcium, zinc, and magnesium, providing a multi-nutrient substrate for leaf development (Xu et al., 2012). The negative control consistently recorded the lowest values across all leaf parameters (6.3 ± 1.3 leaves; 18.2 ± 2.6 cm length; 9.2 ± 1.7 cm width), underscoring the critical role of supplemental fertilization in pechay leaf development.

Stem Length and Circumference

Kangkong FPJ produced the longest stems among FPJ groups (4.86 ± 1.26 cm), closely approaching and not statistically differing from the positive control (4.78 ± 0.92 cm; LSD p = 0.771). Stem circumference was greatest under the KKA combined FPJ (2.83 ± 0.21 cm), marginally exceeding the positive control (2.79 ± 0.56 cm) with no significant difference (LSD p = 0.987). These findings suggest that the combined FPJ formulation, despite its limitations in height promotion, confers measurable structural advantages in stem girth, a parameter linked to mechanical strength and vascular capacity for nutrient transport (Marschner, 2012). The dominance of nitrogen as the primary driver of stem elongation (Xu et al., 2012) likely explains the performance of Kangkong FPJ in this parameter, while the broader mineral profile of the combined KKA formulation may explain its superior circumference.

Root Development

Root development, assessed by root length, was most pronounced under the KKA combined FPJ (16.8 ± 3.0 cm), significantly exceeding all other treatments and the positive control (13.6 ± 3.4 cm; LSD $p < 0.05$), with the exception of Alugbati FPJ (14.9 ± 3.1 cm; LSD p = 0.053). Kangkong FPJ, Kulitis FPJ, and Alugbati FPJ recorded root lengths statistically equivalent to the positive control (LSD p = 0.476, 0.939, and 0.217,

respectively). The superiority of KKA FPJ in root development, despite its lower performance in above-ground parameters, is consistent with the root-to-shoot ratio concept described by Sakimin et al. (2017) and Wira et al. (2011): higher root length in the combined FPJ treatment may reflect a compensatory below-ground allocation strategy in response to comparatively reduced shoot growth, ensuring adequate water and nutrient acquisition. It is also plausible that the combined phytochemical and microbial profile of the three-source FPJ may have enriched the rhizosphere with auxin-producing microorganisms, as indole acetic acid (IAA) production by plant growth-promoting bacteria (PGPB) is among the best-characterized mechanisms of root system stimulation (Glick, 2012). All FPJ treatments significantly outperformed the negative control in root length ($p < 0.05$), with the negative control yielding the shortest roots (8.0 ± 2.2 cm).

Biomass: Wet and Dry Weight

Kangkong FPJ produced the highest fresh and dry biomass among FPJ groups (105.0 ± 37.7 g wet; 19.8 ± 10.5 g dry), followed by Kulitis FPJ (100.0 ± 35.4 g; 17.2 ± 11.0 g) and KKA FPJ (94.5 ± 34.9 g; 16.2 ± 13.3 g). The value of wet and dry biomass in pechay reflects the cumulative effect of nutrient-driven seedling development throughout the growing period (Gonzales et al., 2015), while dry biomass specifically represents the plant's capacity to synthesize and accumulate organic compounds through photosynthesis (Marschner, 2012). Alugbati FPJ recorded the lowest fresh and dry weight among FPJ groups (81.8 ± 18.7 g; 11.4 ± 5.0 g), despite its strong performance in height and leaf length, suggesting that the nutrient composition of *Basella alba*-derived FPJ may preferentially support elongation over biomass accumulation at the dilution rate applied. Importantly, no significant differences were detected among any FPJ-to-FPJ pairwise comparisons for biomass (LSD $p > 0.05$ for all FPJ pairs), indicating broadly equivalent biomass-stimulating capacity across the four formulations.

