

Chemical Composition and Structural Characterization of a Basil (*Ocimum basilicum* L.) Leaf Extract–Bionutrient S-367B Composite

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Abstract

The excessive use of synthetic pesticides in horticultural production poses serious environmental and health concerns, necessitating the development of eco-friendly alternatives. This study evaluates the effectiveness of a composite biopesticide formulated from basil leaf (*Ocimum basilicum* L.) extract and Bionutrient S-367B on the functional response and yield of broccoli (*Brassica oleracea* var. *italica*). The extract was composited with Bionutrient S-367B at concentrations of 25%, 50%, and 75% and applied at dosages of 5, 7.5, and 10 mL/L. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, saponins, and tannins, with a total phenolic content of 27.624 ± 1.468 mg GAE/g. FTIR characterization revealed key functional groups, including hydroxyl (–OH), aliphatic C–H, carbonyl (C=O), aromatic C=C, and C–N groups, indicating the presence of phenolic and non-phenolic bioactive compounds. The composite treatment showed improved functional response and yield of broccoli, particularly at a 50% concentration with a dosage of 7.5 mL/L, which produced the highest plant height, leaf dimensions, growth rate constant, and harvested biomass. However, statistical analysis indicated that these differences were not significant at the 95% confidence level ($P > 0.05$) across most measured parameters. These findings suggest that the integration of plant-based biopesticides with bionutrients has the potential to enhance crop performance while maintaining environmental sustainability. This study provides an integrated chemical and agronomic perspective on the application of basil leaf extract–Bionutrient S-367B composites as a promising eco-friendly strategy for sustainable broccoli production.

Keywords: basil leaf extract, bionutrient S-367B, biopesticide, broccoli growth, sustainable agriculture

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1. Introduction

The use of synthetic chemical pesticides in modern agriculture remains the dominant practice for controlling plant pests and diseases, particularly in high-value horticultural commodities like broccoli. However, long-term dependence on chemical pesticides has given rise to various global issues, including environmental pollution, soil degradation, pest resistance, and health risks

to humans and non-target organisms (Jote, 2023). Issues of sustainability and food safety are driving a paradigm shift toward environmentally friendly agricultural systems based on biopesticides derived from natural compounds (Ramya & Kumar, 2024; Akude et al., 2024; Juliana et al., 2025).

Plant-based biopesticides are a promising alternative because they contain secondary metabolites such as alkaloids, flavonoids,

phenolics, saponins, and tannins, which have insecticidal, antifungal, and antimicrobial activities. One plant with high potential is basil (*Ocimum basilicum* L.), which has been reported to have broad biological activities due to its essential oil and phenolic compounds (Bernhardt et al., 2015; Diba et al., 2022). Various studies have shown that basil leaf extract can effectively inhibit the growth of plant pathogens and insect pests without causing significant phytotoxic effects (Son et al., 2018; Sa'adah et al., 2023).

Empirically, previous research has focused more on the effectiveness of *Ocimum basilicum* extract as a single agent, both in controlling pathogenic fungi and insect pests. Babayan (2023) reported the antimicrobial and antioxidant activity of basil leaf ethanol extract, while Sa'adah et al. (2023) confirmed the toxicity of basil extract against *Spodoptera exigua*. However, most of these studies are still limited to laboratory tests or toxicity observations, and have not integrated aspects of plant nutrition and growth responses comprehensively in the field.

On the other hand, increasing plant productivity is determined not only by pest control but also by adequate nutrition and plant physiological conditions. Bionutrient S-367B has been reported to increase vegetative growth and yield in various commodities, such as oil palm and cayenne pepper (Dharani et al., 2023; Monib et al., 2023; Panchal & Maitreya, 2023). However, the application of bionutrients as nutritional supplements is generally still treated separately from pest control strategies, so the synergistic potential between biopesticides and bionutrients has not been fully explored.

Bionutrient S-367B is not merely a growth supplement but a complex formulation containing essential macro- and micronutrients, as well as organic compounds that play important roles in plant metabolism. In general, such bionutrients contain nitrogen (N), phosphorus (P), potassium (K), trace elements, and organic constituents such as amino acids and humic-like substances that enhance nutrient uptake efficiency and

stimulate plant physiological processes. These components contribute to improving chlorophyll formation, enzymatic activity, and overall plant vigor.

The integration of Bionutrient S-367B with basil leaf extract is therefore scientifically relevant, as it combines two complementary mechanisms: bioactive secondary metabolites from *Ocimum basilicum* that act as natural biopesticides, and nutrient-driven metabolic enhancement provided by the bionutrient. This synergistic interaction enables simultaneous pest suppression and growth optimization, which cannot be achieved when each component is applied separately.

Several recent studies have begun to examine the composite approach between plant extracts and bionutrients. Damanik (2023) and Madani (2023) reported that the combination of bitter leaf extract and neem with the bionutrient S-367B increased broccoli growth and yield compared to the control. However, to date, no scientific report has specifically examined the composite of basil (*Ocimum basilicum* L.) leaf extract with the bionutrient S-367B, particularly using an analytical chemistry approach involving the characterization of active compounds and their correlation with plant growth responses. The research gap in this study lies in the absence of an integrated study that combines (1) chemical analysis of basil leaf extract through phytochemical tests, total phenolic content, and FTIR, with (2) the application of biopesticide-bionutrient composites on broccoli growth parameters and harvest yields. In addition, the effect of variations in the concentration and dose of the composite on plant growth rates and harvest mass has not been systematically documented in the literature.

The urgency of this research is increasing considering that broccoli (*Brassica oleracea* var. *italica*) is a functional vegetable rich in bioactive compounds such as flavonoids, polyphenols, and glucosinolates, yet it is highly susceptible to pests and diseases (Nagraj et al., 2020; Lippens et al., 2022; Nagi et al., 2024). In Indonesia, especially in West

Java, broccoli production plays an important role in the horticultural sector. However, its cultivation still depends heavily on chemical pesticides (Kashyap et al., 2023). Therefore, safe, effective, and sustainable solutions for pest control and productivity improvement are needed.

Based on the current state of the art, this study introduces a novel approach through the development and application of a composite biopesticide derived from basil (*Ocimum basilicum* L.) leaf extract and Bionutrient S-367B. This approach integrates chemical and agronomic perspectives, aiming not only to suppress pests and diseases but also to improve the growth performance and quality of broccoli yields, thereby contributing to the advancement of sustainable agricultural systems based on locally available biological resources.

In this context, the study emphasizes a systematic integration of chemical characterization and field-based evaluation. The chemical properties of the composite are first established through phytochemical screening, total phenolic content determination, and FTIR-based functional group analysis, which provide a scientific basis for understanding the activity of the bioactive compounds. Subsequently, field observations are conducted to assess the functional performance of the composite in a biological system.

Therefore, this study aims to investigate the chemical characteristics and functional properties of basil leaf extract–Bionutrient S-367B composites, with biological observations serving as supporting validation of the chemical activity rather than the primary focus of the research.

2. Research Method

2.1. Materials

Fresh basil leaves (*Ocimum basilicum* L.) were collected from Cibodas Village, Lembang District, West Bandung Regency, Indonesia, on March 9, 2025. Approximately 5 kg of fresh leaves were used as raw material. Ethanol

(96%, technical grade) and distilled water were employed as extraction solvents. Reagents used for phytochemical screening included Dragendorff reagent, magnesium ribbon (Mg), concentrated hydrochloric acid (HCl), and ferric chloride solution (FeCl₃, 5%). Total phenolic content analysis utilized gallic acid standard, Folin–Ciocalteu reagent (10%), and sodium carbonate (Na₂CO₃, 7%). Additional materials included Bionutrient S-367B and water for field application.

