



Effect of Additive Ingredients in Papaya Leaf-Based Botanical Pesticides on the Vegetative Growth of Water Spinach (*Ipomoea reptans* Poir)

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Abstract: This study evaluated the effect of additives in papaya leaf-based botanical pesticides on the vegetative growth of water spinach (*Ipomoea reptans* Poir). The experiment was arranged using a Randomized Complete Block Design (RCBD) with five treatments and five replications: P0 (control), P1 (papaya leaves), P2 (papaya leaves + garlic), P3 (papaya leaves + lemongrass), and P4 (papaya leaves + soursop leaves). Observed parameters included plant height, root length, number of leaves, and fresh weight. Data were analyzed using analysis of variance (ANOVA), Duncan's Multiple Range Test (DMRT) at 5%, correlation, and multiple linear regression analyses. The treatments significantly affected most growth parameters. P3 (papaya leaves + lemongrass) produced the best performance, with plant height of 24.35 cm, root length of 22.58 cm, approximately 18 leaves, and fresh weight of 14 g. Correlation analysis showed strong positive relationships between plant height and number of leaves ($r = 0.890$), and between root length and fresh weight ($r = 0.764$). Regression analysis identified number of leaves as the strongest predictor of fresh weight ($\beta = 1.015$), followed by root length ($\beta = 0.528$), whereas plant height showed a negative effect ($\beta = -0.891$). Overall, the addition of lemongrass to papaya leaf-based botanical pesticides enhanced vegetative growth and biomass accumulation in water spinach plants.

Keywords: Botanical pesticides; Lemongrass; Papaya leaf extract; Pest control; Water spinach.

Introduction

The control of plant pests and diseases (PPDs) in vegetable cultivation systems is still predominantly dependent on synthetic pesticides due to their rapid and practical effectiveness. However, intensive and repeated applications have been associated with negative impacts such as pest resistance, environmental contamination, pesticide residues, and risks to human health (Ahmad et al., 2024; Beyuo et al., 2024; Klátyik et al., 2023; Mota et al., 2024). In addition, long-term use may disrupt agroecosystem stability and reduce

production sustainability (Li et al., 2024; Parven et al., 2025; Sarker et al., 2024). Therefore, safer and more sustainable alternatives are required.

Water spinach (*Ipomoea reptans* Poir) is a fast-growing leafy vegetable with high economic value; however, its vegetative growth is highly sensitive to pest attacks during early developmental stages. Herbivorous pest activity can reduce leaf area, inhibit photosynthetic efficiency, and disrupt metabolic processes, ultimately limiting biomass accumulation (Chávez-Arias et al., 2021). These physiological disturbances directly affect vegetative growth

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performance, which is a key indicator of crop productivity.

In this context, pest management is essential not only to suppress pest presence but also to maintain optimal vegetative growth. Integrated pest management has been widely promoted as a sustainable approach; however, its field implementation still faces practical constraints (Deguine et al., 2021). Alternative ecological approaches such as trap cropping and environmentally based crop protection strategies have also been developed to support plant growth under pest pressure (Meyer et al., 2021; Panwar et al., 2021; Zhang et al., 2021).

Botanical pesticides have gained attention as eco-friendly alternatives due to their biodegradability and compatibility with sustainable agriculture systems. Plant-derived compounds contain secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, and phenolics, which exhibit antifeedant, repellent, and growth-inhibiting properties (Chaudhary et al., 2024; Guo et al., 2023; Lengai et al., 2020). By reducing pest damage, these compounds indirectly contribute to maintaining plant physiological performance.

Among botanical sources, papaya (*Carica papaya* L.) has been widely studied for its pesticidal properties. Papaya leaf extracts have shown bioactivity against several insect pests, including *Spodoptera litura*, resulting in reduced plant damage in crop systems (Fachraniah et al., 2023; Rahayu et al., 2023). These bioactivities are associated with compounds such as flavonoids, saponins, alkaloids, phenolics, and papain enzymes, which interfere with insect physiological systems (Ilham et al., 2019).