The positive control (129.2 ± 45.2 g wet; 27.4 ± 13.3 g dry) significantly outperformed all FPJ treatments in both biomass parameters (LSD $p < 0.05$ for all comparisons). This disparity is mechanistically explained by the contrasting nutrient release dynamics of inorganic versus organic fertilizers: the Triple 14 formulation provides immediately plant-available NH_4^+ and NO_3^- nitrogen at a guaranteed 14% concentration, whereas FPJ delivers nutrients through microbially mediated mineralization of organic nitrogen, a process subject to lag phases and variable conversion rates depending on soil temperature, moisture, and microbial community structure (Geisseler et al., 2021). In short-cycle crops such as pechay with a 35-day harvest window, this mineralization lag likely restricted the total quantity of plant-available nitrogen released from FPJ during the critical leaf expansion and biomass accumulation phases, while the immediately available inorganic nitrogen in the positive control was fully exploited throughout the growing period. This mechanistic explanation is consistent with the characterization of fermented plant juices as soil organic conditioners with moderately low macronutrient concentrations approximately 90–95% organic content that function primarily as nutrient supplements rather than primary fertilizers (Zuraihah et al., 2012; Zamora & Calub, 2016).

These results align with Omidire et al. (2015), who reported that inorganic fertilizers consistently produced higher yield measurements than organic alternatives under comparable conditions. However, they contrast with findings by Bercero et al. (2014) and Pascual et al. (2013), who reported that pechay grown under organic fertilization demonstrated comparative or superior performance to commercial fertilizers. This discrepancy is likely attributable to several interacting factors: differences in soil baseline fertility and pH across study sites, which substantially influence organic nitrogen mineralization rates; the type and age of organic amendment used like compost and fermented activators in Pascual et al. (2013) as compared to FPJ in the present study; application rates and frequencies; and inherent variability in FPJ nutrient content across production batches (Chontal et al., 2019). The absence of nutrient characterization data for the FPJ formulations used in this study, identified as a priority for future research limits, direct comparison across studies and constitutes a recognized limitation of the current work.

Multivariate Analysis of Variance

A one-way MANOVA revealed a statistically significant overall treatment effect on the combined set of ten growth parameters (Wilks' $\lambda = 0.097$, $F(50, 482.238) = 6.459$, $p < .001$, partial $\eta^2 = 0.374$), indicating that treatment identity accounted for approximately 37.4% of the multivariate variance in pechay growth, a large effect size by conventional benchmarks. The Wilks' lambda value of 0.097, approaching zero, reflects strong

group separation in the multivariate space, confirming that the six treatment groups produced meaningfully distinct growth profiles across the full parameter set (Table 2).

Table 2. MANOVA multivariate test results for the effect of FPJ treatments on the combined growth parameters of pechay *(Brassica rapa* L.).

Effect	Wilks' λ	F	Hyp. df	Error df	p	Partial η²
Intercept	0.006	1718.07	10.000	105.000	< .001	0.994
Treatment	0.097	6.459	50.000	482.238	< .001	0.374

Tests of Between-Subjects Effects

Tests of between-subjects effects confirmed that treatment significantly influenced every individual growth parameter at $\alpha = 0.05$ (Table 3). Partial η^2 values ranged from 0.183 (stem length) to 0.602 (leaf width), indicating that the proportion of variance attributable to treatment was large across all parameters. Leaf width demonstrated the strongest treatment dependency ($F(5, 114) = 34.484$, $p < .001$, $\eta^2 = 0.602$), followed by wet weight ($F(5, 114) = 23.198$, $p < .001$, $\eta^2 = 0.504$) and stem circumference ($F(5, 114) = 17.671$, $p < .001$, $\eta^2 = 0.437$). Stem length showed the smallest treatment effect ($F(5, 114) = 5.111$, $p < .001$, $\eta^2 = 0.183$), though still statistically significant, suggesting that stem elongation is comparatively less responsive to fertilizer type across the range of treatments applied. Collectively, the significant F-values and substantial η^2 values across all ten parameters confirm that observed growth differences were driven by treatment identity rather than random variation.