2.2. Extraction of Basil Leaves

Fresh basil leaves were washed thoroughly and air-dried at room temperature for 15 days until the leaves turned brownish and slightly shriveled. The dried leaves were selected for quality and ground into powder using a blender, yielding approximately 500 g of basil leaf powder. The powder was macerated with 96% ethanol using a solvent volume of 1.5 L per day for 3 × 24 h at room temperature. The resulting filtrate was concentrated using a rotary vacuum evaporator at 60 °C to obtain 1 L of basil leaf extract. A total of 900 mL of the extract was diluted into three concentration levels (25%, 50%, and 75%) in 1000 mL solutions and subsequently composited with Bionutrient S-367B. The remaining 100 mL was further evaporated to obtain a concentrated extract for chemical analysis.

In this study, the percentages of 25%, 50%, and 75% represent the dilution levels of the pre-formulated basil extract–Bionutrient S-367B composite and do not indicate the mixing proportions between basil extract and Bionutrient S-367B as separate components.

2.3. Phytochemical Screening

Qualitative phytochemical screening was conducted on the concentrated basil leaf extract to identify secondary metabolites, including alkaloids, flavonoids, saponins, and tannins, following standard procedures adapted from Bahri et al. (2025). Alkaloids were detected by adding 3–5 drops of Dragendorff reagent to 1 mL of extract, with the formation of an orange-red precipitate indicating a positive result. Flavonoids were identified using the magnesium–hydrochloric acid test, indicated by the appearance of a

dark red coloration. Saponins were confirmed by vigorous shaking of the extract with distilled water, where stable foam persisting for approximately 15 min indicated a positive result. Tannins were detected by the addition of FeCl_3 5%, producing a greenish-black or bluish coloration.

2.4. Determination of Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method with a UV–Visible spectrophotometer. One milliliter of extract was mixed with 5 mL of Folin–Ciocalteu reagent (10%) and allowed to stand for 8 min, followed by the addition of 4 mL sodium carbonate solution (Na_2CO_3 , 7%). The mixture was incubated at room temperature in the dark for 60 min before measuring the absorbance. The mixture was incubated at room temperature in the dark for 60 min. The absorbance of the reaction mixture was measured at 765 nm using a UV–Vis spectrophotometer, which corresponds to the maximum absorption wavelength of the Folin–Ciocalteu chromophore complex. The TPC was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g), with a calibration curve exhibiting a regression coefficient (R^2) greater than 0.99.

2.5. FTIR Analysis

Functional group characterization of the basil leaf extract was performed using Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu IRTracer-100, Japan). FTIR spectra were recorded in the wavenumber range of $4000\text{--}400\text{ cm}^{-1}$ with a resolution of 4 cm^{-1} and 32 scanning accumulations. The sample was prepared and exposed to infrared radiation, and the resulting absorption spectra were recorded as relative transmittance (%) versus wavenumber (cm^{-1}). The spectral peaks were interpreted to identify characteristic functional groups associated with phenolic and non-phenolic compounds, following established analytical principles (Nandiyanto et al., 2023).

2.6. Preparation and Application of Composite Treatments

The composite formulations were prepared using basil leaf extract concentrations of 25%, 50%, and 75% (w/v), while the volume of

Bionutrient S-367B was kept constant for all treatments. Each concentration was applied at three dosage levels: 5 mL/L, 7.5 mL/L, and 10 mL/L. Applications were performed once a week in the morning by spraying both the upper and lower surfaces of broccoli leaves. A solvent control treatment containing the same ethanol–water ratio without extract and bionutrient was included for comparison.

The composite formulations were prepared using basil leaf extract concentrations of 25%, 50%, and 75% (w/v), each combined with a constant volume of Bionutrient S-367B. Therefore, the percentages used in this study represent variations in basil leaf extract concentration rather than the mixing proportions between basil extract and Bionutrient S-367B.

2.7. Observation of Broccoli Growth Parameters

Growth observations included soil pH, soil moisture, plant height, leaf length, leaf width, growth rate, and harvested biomass. Soil pH and moisture were measured using an ETP306 3-in-1 soil pH meter. Plant height and leaf dimensions were recorded weekly in the morning using a ruler or measuring tape. Harvested biomass was measured using a digital balance at the end of the cultivation period.

The growth rate of broccoli plants was estimated using the first-order growth model based on the natural logarithm of plant height measurements. The growth rate constant (k) was calculated according to the following equation:

$$K = \frac{\ln Nt - \ln N_0}{t - t_0} \quad (1)$$

where k is the growth rate constant (week^{-1}), N_0 is the initial plant height, Nt is the plant height at time t , and $(t - t_0)$ represents the observation period in weeks. Linear regression of \ln plant height versus time was subsequently performed to determine the growth rate constant and regression coefficient (R^2).

2.8. Experimental Design

The field experiment was arranged using a Completely Randomized Design (CRD). The treatments consisted of three concentration levels of basil leaf extract–Bionutrient S-367B composite (25%, 50%, and 75%) combined with three dosage levels (5, 7.5, and 10 mL/L), along with a solvent control. Each treatment was replicated three times, resulting in a total of experimental units that allowed statistical analysis of treatment effects. The assignment of treatments to experimental units was conducted randomly to minimize bias and ensure the validity of the results.

2.9. Statistical Analysis

All experimental data were expressed as mean \pm standard deviation (SD) of three replications. Statistical analyses were performed using one-way Analysis of Variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$) to evaluate differences between composite treatments and solvent controls. A P-value of less than 0.05 ($P < 0.05$) was considered statistically significant, whereas $P > 0.05$ indicated no significant difference among treatments. Descriptive and regression analyses were also employed to interpret plant growth trends and treatment responses.

To quantify the vegetative growth dynamics of broccoli plants under different treatments, the growth rate constant (k) was estimated using a first-order growth model based on changes in the natural logarithm of plant height over time. This approach assumes that plant growth follows an exponential pattern during the vegetative phase and allows for comparison of growth performance among treatment groups.

3. Result and Discussion

3.1. Basil Leaf Extract (*Ocimum basilicum* L.)

The first stage in this research was the extraction of basil leaf samples. Table 1 shows the results of the basil leaf sample extraction stage.

Table 1. Basil Leaf Extraction Results

Information	Mass or Volume
Wet mass of basil leaves	5000 grams
Basil leaf powder mass	500 grams
Maceration filtrate	4500 mL
Evaporation filtrate	1000 mL
Volume of thick extract	10 mL

The liquid extract (evaporated filtrate) was used to make a composite with S-367B bionutrients to be applied to broccoli plants, while the thick extract was used for phytochemical test analysis, determination of total phenolic content and characterization of functional groups of basil leaf extract.

3.2. Phytochemical Test

In this study, phytochemical tests were conducted on the active compound alkaloids, flavonoids, saponins, and tannins to determine the secondary metabolite content of basil leaf extract. The results of the phytochemical tests are as follows.

Table 2. Phytochemical Test Results of Basil Leaf Extract

No	Contents	Information
1	Alkaloid	There is orange sediment
2	Flavonoid	Red colored solution
3	Saponin	Foam formed
4	Tannin	Blackish green solution

Description: (+) = positive active compound

3.3. Determination of Total Phenolic Content Using UV-Vis Spectrophotometry

Based on the standard curve, gallic acid exhibited a linear regression equation of $y = 0.0047x + 0.0578$ with a regression coefficient (R^2) of 0.9989, where y represents the absorbance measured at 765 nm and x represents the concentration of gallic acid standard (mg/L). The high regression coefficient, approaching unity, indicates excellent linearity of the calibration curve. The absorbance values obtained from the basil leaf extract samples were subsequently substituted into the regression equation to determine the total phenolic content. The results of the total phenolic content measurement of basil leaf extract are presented in Table 3.