Recent studies indicate that botanical extracts act through multiple mechanisms, including feeding deterrence and physiological disruption in insects, thereby reducing plant damage pressure (Chaudhary et al., 2024; Guo et al., 2023). In vegetable crops, papaya-based biopesticides have been reported to help maintain leaf integrity and support plant physiological stability (Tanor & Sumayku, 2023), which is directly related to vegetative growth performance.

Nevertheless, single botanical formulations often show inconsistent performance due to rapid degradation of active compounds and limited persistence in field conditions (Abdel-Hakim et al., 2021; Dougoud et al., 2019; Ullah et al., 2025). This limitation highlights the need for formulation improvements using additive combinations of botanical materials.

Plants such as lemongrass (*Cymbopogon citratus*), garlic (*Allium sativum*), and soursop (*Annona muricata*)

contain bioactive compounds that may enhance the overall effectiveness of botanical formulations. Soursop contains acetogenins with strong biological activity (Ramadan & Yuliani, 2025; Salessy et al., 2022; Sulaminingsih, 2025; Syahputra & Ginting, 2024), while lemongrass essential oil exhibits bioactive properties that influence insect behavior and physiology (Abdel-Hakim et al., 2021; Han et al., 2025; Moustafa et al., 2024). Garlic contributes sulfur-based compounds that disrupt insect metabolic processes (Abdel-Hakim et al., 2021). Combined formulations of these plants have been reported to improve overall bioactivity through synergistic interactions (Firdausi et al., 2014; Moustafa et al., 2024; Wattimena et al., 2020).

Despite these findings, most studies have focused on insecticidal efficacy parameters such as mortality or feeding inhibition (Ramadan & Yuliani, 2025; Salessy et al., 2022; Syahputra & Ginting, 2024; Ullah et al., 2025). In contrast, the effects of botanical pesticide formulations on vegetative growth as a plant performance indicator remain less explored.

This indicates a research gap in understanding how botanical pesticide formulations, particularly papaya leaf-based systems enriched with additional botanical additives, influence vegetative growth performance in leafy vegetables. Therefore, this study aims to evaluate the effect of additive combinations in papaya leaf-based botanical pesticides on vegetative growth of water spinach. It is hypothesized that additive combinations may support improved vegetative growth by reducing pest-induced stress and maintaining physiological efficiency. A controlled experimental design was applied using several formulation treatments, and vegetative growth parameters were used as the main evaluation indicators.

Method

Experimental Design

The study was conducted in an open-field experimental area in Tarakan. The experiment was arranged using a Randomized Complete Block Design (RCBD) consisting of five treatments with five replications. Each treatment was applied to experimental units randomly arranged within each block to minimize the effects of environmental heterogeneity (Figure 1). The treatments tested were as follows: P0 (control without pesticide), P1 (papaya leaf extract), P2 (papaya leaf extract + garlic), P3 (papaya leaf extract + lemongrass), and P4 (papaya leaf extract + soursop leaf extract).

Plant Cultivation

Water spinach was cultivated on raised beds containing prepared soil media. The seeds were directly sown using uniform planting spacing. Plant maintenance included watering and weeding, which were carried out according to field conditions to ensure optimal plant growth.