Table 3. Tests of between-subjects effects (Treatment source, df = 5) for each growth parameter of pechay (Brassica rapa L.) under six FPJ treatment groups.

Dependent Variable	Type III SS	df	MS	F	p	Partial η²
Plant Height	937.999	5	187.600	11.915	< .001	0.343
No. of Leaves	102.967	5	20.593	9.138	< .001	0.286
Leaf Length	531.333	5	106.267	12.378	< .001	0.352
Leaf Width	649.254	5	129.851	34.484	< .001	0.602
Stem Length	24.345	5	4.869	5.111	< .001	0.183
Stem Circumference	21.582	5	4.316	17.671	< .001	0.437
Root Length	854.247	5	170.849	17.867	< .001	0.439
Wet Weight	124,330.400	5	24,866.080	23.198	< .001	0.504
Dry Weight	6,591.342	5	1,318.268	15.649	< .001	0.407
Height-based GR	15,288.892	5	3,057.778	11.582	< .001	0.337

Post-Hoc LSD Pairwise Comparisons

Post-hoc LSD comparisons confirmed that the growth differentiation observed descriptively was statistically robust across the majority of parameter-treatment combinations (Table 4). Broadly, all FPJ treatments significantly exceeded the negative control for most parameters ($p < 0.05$), affirming the growth-promoting capacity of FPJ regardless of source plant. The positive control significantly outperformed FPJ treatments in plant height, leaf length, biomass, and growth index, consistent with its higher and immediately bioavailable nutrient concentrations.

Notably, non-significant differences between FPJ treatments and the positive control were recorded in several parameters: Kangkong FPJ in stem length ($p = 0.771$) and height-based growth index ($p = 0.061$); KKA FPJ in

leaf width (p = 0.987) and stem circumference; and Kangkong, Kulitis, and Alugbati FPJ in root length (p = 0.476, 0.939, and 0.217, respectively). These findings indicate that select FPJ formulations can approach the efficacy of commercial inorganic fertilizers in specific morphological parameters, particularly those governing structural development (stem, root) and leaf expansion. Among FPJ-to-FPJ comparisons, no significant differences were detected for biomass parameters (all p > 0.05), indicating that the four FPJ formulations stimulated fresh and dry matter accumulation to a statistically equivalent degree. This equivalence in biomass response, despite apparent differences in foliar and structural parameters, suggests that all four source plants provided sufficient nutrients for comparable biomass production at the application rates used.

Table 4. Summary of LSD post-hoc pairwise comparisons among treatments for each growth parameter of pechay (Brassica rapa L.) at α = 0.05. KKA FPJ = Kangkong + Kulitis + Alugbati combined FPJ; GR = height-based growth index; ns = not significant.