Table 3. Total Phenolic Content of Basil Leaf Extract (mg GAE/g)

Information	Treatment of Basil Leaf Extract Samples	
	Simple	Duplo
Sample Absorbance	0.191	0.182
Average Abs	0.186	
Sample Concentration (mg/L)	28.663	26.586
Average Concentration	27.624	
Total Phenol Content ± Standard Deviation (mg GAE/g)	27,624 ± 1,468	

3.4. Characterization of Functional Groups by FTIR Spectrophotometry

Characterization was performed using an FTIR spectrophotometer on basil leaf extract to determine the functional groups present in the extract. The FTIR spectrum results can be seen in Figure 1.

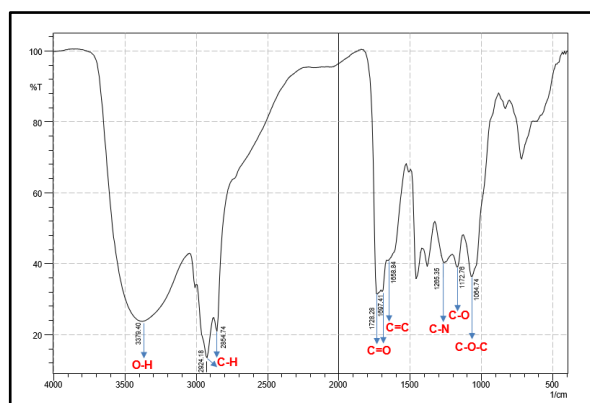


Figure 1. FTIR Spectrum of Basil Leaf Extract

The results of the FTIR spectrum measurement of basil leaf extract in Table 4, show the presence of absorption in the wave number

region of 3379.4 cm^{-1} with a broad shape which is characteristic of the hydroxyl group (OH). This group strengthens the phytochemical test of phenolic compounds such as flavonoids and tannins. Peak broadening is caused by the formation of hydrogen bonds between molecules, resulting in a shift to a lower wave number. There are also wave numbers of 2924.18 cm^{-1} and 2854.74 cm^{-1} which are aliphatic -CH groups which indicate the presence of methylene (CH_2), and methyl (CH_3), chains in the framework of terpenoid molecules such as linalool, the appearance of both aliphatic -CH groups which show stretching (stretching) and deformation (bending) can strengthen the suspicion of the presence of saponin compounds. The wave number of 1728.28 cm^{-1} is a C=O functional group, which indicates the presence of ester bonds, such as ferulic acid. The wave number 1697.41 cm^{-1} is a conjugated C=O functional group, which indicates conjugated phenolic acid (such as rosmarinic acid). The wave number 1572.76 cm^{-1} is an aromatic C=C functional group indicating the presence of a carbon double bond in the aromatic ring. This group is a characteristic of the flavonoid structure and is also found in tannin compounds. The wave number 1265.35 cm^{-1} is a CN functional group indicating the presence of alkaloids, and the wave number 1172.76 cm^{-1} is a CO stretch group indicating the presence of phenol and phenolic ether (supporting the presence of eugenol and estragol in basil leaf extract), and the wave number 1064.74 cm^{-1} is a COC functional group indicating the presence of saponins due to the characteristic of glycosidic.

Table 4. Results of FTIR Spectrum Analysis of Basil Leaf Extract Based on Literature

Wave Number (cm^{-1})		Vibration Mode/Functional Group	Intensity	References
Experiment	Literature			
3379.4	3600-3200	OH stretch (alcohol/phenol)	Strong	(Kassem et al., 2023)
2924.18	2990-2850	C-H symmetric stretch & CH_2CH_3 (aliphatic)	Medium-Strong	(Stuart, 2004; Socrates, 2004; Shurvell, 2006)

Wave Number (cm ⁻¹)	Vibration Mode/Functional Group	Intensity	References
2854.74	2990-2850 C-H symmetric stretch & CH ₂ CH ₃ (aliphatic)	Medium-Strong	
1728.28	1740-1720 C=O stretch (aldehyde)	Strong	
1697.41	1710-1690 C=O stretch (carboxylic acid)	Strong	(Stuart, 2004;
1658.84	1680-1630 C=C stretch (alkene/aromatic ring)	Medium-Strong	Socrates, 2004;
1265.35	1280-1180 CN stretch (aromatic amine)	Strong	Shurvell, 2006)
1172.76	1200-1015 CO stretch (alcohol)	Very Strong	
1064.74	1240-1070 COC asymmetric stretch (aliphatic ether)	Strong-Very Strong	

3.5. Effect of Basil Leaf Extract and Bionutrient S-367B Composite on Soil pH

Based on the graph, it can be seen that the soil pH at concentrations of 25%, 50% and 75% (doses of 5 mL/L, 7.5 mL/L, and 10 mL/L) of the composite treatment group and the solvent control group did not experience an increase or decrease from the first week to the eighth week, the soil pH was constant at 8.

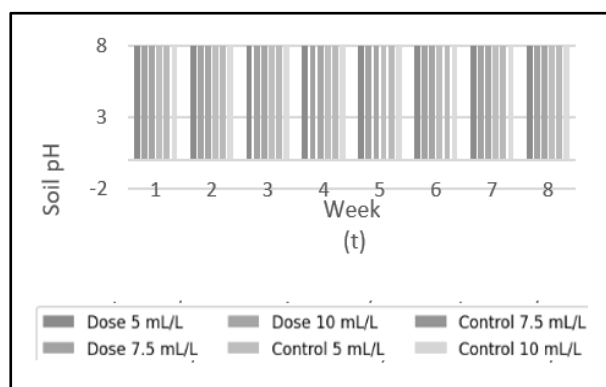


Figure 2. Soil pH Treatment Concentration 25%, 50%, 75% Composite and Control

The relatively constant soil pH observed throughout the eight-week cultivation period indicates that the application of basil leaf extract–Bionutrient S-367B composites did not substantially alter the chemical properties of the growth medium. This stability may be attributed to the buffering capacity of the soil, which can resist changes in acidity and alkalinity despite the addition of external organic inputs. Furthermore, the concentrations and dosages of the composite formulations applied in this study were relatively low and therefore insufficient to induce significant acidification or alkalization

processes. The absence of considerable changes in microbial decomposition products and ion exchange processes may have further contributed to maintaining the soil pH at approximately 8 in both the treatment and control groups.

3.6. Effect of Basil Leaf Extract and Bionutrient S-367B Composite on Soil Moisture

In Figure 3, it shows that the highest humidity was 8 which occurred in the control treatment group with a solvent dose of 5 mL/L and 10 mL/L in weeks 4 and 6.

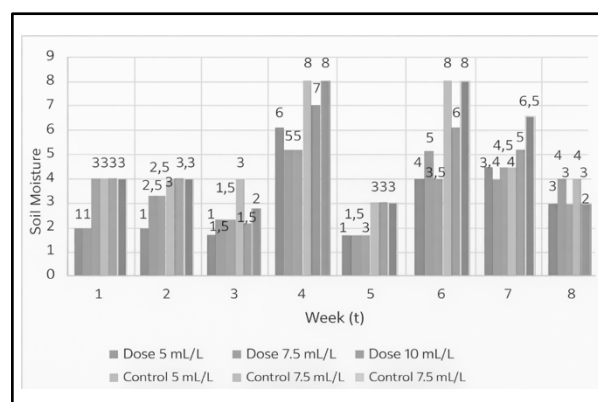


Figure 3. Soil Moisture of 25% Composite Treatment and Control

Based on the ANOVA variance test with a 95% confidence level ($P > 0.05$), the P values were 0.181 (dose 5 mL/L); 0.517 (dose 7.5 mL/L); and 0.281 (dose 10 mL/L), which showed no significant difference in soil moisture between the treatment groups of basil leaf extract biopesticide composite with 25% S-367B bionutrient and solvent control.