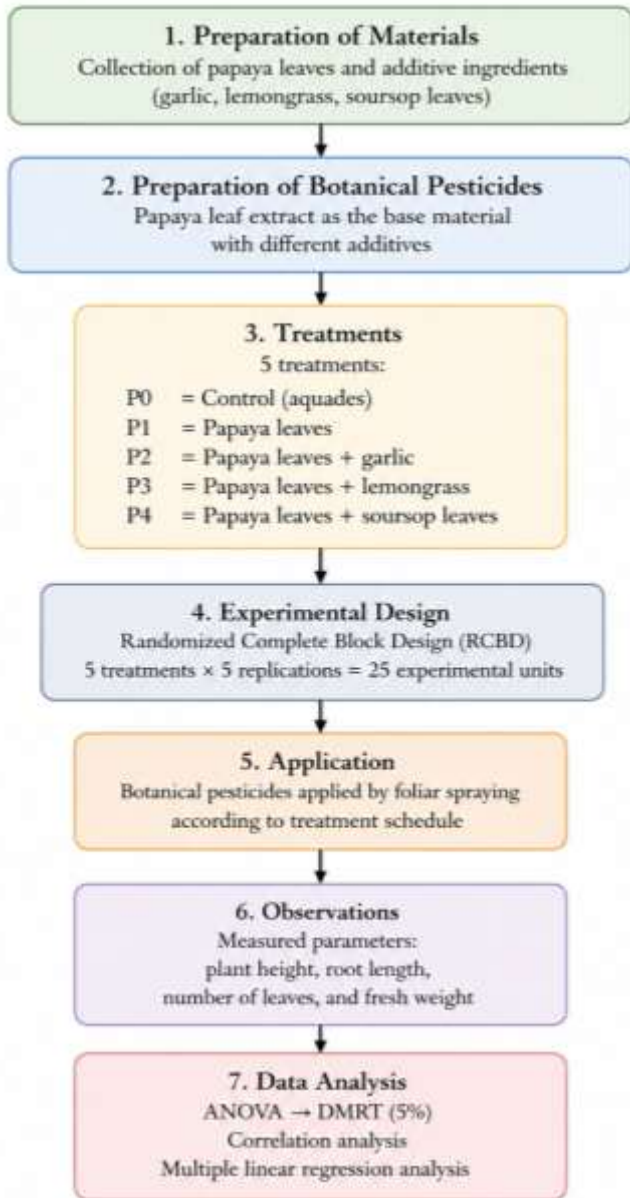


Figure 1. Research procedure stages

Preparation of Botanical Pesticides

Fresh plant materials used as sources of botanical pesticides were crushed until homogeneous and then mixed with water as a solvent. The mixture was stored in a closed container for approximately 3 days to allow the extraction of active compounds. Afterward, the

solution was filtered to separate the residues and obtain a filtrate ready for use as a pesticide solution.

Application of Treatments

The botanical pesticides were applied by evenly spraying all parts of the plants. Treatments were administered periodically throughout the plant growth period according to the experimental design. The control group received only water without any added active ingredients.

Observation Parameters

Observations were conducted to evaluate vegetative growth responses and pest attack intensity. Growth parameters included plant height (cm), root length (cm), number of leaves, and plant fresh weight (g). In addition, the number of pests and the level of leaf damage were also observed as indicators of pest attack intensity.

Statistical Analysis

The observational data were analyzed using analysis of variance (ANOVA) to determine the effects of the treatments. When significant differences were detected, the analysis was followed by Duncan’s Multiple Range Test (DMRT) at the 5% significance level to compare differences among treatments. In addition to ANOVA, Pearson correlation analysis was performed to examine the relationships among growth parameters. Furthermore, multiple linear regression analysis was used to identify the effects of growth parameters (root length, plant height, and number of leaves) on plant fresh weight. All analyses were conducted at a 95% confidence level.

Result and Discussion

The results showed that the application of papaya leaf-based botanical pesticides with different additives produced varying responses in the vegetative growth of water spinach and the intensity of pest attacks. In general, the combination treatment of papaya leaves and lemongrass (P3) demonstrated the most consistent performance in enhancing plant growth compared with the other treatments.

The superior plant height observed in P3 (papaya leaves + lemongrass) was likely attributed to the synergistic interaction of bioactive compounds that enhanced vegetative growth while reducing pest pressure. This combined botanical formulation may therefore provide both protective and growth-supporting effects on water spinach plants (Figure 2).