Parameter	**Pairwise Comparison Summary (LSD, α = 0.05; ns = not significant)**
Plant Height	All FPJ treatments vs. Negative Control: p < 0.05. Positive Control vs. Kangkong FPJ: p = 0.013; vs. Kulitis FPJ: p = 0.024; vs. Alugbati FPJ: p = 0.048; vs. KKA FPJ: p < 0.001. Alugbati FPJ vs. KKA FPJ: p = 0.041. Kangkong FPJ vs. KKA FPJ: p = 0.041.
No. of Leaves	All FPJ treatments and Positive Control vs. Negative Control: p < 0.05. Non-significant: Kangkong vs. KKA FPJ (p = 0.599); Kulitis vs. Alugbati FPJ (p = 0.834); Kulitis vs. Positive Control (p = 0.599); Alugbati vs. Positive Control (p = 0.753).
Leaf Length	All FPJ treatments vs. Negative Control: p < 0.05. Positive Control vs. Kangkong FPJ: p = 0.013; vs. Kulitis FPJ: p = 0.024; vs. KKA FPJ: p < 0.001. Alugbati FPJ vs. KKA FPJ: p = 0.041; vs. Positive Control: p = 0.048.
Leaf Width	Most treatments vs. Negative Control: p < 0.05. Non-significant: Kangkong vs. Kulitis FPJ (p = 0.505); Kangkong vs. Alugbati FPJ (p = 0.399); Kulitis vs. Alugbati FPJ (p = 0.132); Kulitis vs. KKA FPJ (p = 0.132); Kulitis vs. Positive Control (p = 0.137); KKA FPJ vs. Positive Control (p = 0.987).
Stem Length	Kangkong FPJ vs. Positive Control: p = 0.771 (ns); vs. Negative Control: p < 0.05. Kulitis vs. Alugbati FPJ (p = 0.358); Kulitis vs. KKA FPJ (p = 0.821); Kulitis vs. Negative Control (p = 0.148). Alugbati vs. KKA FPJ (p = 0.487); Alugbati vs. Positive Control (p = 0.123). KKA FPJ vs. Negative Control (p = 0.095).
Stem Circumference	Kangkong FPJ vs. Positive Control: p = 0.771 (ns). KKA FPJ vs. Positive Control: p = 0.987 (ns). Kulitis, Alugbati, and KKA FPJ mutually non-significant. All FPJ treatments vs. Negative Control: p < 0.05 except Kulitis (p = 0.148) and KKA FPJ (p = 0.095).
Root Length	KKA FPJ vs. Alugbati FPJ: p = 0.053 (ns); KKA FPJ vs. all others: p < 0.05. Kangkong, Kulitis, and Alugbati FPJ vs. Positive Control: all non-significant (p = 0.476; 0.939; 0.217). All FPJ treatments vs. Negative Control: p < 0.05.
Wet Weight	Positive Control vs. all FPJ treatments: p < 0.05; vs. Negative Control: p < 0.05. All FPJ treatments vs. Negative Control: p < 0.05. Non-significant among FPJ: Kangkong vs. Kulitis (p = 0.654); Kangkong vs. KKA (p = 0.310); Kulitis vs. KKA (p = 0.570); Kulitis vs. Alugbati (p = 0.076); Alugbati vs. KKA (p = 0.224).
Dry Weight	Positive Control vs. all FPJ treatments: p < 0.05. All FPJ treatments vs. Negative Control: p < 0.05. Non-significant among FPJ: Kangkong vs. Kulitis (p = 0.381); Kangkong vs. KKA (p = 0.224); Kulitis vs. KKA (p = 0.731); Alugbati vs. KKA (p = 0.097).
Height-based GR	Positive Control vs. KKA FPJ: p < 0.001. Kangkong FPJ vs. Positive Control: p = 0.061 (ns). All FPJ treatments vs. Negative Control: p < 0.05 except Kangkong (p = 0.061). Alugbati FPJ vs. KKA FPJ: p = 0.021.

Mechanistic Basis of FPJ Biostimulant Activity

The growth-promoting effects observed across all FPJ treatments are most parsimoniously explained by the biological activity of the fermentation-associated microbial community rather than direct nutrient supply alone. Leaf surfaces of healthy plants harbor approximately 100,000 to 150,000 microbial cells per square centimeter (Barna et al., 2021), and these epiphytic organisms are not removed during FPJ preparation since plant materials are deliberately left unwashed (Miller et al., 2013). Microbiological characterization of FPJ by Sulok et al. (2021) identified Lactobacillus, Bacillus, Sporosarcina, Anaerobacillus, Aspergillus, Trichococcus, Talaromyces, and Penicillium species within the extract, organisms collectively associated with lactic acid and yeast-type fermentation (Bercero et al., 2014).