The relatively uniform fluctuations in soil moisture observed across all treatment groups indicate that the changes were not primarily

caused by the basil leaf extract–Bionutrient S-367B composite formulations. Instead, these fluctuations were more likely influenced by external environmental factors, including irrigation frequency, evapotranspiration, ambient temperature, and natural variations in water availability throughout the cultivation period. Since all experimental units were maintained under identical environmental and management conditions, similar patterns of soil moisture dynamics were observed in both the treatment and control groups. Therefore, the fluctuations in soil moisture can be considered a consequence of common environmental influences rather than a direct effect of the composite formulations on the water-holding capacity of the soil.

Figure 4 shows that the highest humidity was 6.5 which occurred in the composite treatment group of Basil leaf extract with S-367B bionutrient at a dose of 5 mL/L in the fourth week.

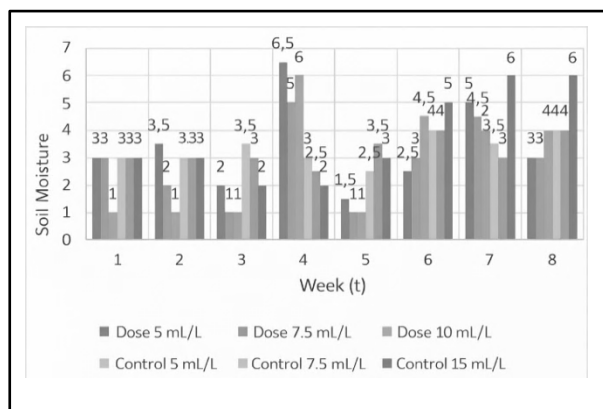


Figure 4. Soil Moisture of 50% Composite Treatment and Control

Based on the ANOVA variance test with a 95% confidence level ($P > 0.05$), the P values were 0.648 (dose 5 mL/L); 0.514 (dose 7.5 mL/L); and 0.367 (dose 10 mL/L), which showed no significant difference in soil moisture between the treatment groups of basil leaf extract biopesticide composite with 50% S-367B bionutrient and solvent control.

In Figure 5, it shows that the highest humidity was 8 which occurred in the control treatment group with a solvent dose of 10 mL/L in the third week.

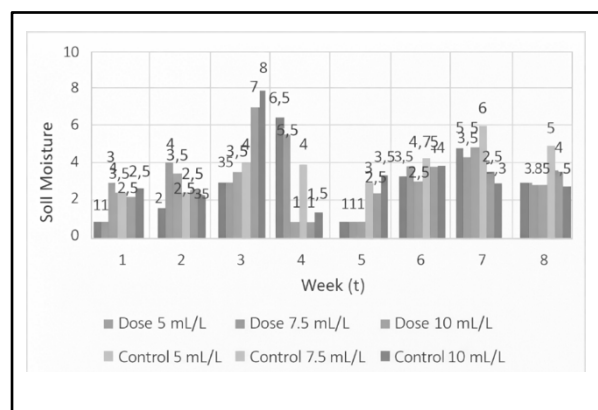


Figure 5. Soil Moisture of 75% Composite Treatment and Control

The results indicate that soil pH remained stable at pH 8 across all treatments, and statistical analysis showed no significant differences between treatment and control groups ($P > 0.05$). Similarly, soil moisture did not differ significantly among treatments, suggesting that the application of the basil leaf extract–Bionutrient S-367B composite did not alter soil physicochemical properties. This indicates that the observed plant growth responses were not influenced by changes in soil conditions but were more likely associated with physiological and biochemical effects of the composite treatment.

3.7. The Effect of Basil Leaf Extract Composite (*Ocimum basilicum* L.) with Bionutrient S-367B on Broccoli Plant Growth Broccoli Plant Height

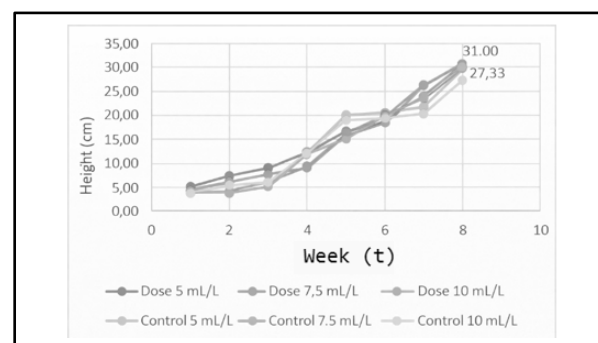


Figure 6. Height of Broccoli Plants with 25% Composite and Control

In Figure 6, it shows that in the eighth week the highest plant growth occurred at a dose of 10 mL/L with a height of 31 cm.

Based on the ANOVA variance test on the height of the plants in the 25% concentration treatment group of basil leaf extract composite with S-367B bionutrient, and the height of the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.930 (dose 5 mL/L); 0.989 (dose 7.5 mL/L); and 0.903 (dose 10 mL/L) which showed no significant difference in the height of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 25% S-367B bionutrient and the solvent control.

In Figure 7, it shows that in the eighth week the highest plant growth occurred at a dose of 7.5 mL/L with a height of 33.85 cm.

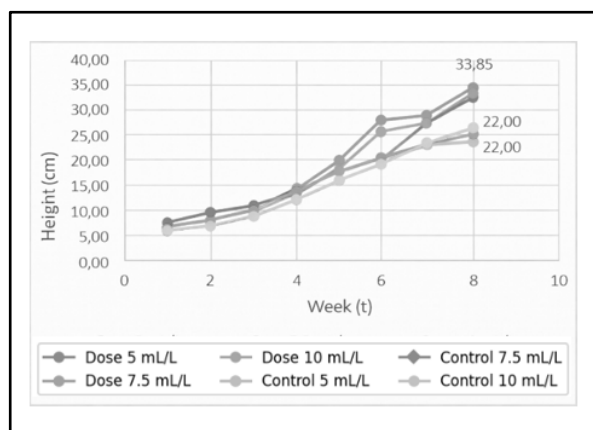


Figure 7. Height of Broccoli Plants with 50% Composite and Control

Based on the ANOVA variance test on the height of the plants in the 50% concentration treatment group of basil leaf extract composite with S-367B bionutrient, and the height of the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.596 (dose 5 mL/L); 0.961 (dose 7.5 mL/L); and 0.654 (dose 10 mL/L) which showed no significant difference in the height of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 50% S-367B bionutrient and the solvent control.

Figure 8 shows that in the eighth week the highest plant growth occurred at a dose of 7.5 mL/L with a height of 30.80 cm.

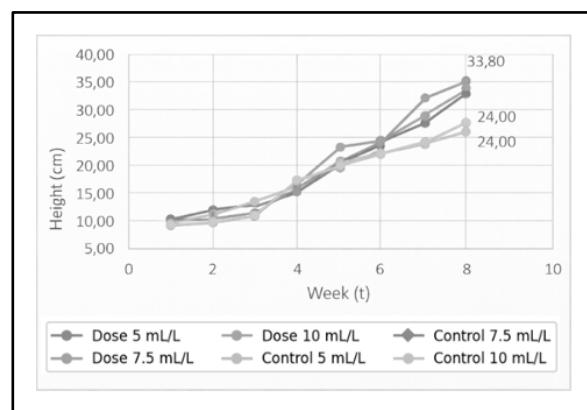


Figure 8. Height of Broccoli Plants Composite 75% and Control

Based on the ANOVA variance test on the height of the plants in the 75% concentration treatment group of basil leaf extract composite with S-367B bionutrient, and the height of the plants in the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.743 (dose 5 mL/L); 0.666 (dose 7.5 mL/L); and 0.633 (dose 10 mL/L) which showed no significant difference in the height of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 75% S-367B bionutrient and the solvent control. This means that statistically the height of broccoli plants in the treatment group of basil leaf extract biopesticide composite with 75% S-367B bionutrient and the solvent control did not have a significant difference.