Lemongrass (*Cymbopogon citratus*) contains bioactive compounds such as citral and geraniol that

exhibit antimicrobial, biostimulant, and insect-repellent properties (Mukarram et al., 2021; Olorunnisola et al., 2014). These compounds may improve nutrient uptake efficiency and support vegetative development, thereby contributing to increased plant height. In addition, lemongrass essential oil has been widely reported as a natural biopesticide and plant growth enhancer (Wan & Xing, 2025).

Papaya leaves also contribute pesticidal activity through papain and alkaloid compounds capable of inhibiting insect development and reducing pest populations (Purba & Muliarta, 2024; Siahaya & Rumthe, 2014). Previous studies further reported that the combined application of papaya leaves and lemongrass improved biopesticidal effectiveness and crop protection in horticultural systems (Tambun et al., 2025; Wakhidya et al., 2024).

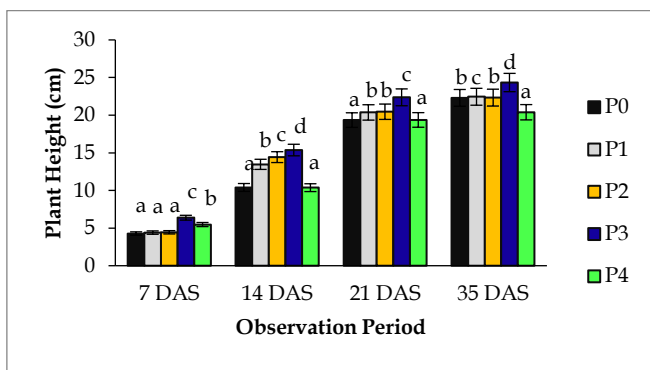


Figure 2. Effect of botanical pesticide treatments on the plant height of water spinach. P0 = control; P1 = papaya leaves; P2 = papaya leaves + garlic; P3 = papaya leaves + lemongrass; P4 = papaya leaves + soursop leaves; DAS = days after sowing. Bars followed by different letters indicate significant differences according to Duncan’s Multiple Range Test (DMRT) at $p < 0.05$.

The reduced pest pressure likely minimized biotic stress, thereby supporting continuous vegetative growth throughout the observation period. Previous studies reported that herbivore attack can negatively affect plant physiological activity, photosynthetic performance, stem development, and overall plant growth. (Haile & Higley, 2003) demonstrated that insect injury reduced gas-exchange activity in soybean plants, while Stephens & Westoby (2015) reported that insect attack suppressed plant growth and physiological performance. In addition, Alves-Silva & Del-Claro (2016) showed that herbivore damage during early growth stages caused instability in leaf development. Herbivory and plant damage have also been associated with reduced vegetative performance and altered plant growth responses under stressful conditions (Egerer et al., 2020; Venâncio et al., 2026). Therefore, lower pest disturbance in the P3 treatment

likely allowed water spinach plants to maintain better stem elongation and vegetative development. Moreover, botanical extracts may improve soil microenvironment conditions, including nutrient availability and water retention, thereby supporting sustained plant growth throughout the observation period. These synergistic effects plausibly explain the consistently superior growth performance observed in the P3 treatment.

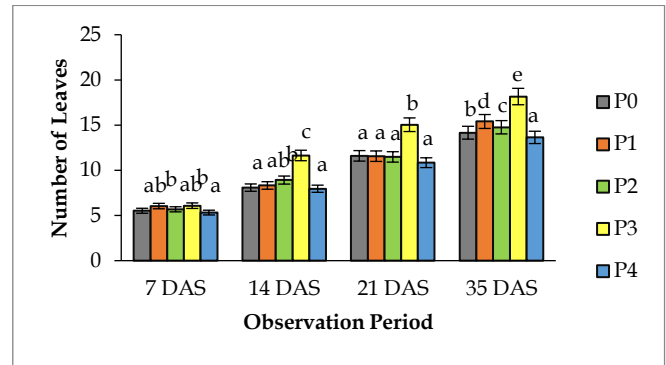


Figure 3. Observation of leaf number in water spinach plants. Note: P0 = control; P1 = papaya leaves; P2 = papaya leaves + garlic; P3 = papaya leaves + lemongrass; P4 = papaya leaves + soursop leaves; DAS = days after sowing. Bars followed by different letters indicate significant differences according to Duncan’s Multiple Range Test (DMRT) at $p < 0.05$.