These microorganisms contribute to plant growth through multiple mechanisms. Bacillus and Aspergillus species are well-documented phosphate solubilizers, converting insoluble soil phosphorus into plant-available orthophosphate, while selected Lactobacillus strains facilitate potassium and zinc solubilization (Glick, 2012; Sulok et al., 2021). Nitrogen fixation by free-living soil bacteria introduced through FPJ application further supplements plant-available nitrogen beyond the organic nitrogen content of the extract itself. Critically, plant growth-promoting bacteria (PGPB) associated with FPJ produce indole acetic acid (IAA), cytokinins, and gibberellic acids, phytohormones that directly stimulate cell division, root elongation, and shoot growth (Glick, 2012). IAA production, in particular, is the most widely studied PGPB mechanism and has been demonstrated to enhance both lateral root initiation and root hair proliferation, consistent with the superior root lengths observed under FPJ treatments relative to the negative control in this study. The observed superiority of KKA combined FPJ in root length may reflect a more diverse microbial inoculant derived from three distinct plant microbiomes, potentially enriching the rhizosphere with a broader spectrum of IAA-producing bacteria (Glick, 2012; Sulok et al., 2021).

Beyond direct growth stimulation, the microbial community introduced by FPJ contributes to broader soil health functions including organic matter cycling, suppression of soil-borne pathogens, and enhancement of soil biodiversity (Sulok et al., 2021). The combined soil drench and foliar application method employed in this study maximizes the delivery of these biological agents to both the rhizosphere and phyllosphere, and has been consistently recommended to increase vegetable crop productivity relative to either method applied in isolation (Bandera, 2020; Rahman et al., 2015). FPJ's role as a soil organic conditioner, characterized by high organic content (90–95%) and moderate macronutrient concentrations, complements its biostimulant function by improving soil physical, chemical, and biological conditions over successive applications (Zuraihah et al., 2012; Zamora & Calub, 2016; Pascual et al., 2013). These combined attributes position FPJ as a multi-functional organic input with agronomic value extending beyond the single growing cycle evaluated in this study.

CONCLUSIONS

Fermented plant juice (FPJ) derived from kangkong (*Ipomoea aquatica*), alugbati (*Basella alba*), kulitis (*Amaranthus spinosus*), and their combination significantly stimulated the growth and development of pechay (*Brassica rapa*) across all ten measured growth parameters. Among the FPJ formulations, Kangkong FPJ led in leaf number, stem length, and biomass production, while Alugbati FPJ recorded the highest plant height and height-based growth index. The combined Kangkong-Kulitis-Alugbati (KKA) FPJ produced the greatest root length and stem circumference among all treatments, including the positive control, and matched the commercial fertilizer in leaf width, suggesting an additive effect on structural development. While the Triple 14 commercial fertilizer retained a significant advantage in overall biomass and growth rate, the narrow performance gap observed in several morphological parameters, particularly stem and foliar traits, underscores the viability of select FPJ formulations as organic alternatives or supplements to synthetic fertilizers in pechay production.

The growth-stimulating effects of FPJ are attributed to its content of beneficial microorganisms that facilitate phosphorus solubilization, nitrogen fixation, and the biosynthesis of plant growth regulators such as indole acetic acid, cytokinins, and gibberellic acids, in addition to contributing to soil health through nutrient cycling and pathogen suppression. These multifunctional properties, combined with its low production cost and local sourcing, position FPJ as an ecologically viable soil alternative for resource-limited agricultural settings. Future studies should optimize FPJ dilution rates, application frequencies, and combination ratios, characterize the NPK

nutrient profiles of each FPJ formulation to enable precise attribution of growth responses to specific nutrient fractions, and conduct open-field, multi-season trials to validate and extend the findings of this study toward large-scale practical application.

CRediT Authorship Contribution Statement

John Paul Cordero Secando: Conceptualization, Methodology, Investigation, Resources, Writing- original draft, Visualization. Regina Embon Samillano: Conceptualization, Writing- review & editing, Supervision, Project administration. Ghison Riuben Embon Samillano: Conceptualization, Methodology, Investigation, Resources, Writing- original draft, Visualization.

Declaration Of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript. No funding was received from any commercial, governmental, or non-governmental organization that could be construed as a potential conflict of interest in relation to the design, execution, analysis, interpretation, or publication of this study.

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