Based on the plant growth graph regarding plant height in the three concentration treatments of the basil leaf extract biopesticide composite with S-367B bionutrient, it can be concluded that each dose at a certain concentration has a different plant height. The highest broccoli plant height occurred in the 50% concentration treatment of the basil leaf extract biopesticide composite with S-367B bionutrient at a dose of 7.5 mL/L, which reached 33.85 cm.

Although the composite treatments generally showed higher values in plant height, leaf length, and leaf width compared to the control, statistical analysis indicated that these differences were not significant at the 95%

confidence level ($P > 0.05$) for several treatment groups. This suggests that the observed increases represent positive growth trends rather than statistically confirmed differences across all treatments.

3.8. Broccoli Leaf Length

Figure 9 shows that in the eighth week, the greatest growth in broccoli leaf length occurred at a dose of 10 mL/L with a leaf length of 37.33 cm.

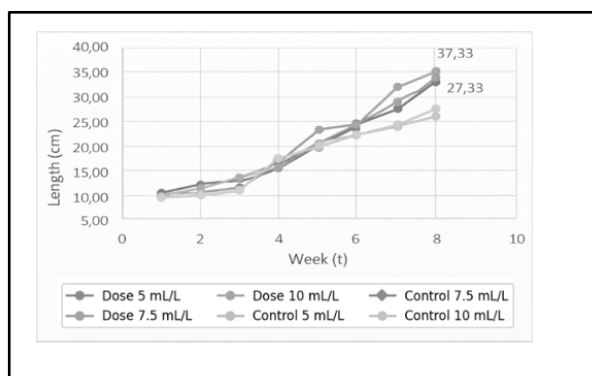


Figure 9. Leaf Length of Broccoli Plants with 25% Composite Treatment and Control

Based on the ANOVA variance test on the leaf length of broccoli plants in the 25% concentration treatment group of basil leaf extract composite with S-367B bionutrient, and the leaf length of broccoli plants in the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.505 (dose 5 mL/L); 0.587 (dose 7.5 mL/L); and 0.493 (dose 10 mL/L) which showed no significant difference in the leaf length of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 25% S-367B bionutrient and the solvent control.

Figure 10 shows that in the eighth week, the greatest growth in broccoli leaf length occurred at a dose of 7.5 mL/L with a maximum leaf length of 38.33 cm.

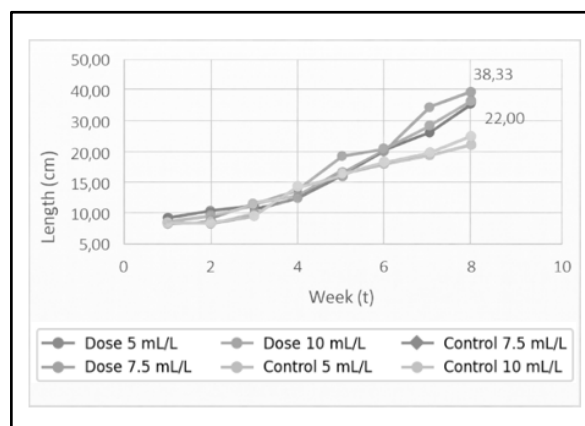


Figure 10. Leaf Length of Broccoli Plants with 50% Composite Treatment and Control

Based on the ANOVA variance test on the leaf length of broccoli plants in the 50% concentration treatment group of basil leaf extract composite with S-367B bionutrient, and the leaf length of broccoli plants in the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.265 (dose 5 mL/L); 0.225 (dose 7.5 mL/L); and 0.209 (dose 10 mL/L) which showed no significant difference in the leaf length of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 50% S-367B bionutrient and the solvent control.

Figure 11 shows that in the eighth week, the greatest growth in broccoli leaf length occurred at a dose of 5 mL/L with a maximum leaf length of 35.40 cm.

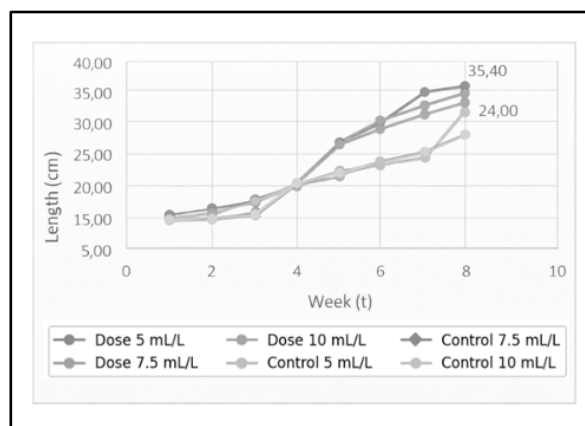


Figure 11. Leaf Length of Broccoli Plants with 75% Composite Treatment and Control

Based on the ANOVA variance test on the leaf length of broccoli plants in the 75% concentration treatment group of Basil leaf extract composite with S-367B bionutrient, and the leaf length of broccoli plants in the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.345 (dose 5 mL/L); 0.292 (dose 7.5 mL/L); and 0.328 (dose 10 mL/L) which indicate there is no significant difference in the leaf length of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 75% S-367B bionutrient and the solvent control. This means that statistically the leaf length of broccoli plants in the treatment group of basil leaf extract biopesticide composite with 75% S-367B bionutrient and the solvent control does not have a significant difference.

Based on the plant growth graph regarding leaf length in the three concentration treatments of the basil leaf extract biopesticide composite with S-367B bionutrient, it can be concluded that each dose at a certain concentration has a different leaf length. The longest broccoli plant leaves occurred in the 50% concentration treatment of the basil leaf extract biopesticide composite with S-367B bionutrient at a dose of 7.5 mL/L, which reached 38.33 cm.

3.9. Broccoli Leaf Width

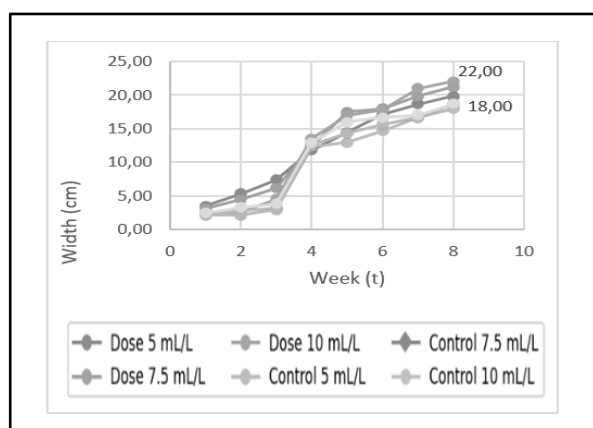


Figure 12. Leaf Width of Broccoli Plants with 25% Composite Treatment and Control

Figure 12 shows that in the eighth week, the greatest growth in broccoli leaf width

occurred at a dose of 10 mL/L with a maximum leaf width of 22 cm.

Based on the ANOVA variance test on the leaf width of broccoli plants in the 25% concentration treatment group of basil leaf extract composite with S-367B bionutrient, and the leaf width of broccoli plants in the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.558 (dose 5 mL/L); 0.549 (dose 7.5 mL/L); and 0.750 (dose 10 mL/L) which showed no significant difference in the leaf width of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 25% S-367B bionutrient and the solvent control.