The number of leaves in water spinach increased progressively throughout the observation period across all treatments (P0–P4) (Figure 3). However, significant differences among treatments were observed from 14 DAS onward. Treatment P3 (papaya leaves + lemongrass) consistently produced the highest number of leaves compared to other treatments, reaching approximately 11.67 leaves at 14 DAS, 15 leaves at 21 DAS, and 18 leaves at 35 DAS. In contrast, P4 (papaya leaves + soursop leaves) generally resulted in the lowest leaf number during the observation period. At 35 DAS, P3 differed significantly from the other treatments according to DMRT analysis ($p < 0.05$), indicating that the papaya leaf and lemongrass formulation was more effective in reducing pest-related stress, thereby supporting leaf development in water spinach plants.

The higher leaf production observed in P3 was likely associated with the synergistic effects of bioactive compounds contained in lemongrass and papaya leaves. Lemongrass (*Cymbopogon citratus*) contains compounds such as citral and geraniol that exhibit biostimulant and antimicrobial activities, which may support nutrient absorption and vegetative development (Mukarram et al., 2021; Olorunnisola et al., 2014). In addition, lemongrass essential oil has been reported to function as a natural biopesticide capable of

reducing pest disturbances and supporting plant growth (Wan & Xing, 2025).

Papaya leaves also contribute bioactive compounds, including papain and alkaloids, which possess insecticidal properties that can suppress pest populations and reduce plant damage (Purba & Muliarta, 2024; Siahaya & Rumthe, 2014). Previous studies further reported that combinations of papaya leaves and lemongrass improved the effectiveness of botanical pesticides and enhanced crop protection in horticultural cultivation systems (Tambun et al., 2025; Wakhidya et al., 2024).

Reduced pest pressure likely allowed plants to allocate more photosynthates toward leaf development and biomass formation. Previous studies reported that herbivore-induced stress can negatively affect plant physiological performance and leaf development. (Haile & Higley, 2003) demonstrated that insect injury reduced gas-exchange activity and photosynthetic performance in soybean plants, while (Alves-Silva & Del-Claro, 2016) reported that herbivore damage during early growth stages caused instability in leaf development. Increased leaf number is closely associated with greater photosynthetic surface area, which contributes to improved plant growth and productivity. Therefore, the superior leaf formation observed in P3 indicates that the combined application of papaya leaves and lemongrass was effective in supporting vegetative growth of water spinach.

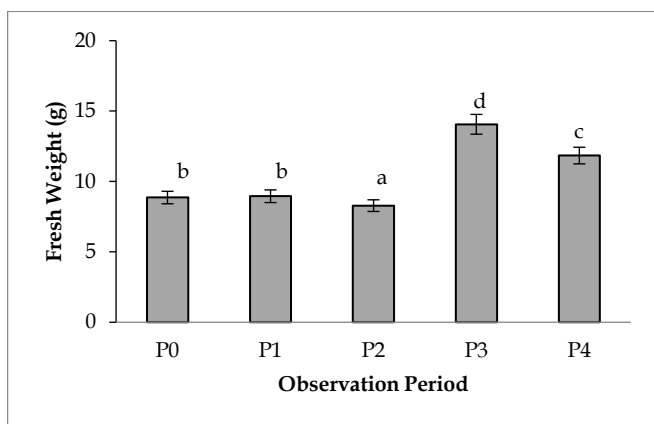


Figure 4. Observation of fresh weight of water spinach at 35 DAS. Note: P0 = control; P1 = papaya leaves; P2 = papaya leaves + garlic; P3 = papaya leaves + lemongrass; P4 = papaya leaves + soursop leaves; DAS = days after sowing. Bars followed by different letters indicate significant differences according to Duncan’s Multiple Range Test (DMRT) at $p < 0.05$.

Fresh weight of water spinach at 35 DAS showed significant differences among treatments (Figure 4). The highest fresh weight was observed in P3 (papaya leaves + lemongrass), reaching 14 g, while the lowest value

was recorded in P2 (papaya leaves + garlic) at approximately 8 g. Treatments P0 and P1 produced relatively similar fresh weights, whereas P4 showed intermediate results. DMRT analysis confirmed that P3 differed significantly from the other treatments ($p < 0.05$). These results indicate that the combined application of papaya leaves and lemongrass was more effective in supporting biomass accumulation in water spinach plants.

The higher fresh weight observed in P3 was likely associated with better vegetative performance and reduced biotic stress during plant growth. Previous studies reported that herbivore attack and plant damage can interfere with physiological activity and reduce plant growth performance (Haile & Higley, 2003; Stephens & Westoby, 2015). Reduced pest disturbance may therefore allow greater assimilate accumulation and biomass formation in plants. In addition, lemongrass and papaya leaf extracts contain various bioactive compounds that may support plant growth indirectly through their biopesticidal activity and protection against pest damage (Mukarram et al., 2021; Purba & Muliarta, 2024). Consequently, lower pest pressure in P3 likely contributed to more efficient biomass accumulation, resulting in higher fresh weight production.

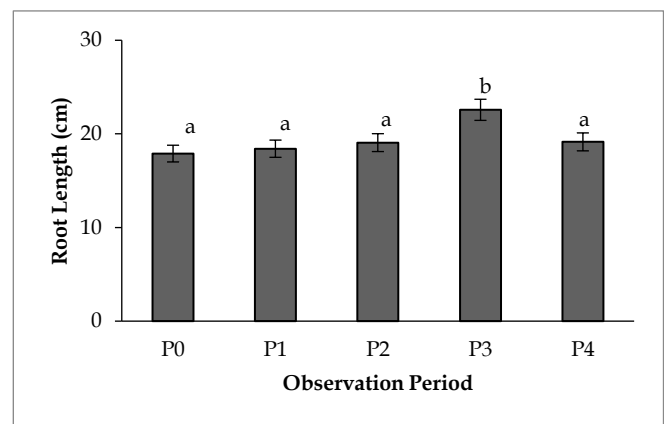


Figure 5. Observation of root length of water spinach at 35 DAS. Note: P0 = control; P1 = papaya leaves; P2 = papaya leaves + garlic; P3 = papaya leaves + lemongrass; P4 = papaya leaves + soursop leaves; DAS = days after sowing. Bars followed by different letters indicate significant differences according to Duncan’s Multiple Range Test (DMRT) at $p < 0.05$.

Root length at 35 DAS showed significant differences among treatments according to DMRT analysis ($p < 0.05$). The highest root length was observed in P3 (22.57 cm), which differed significantly from the control treatment (P0) and several other treatments. In contrast, P0 recorded the lowest root length (17.90 cm) (Figure 5). These results indicate that the combination of papaya leaves and lemongrass was

more effective in supporting root development compared to the other treatments.