Figure 13 shows that in the eighth week, the greatest growth in broccoli leaf width occurred at a dose of 10 mL/L with a maximum leaf width of 23.50 cm.

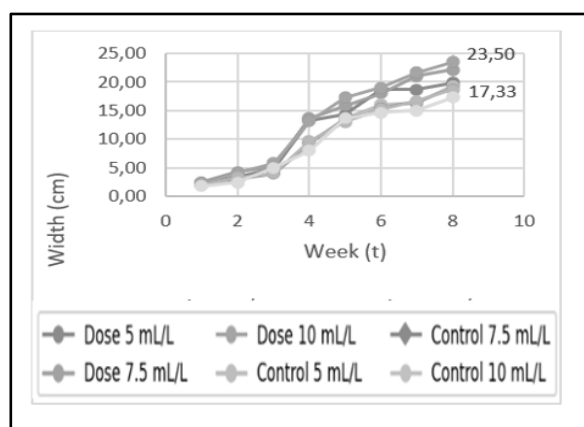


Figure 13. Leaf Width of Broccoli Plants with 50% Composite Treatment and Control

Based on the ANOVA variance test on the leaf width of broccoli plants in the 50% concentration treatment group of basil leaf extract composite with S-367B bionutrient, and the leaf width of broccoli plants in the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.583 (dose 5 mL/L); 0.499 (dose 7.5 mL/L); and 0.338 (dose 10 mL/L) which showed no significant difference in the leaf width of broccoli plants between the treatment group of basil leaf extract biopesticide composite

with 50% S-367B bionutrient and the solvent control.

Figure 14 shows that in the eighth week, the greatest growth in broccoli leaf width occurred at a dose of 7.5 mL/L with a maximum leaf width of 22.17 cm.

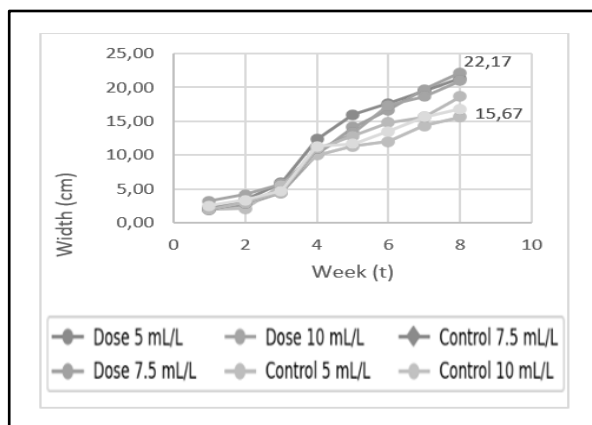


Figure 14. Leaf Width of Broccoli Plants with 75% Composite Treatment and Control

Based on the ANOVA variance test on the leaf width of broccoli plants in the 75% concentration treatment group of Basil leaf extract composite with S-367B bionutrient, and the leaf width of broccoli plants in the solvent control treatment group with a 95% confidence level ($P > 0.05$) obtained P values of 0.344 (dose 5 mL/L); 0.796 (dose 7.5 mL/L); and 0.579 (dose 10 mL/L) which showed no significant difference in the leaf width of broccoli plants between the treatment group of basil leaf extract biopesticide composite with 75% S-367B bionutrient and the solvent control. This means that statistically the leaf width of broccoli plants in the treatment group of basil leaf extract biopesticide composite with 75% S-367B bionutrient and the solvent control did not have a significant difference.

Based on the plant growth graph regarding leaf width in the three concentration treatments of the composite mixture of basil leaf extract biopesticide with 75% S-367B bionutrient, it can be concluded that each dose at a certain concentration has a different leaf width. Similarly, with leaf length, the widest leaves occurred in the concentration

treatment of the composite basil leaf extract biopesticide with 50% S-367B bionutrient, but with a different dose, namely a dose of 10 mL/L which reached 23.50 cm.

3.10. The Effect of Basil Leaf Extract Composite (*Ocimum basilicum* L.) with Bionutrient S-367B on Broccoli Growth Rate

The growth rate constant and regression coefficient in this study are shown in Figure 15, which shows that the plant with the highest growth rate constant in broccoli plants is the plant treated with the composite biopesticide extra basil leaves with 50% bionutrient S-367B at a dose of 7.5 mL/L with a growth rate constant of 0.4862. This value is obtained from the linear equation, namely $y = 0.4862x + 0.4059$ with a regression value of 0.9989.

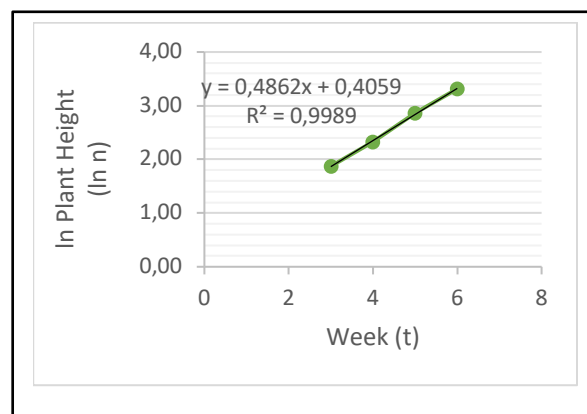


Figure 15. Growth Rate of Broccoli Plants Treated with 50% Composite at a dose of 7.5 mL/L.

Meanwhile, the highest constant growth rate of solvent control plants was at a concentration of 75% dose of 7.5 mL/L of 0.4626 obtained from the linear equation, namely $y = 0.4626x + 0.2789$ with a regression value of 0.8015. Several plants treated with the composite biopesticide of basil leaf extract with bionutrient S-367B had a higher constant value than the solvent control, except for the composite treatment of biopesticide of basil leaf extract with bionutrient S-367B 25% dose of 5 mL/L and 7.5 mL/L, 50% dose of 5 mL/L, and 75% dose of 7.5 mL/L.

3.11. Effectiveness of Basil Leaf Extract Composite (*Ocimum basilicum* L.) with Bionutrient S-367B against Pests and Diseases in Broccoli

The results of the comparison of broccoli plant growth in the composite treatment group of basil leaf extract biopesticide with S-367B bionutrient, solvent control, and without treatment are shown in Figure 16.

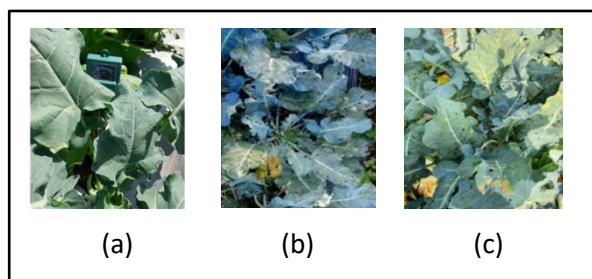


Figure 16. Broccoli Plant Growth Results

(a) Composite treatment of basil leaf extract biopesticide with bionutrient S-367B, (b) Solvent Control Treatment, (c) No Treatment.

Figure 16 shows that broccoli plants in the composite treatment group of basil leaf extract with bionutrient S-367B showed significant differences in results with broccoli plants in the solvent control treatment group and without treatment. Physically, in the composite treatment group of basil leaf extract with bionutrient S-367B, the broccoli plant leaves were fresh, without holes and spots, and had a shiny bluish green color, the growth of broccoli plant height, length and width of the leaves produced were also higher and larger than the solvent control treatment group and without treatment.