Improved root development in P3 may be associated with reduced biotic stress and better physiological performance during plant growth. Root systems play an important role in water and nutrient uptake, which subsequently supports shoot growth and biomass accumulation. Previous studies reported that herbivory can alter root morphology and belowground plant responses, ultimately affecting overall plant growth and performance (Johnson et al., 2016). Heinze (2020) further demonstrated that aboveground insect herbivory significantly influenced root morphological traits, while Heinze et al. (2022) reported that shoot herbivory produced stronger effects on root morphology than mechanical damage. In a broader context, Myers & Sarfraz (2017) explained that insect herbivores may reduce plant growth and vegetative performance through continuous biotic stress and tissue damage. Therefore, reduced pest pressure in the P3 treatment likely enabled water spinach plants to maintain more stable root development and vegetative growth. These findings suggest that the synergistic interaction between papaya leaves and lemongrass contributed to the superior root growth performance observed in the P3 treatment.

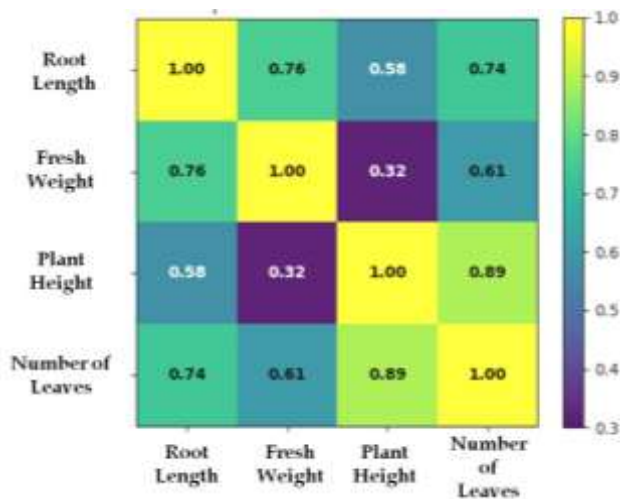


Figure 6. Correlation heatmap among growth parameters of water spinach plants. Note: Colors indicate the strength of the relationship (Pearson correlation coefficient), with brighter colors representing higher correlations. Correlation values are displayed in each cell to facilitate interpretation of the relationships among parameters.

Correlation analysis revealed different levels of association among the growth parameters of water spinach plants (Figure 6). The strongest positive correlation was observed between plant height and number of leaves ($r = 0.890$; $p < 0.01$), indicating that increased stem elongation was closely followed by leaf

formation during vegetative growth. This relationship reflects the strong coordination between canopy development and photosynthetic capacity in supporting plant productivity. Previous studies reported that leaf development is closely associated with photosynthetic activity and assimilate production, which are important determinants of plant growth performance (Hu et al., 2020; Yang et al., 2025).

Root length also showed strong positive correlations with fresh weight ($r = 0.764$; $p < 0.01$) and number of leaves ($r = 0.739$; $p < 0.01$), suggesting that improved root development contributed to more efficient water and nutrient uptake, which subsequently enhanced vegetative performance and biomass accumulation. Root systems are known to regulate nutrient absorption and plant growth responses through root plasticity and physiological adaptation mechanisms (Jia et al., 2022; Siqueira et al., 2022).

In contrast, plant height showed a weak and non-significant correlation with fresh weight ($r = 0.317$; $p > 0.05$), indicating that stem elongation alone did not necessarily reflect biomass production. This finding suggests that biomass accumulation was more strongly associated with leaf development and root function than with plant height itself.

Overall, the correlation pattern demonstrates that vegetative growth in water spinach is strongly regulated by interactions among root development, leaf formation, and biomass accumulation. Under botanical pesticide application, reduced pest pressure likely supported more stable physiological activity, thereby promoting more efficient plant growth and resource allocation. Previous studies reported that herbivore attack may alter plant physiological performance, photosynthetic activity, and vegetative responses, ultimately affecting plant growth and biomass production (Haile & Higley, 2003; Myers & Sarfraz, 2017).