The bluish-green color of broccoli leaves in the basil leaf extract composite treatment group with S-367B bionutrient indicates a high content of type a chlorophyll. Type a chlorophyll is the main pigment that captures light energy for photosynthesis, so high levels indicate active and efficient photosynthetic activity. This affects plant health and productivity. Meanwhile, type b chlorophyll acts as a companion pigment in increasing the efficiency of light absorption by leaves. With a high concentration of chlorophyll in the leaves, plants can optimize light absorption,

increase biomass accumulation, and accelerate vegetative growth.

Meanwhile, in the solvent control treatment group, the broccoli leaves were physically unsightly, had many holes, were dull green, and some were brownish-yellow, and had more blackish-white spots than in the untreated group. The untreated group was also not much different from the solvent control treatment group, only there were fewer spots and broccoli plant growth was better than in the solvent control treatment group. This occurred because the high ethanol content in the solvent control treatment reduced nutrient availability in broccoli plants, thus inhibiting their growth. High ethanol concentrations (6%) can be toxic to plants because they can induce osmotic stress in the roots, the decrease in osmotic potential will result in lower water and nutrient absorption (Sillmann & Mattiuz, 2024). Therefore, it can be said that ethanol has an effect on the cause of the inhibited growth of broccoli plants in the solvent control treatment group.

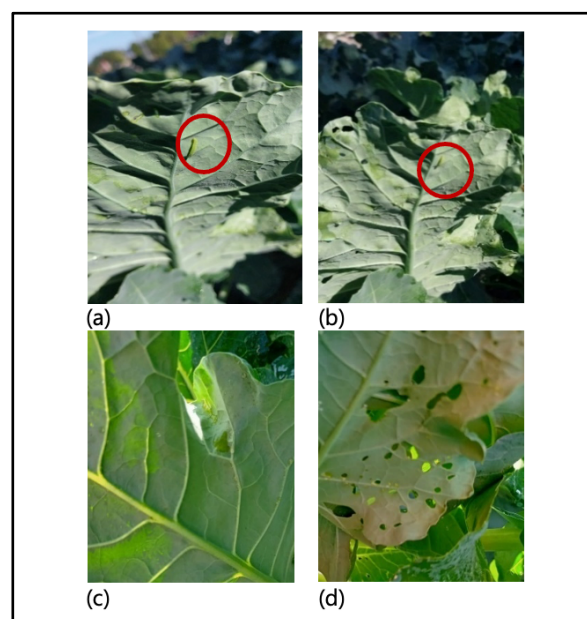


Figure 17. Broccoli plants are affected by leaf caterpillar pests (*Plutella xylostella*)

(a) Pest Attacks on the Composite Treatment of Basil Leaf Extract Biopesticide with 25% Bionutrient S-367B in the Fourth Week, (b) Pest Attacks on the Solvent Control Treatment and No Treatment in the Fourth Week, (c)

Leaves Without Holes in the Composite Treatment and 25% Bionutrient S-367B in the Fifth Week, (d) Leaves with Holes in the Solvent Control Treatment and No Treatment in the Fifth Week.

Figure 17 shows the presence of leaf caterpillar pests (*Plutella Xylostella*) on the underside of broccoli plants in the treatment group of basil leaf extract composite with 25% S-367B bionutrient, solvent control, and without treatment in the fourth week because they had not sprayed the underside of the leaves. After spraying the underside of the leaves, it was seen in the fifth week that the broccoli plants in the treatment group of basil leaf extract composite with 25% S-367B bionutrient had no more leaf caterpillars (*Plutella Xylostella*) and there were no holes in the leaves, while in the solvent control treatment group and without treatment, there were many holes in the leaves of broccoli plants. Therefore, it can be concluded that the treatment group of basil leaf extract composite with S-367B bionutrient on broccoli plants showed the ability as an effective biopesticide to reduce leaf damage in broccoli plants caused by leaf caterpillars (*Plutella Xylostella*).

This is in line with the results of phytochemical tests, total phenolic content with UV-Vis spectrophotometer, and functional group characterization with FTIR spectrophotometry. The test results showed that there were active compounds of alkaloids, flavonoids, saponins, and tannins in basil leaves. Research by Sanjaya et al., (2022) supports these test results, because the results of Sanjaya et al., (2022) showed that basil leaf extract contains active compounds of alkaloids, flavonoids, saponins, tannins, glycosides, and steroids or triterpenoids which act as antibacterials, antifungals, insecticides, antimicrobials, and antioxidants. This proves that basil leaf extract has great potential as an environmentally friendly biopesticide for plant protection against insect pests, one of which is the leaf caterpillar pest (*Plutella Xylostella*).

In addition to being attacked by leaf caterpillar pests (*Plutella Xylostella*), broccoli plants in

the solvent control and untreated treatment groups were also attacked by leaf spot disease, as shown in Figure 18.

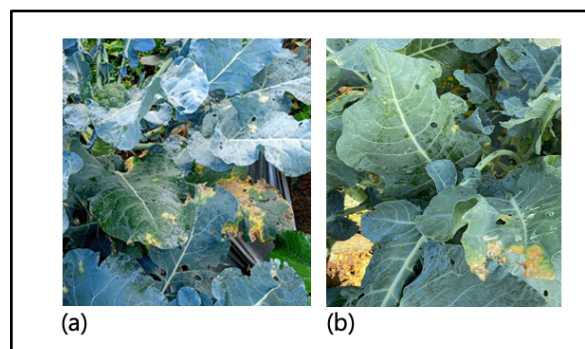


Figure 18. Broccoli plants are affected by leaf spot disease

(a) Leaf Spot Disease Attack in Solvent Control Treatment, (b) Leaf Spot Disease Attack on Untreated Plants.

Figure 18 shows the presence of leaf spot disease in broccoli plants treated with solvent control and no treatment, while broccoli plants treated with the composite biopesticide basil leaf extract with bionutrient S-367B were not affected by leaf spot disease. Leaf spot disease in plants is an important disease of many plant species and has a wide distribution area and continues to grow. The causative agent of leaf spot disease in broccoli plants is *Alternaria brassicae* (Lippens et al., 2022; Nagi et al., 2024). The fungus *Alternaria brassicae* causes the appearance of dark brown to blackish necrotic spots that form circles and can merge, damaging the leaf surface area. This fungus spreads through spores originating from plant debris, infected seeds, rain splashes, and wind. Spores land on damp leaves, then germinate, penetrate through the stomata, then release cell wall-degrading enzymes and toxins that kill leaf cells, forming necrotic spots. The significance of leaf spot disease in plants lies in the fact that severe leaf infections can reduce plant vitality, make the crop unmarketable, cause cankers on the stems, and premature defoliation, which can lead to total plant destruction. Therefore, it is crucial to find environmentally friendly alternatives for fungal control.

Based on the results of this study, it was found that broccoli plants treated with a composite

biopesticide of basil leaf extract with bionutrient S-367B could prevent leaf spot disease as evidenced by the absence of spots on broccoli plant leaves. Therefore, it can be said that the composite biopesticide of basil leaf extract with bionutrient S-367B has great potential as an environmentally friendly biopesticide for plant protection against leaf spot disease in broccoli plants.

The visual observations presented in Figures 16–18 provide qualitative support for the effectiveness of the composite treatment. Broccoli plants treated with the basil leaf extract–Bionutrient S-367B composite exhibited healthier leaf morphology, reduced pest damage from *Plutella xylostella*, and absence of leaf spot symptoms compared to control groups. These findings indicate that, although some quantitative parameters were not statistically significant, the composite

treatment contributed to improved plant health and resistance against biotic stress.