Table 1. Results of multiple linear regression analysis on plant fresh weight

Variable	B	Std. Error	Beta	t	Sig.
Constant	12.276	6.247	-	1.965	0.063
Root Length	0.629	0.203	0.528	3.09	0.006
Plant Height	-1.577	0.447	-0.891	-3.524	0.002
Number of Leaves	1.391	0.42	1.015	3.311	0.003

The multiple linear regression analysis demonstrated that root length, plant height, and number of leaves significantly influenced plant fresh

weight ($p < 0.05$) (Table 1). Root length and number of leaves showed significant positive effects on fresh weight, whereas plant height exhibited a significant negative relationship. Based on the standardized beta coefficients, number of leaves was identified as the most influential variable affecting fresh weight ($\beta = 1.015$), followed by plant height ($\beta = -0.891$) and root length ($\beta = 0.528$). These results indicate that biomass accumulation in water spinach was more strongly associated with leaf formation and root development than with stem elongation alone.

The strong contribution of leaf number to fresh weight is closely related to its role in determining photosynthetic surface area and assimilate production. Increased leaf formation generally enhances photosynthetic capacity, thereby promoting biomass accumulation and vegetative growth (Hu et al., 2020; Yang et al., 2025). Likewise, improved root development may increase water and nutrient uptake efficiency, which supports plant physiological activity and biomass production (Jia et al., 2022; Siqueira et al., 2022).

The superior performance of the P3 treatment was likely associated with reduced pest disturbance due to the synergistic action of papaya leaves and lemongrass as botanical pesticide components. Papaya leaves contain papain and alkaloid compounds with insecticidal properties, whereas lemongrass contains essential oils such as citronellal, citral, and geraniol that function as natural repellents and biopesticides; et al., 2021; 2024) (Mukarram et al., 2021; Olorunnisola et al., 2014; Purba & Muliarta, 2024). Reduced pest pressure likely minimized biotic stress and leaf damage, thereby allowing more efficient photosynthate allocation toward vegetative growth and biomass accumulation.

Previous studies also reported that herbivore attack may suppress plant physiological performance and alter growth allocation patterns. (Haile & Higley, 2003) demonstrated that insect injury reduced gas-exchange activity and photosynthetic performance, while Myers & Sarfraz (2017) reported that herbivory negatively affected plant growth and productivity. Therefore, the reduced pest disturbance in P3 likely contributed to better vegetative performance compared with the other treatments.

Overall, P3 consistently produced the best growth performance across most observed parameters, indicating that the addition of lemongrass to papaya leaf-based botanical pesticide formulations improved not only pest management effectiveness but also supported vegetative growth and biomass production in water spinach plants.

Conclusion

Additives in papaya leaf-based botanical pesticides significantly affected the vegetative growth of water spinach plants. The combination of papaya leaves and lemongrass (P3) produced the best results among all treatments, with a plant height of 24.34 cm, root length of 22.58 cm, 18 leaves, and a fresh weight of 14 g. Correlation analysis revealed a strong relationship between plant height and number of leaves ($r = 0.890$; $p < 0.01$), as well as between root length and fresh weight ($r = 0.764$; $p < 0.01$), indicating a close association between vegetative growth and biomass accumulation. Multiple linear regression analysis showed that the number of leaves was the most dominant factor determining fresh weight ($\beta = 1.015$; $p = 0.003$), followed by root length ($\beta = 0.528$; $p = 0.006$), whereas plant height had a significant negative effect ($\beta = -0.891$; $p = 0.002$). Overall, these findings demonstrate that an appropriate botanical pesticide-based pest management strategy not only provides protective effects against pests but also improves plant growth efficiency toward more optimal biomass formation.

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Author Contributions

Conceptualization, N.C.; methodology, N.C., I., and D.S.; validation, N.C., D.S., and N.I.M.; formal analysis, N.C.; investigation, N.C., N., M.A., and M.; data curation, N.C.; writing—original draft preparation, N.C.; writing—review and editing, N.C., I., and D.S.; visualization, N.C.; supervision, D.S. and I.; project administration, N.C.; funding acquisition, N.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this manuscript. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. All authors have approved

the final version of the manuscript and agree to its submission for publication.

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