3.12. The Effect of Basil Leaf Extract Biopesticide Composite with Bionutrient S-367B on Broccoli Plant Harvest Yield

In this study, the largest harvest mass was obtained in the composite treatment group of basil leaf extract biopesticide with 50% S-367B bionutrient at a dose of 7.5 mL/L, obtaining a broccoli harvest mass of 921.33 ± 64.89 . The average harvest mass at the three doses of basil leaf extract biopesticide composite treatment with S-367B bionutrient at concentrations of 25%, 50%, and 75% was greater than the solvent control treatment group.

Table 5. Broccoli Harvest Mass

Broccoli Plant Treatment	Dose (mL/L)	Mass of Harvested Plants (grams)			Average Mass of Broccoli \pm Standard Deviation (grams)
		1	2	3	
Composite 25%	5	458	450	422	443.33 ± 64.89
	7.5	609	595	590	598.00 ± 8.04
	10	581	580	579	580.00 ± 0.82
Control 25%	5	298	274	237	269.67 ± 25.09
	7.5	586	443	523	517.33 ± 58.52
	10	394	393	325	370.67 ± 32.29
Composite 50%	5	684	657	656	665.67 ± 12.97
	7.5	1009	901	854	921.33 ± 64.89
	10	901	819	747	822.33 ± 62.91
50% Control	5	395	399	375	389.67 ± 10.50
	7.5	500	411	380	430.33 ± 50.86
	10	629	500	416	515.00 ± 87.60
Composite 75%	5	690	689	686	688.33 ± 1.70
	7.5	819	793	747	786.33 ± 29.77
	10	649	647	646	647.33 ± 1.25
75% Control	5	437	422	352	403.67 ± 37.04
	7.5	481	455	460	465.33 ± 11.26
	10	460	428	490	459.33 ± 25.32

3.13. Discussion

This study primarily focuses on the chemical characterization of bioactive compounds and their functional implications, with agronomic observations serving as biological validation rather than the primary research focus. Within this framework, the integration of chemical characterization and field-based observations

provides a comprehensive approach to understanding the effectiveness of basil leaf extract–Bionutrient S-367B composites in a biologically relevant system.

The findings demonstrate that basil (*Ocimum basilicum* L.) leaf extract contains diverse bioactive secondary metabolites, including

alkaloids, flavonoids, saponins, and tannins, which are consistent with previous phytochemical reports on *Ocimum* species (Bernhardt et al., 2015; Diba et al., 2022). Furthermore, the relatively high total phenolic content observed in this study highlights the central role of phenolic compounds as key contributors to the biological activity of the extract.

From a chemical perspective, phenolic compounds are widely recognized for their multifunctional bioactivity, including their ability to disrupt insect nervous systems, inhibit microbial enzymatic processes, and induce oxidative stress in pathogenic organisms. These properties provide a strong mechanistic basis for the observed biopesticidal effects in treated broccoli plants (Bahri et al., 2025). This biochemical evidence is further supported by FTIR analysis, which revealed characteristic functional groups associated with phenolic acids, flavonoids, and terpenoid derivatives—classes of compounds frequently reported as key pesticidal and antimicrobial agents in plant-based biopesticides (Sasidharan et al., 2011).

At the molecular level, the antifungal activity of basil (*Ocimum basilicum* L.) extract can be attributed primarily to its phenolic compounds, which interact directly with the fungal cell membrane of *Alternaria brassicae*. Phenolic compounds are known to disrupt membrane integrity by interacting with lipid bilayers, particularly by altering the structure and fluidity of membrane phospholipids. This interaction can lead to increased membrane permeability, resulting in leakage of essential intracellular components such as ions, proteins, and metabolites. In addition, phenolic compounds may interfere with ergosterol, a key sterol component of fungal cell membranes that is essential for maintaining membrane stability and function. Disruption of ergosterol biosynthesis or structure can weaken the membrane and impair fungal growth. Furthermore, phenolics can induce oxidative stress within fungal cells by generating reactive oxygen species (ROS), which damage cellular macromolecules including proteins, lipids, and nucleic acids.

These combined mechanisms ultimately lead to inhibition of fungal growth and development, which explains the absence of leaf spot symptoms observed in broccoli plants treated with the basil extract–Bionutrient S-367B composite. This mechanistic insight supports the role of phenolic-rich plant extracts as effective and environmentally friendly antifungal agents.

Building upon these phytochemical findings, the application of basil extract composited with Bionutrient S-367B translated into measurable agronomic improvements. The composite treatment enhanced vegetative growth parameters, particularly plant height, leaf length, and leaf width, with the most pronounced effects observed at the 50% composite concentration and 7.5 mL/L dosage. This synergistic response aligns with earlier findings that plant-derived biopesticides can promote crop growth indirectly by reducing biotic stress while maintaining physiological balance (Azwanida, 2015). At the same time, the contribution of Bionutrient S-367B as a nutrient supplement likely improved nutrient uptake efficiency and photosynthetic capacity, as reported in previous studies on bionutrient-assisted crop growth (Dharani et al., 2023; Monib et al., 2023; Panchal & Maitreya, 2023). Similar synergistic effects have been documented when botanical extracts were combined with organic or bio-based fertilizers, resulting in enhanced plant vigor and biomass accumulation (Dvořáčková et al., 2022).

Notably, the treatment with a 50% composite concentration at a dosage of 7.5 mL/L consistently exhibited the highest values across multiple growth parameters, including plant height, leaf dimensions, growth rate constant, and harvested biomass. This treatment can therefore be considered the optimal condition within the experimental range. The improved performance at this concentration suggests a balanced interaction between bioactive compounds in basil extract and nutrient availability from Bionutrient S-367B, which enhances plant physiological processes while minimizing potential phytotoxic effects at higher concentrations.

The physiological implications of this synergy were further reflected in the increased growth rate constants and harvested biomass. These results indicate that the composite treatment not only protected plants from pest and disease pressure but also optimized internal growth processes. Reduced pest damage, particularly from leaf-feeding insects, preserves leaf integrity and chlorophyll content, thereby enhancing photosynthetic efficiency and assimilate translocation to edible plant parts (Klatt et al., 2023; Korzin et al., 2022). Comparable outcomes have been reported in broccoli cultivation studies, where eco-friendly pest management strategies significantly increased marketable yield compared to conventional chemical controls (Lippens et al., 2022; Nagi et al., 2024; Barad et al., 2024). Interestingly, the superior performance observed at intermediate composite concentrations suggests that excessively high extract levels may induce mild phytotoxic effects—a phenomenon also noted in other botanical pesticide studies (Humane et al., 2023; Rys et al., 2022)

Taken together, these findings contribute to the growing body of evidence supporting integrated biopesticide–bionutrient systems as sustainable alternatives to synthetic agrochemicals. Unlike conventional pesticides, botanical extracts derived from *Ocimum basilicum* are biodegradable, environmentally benign, and less likely to induce pest resistance (Jote, 2023). When combined with bionutrients, such systems simultaneously address crop protection and nutritional adequacy—an integrated approach that is rarely achieved through single-input strategies. By demonstrating the effectiveness of basil leaf extract–Bionutrient S-367B composites in enhancing broccoli showed a positive growth trend, although the differences were not statistically significant ($P > 0.05$), this study extends previous research on plant-based composite applications and offers a promising strategy for sustainable horticultural production, particularly under tropical and subtropical conditions.

From a theoretical standpoint, this research enriches the development of applied

chemistry and sustainable agriculture by strengthening the concept of synergy between bioactive plant compounds and bionutrients in plant protection systems. The identification of secondary metabolites and functional groups in basil leaf extracts reinforces the theoretical understanding that phenolics, flavonoids, and terpenoids not only function as biopesticidal agents but also indirectly enhance plant physiology by mitigating biotic stress. Furthermore, the results expand the theoretical framework of integrated plant protection and nutrition, which has often been studied separately, by demonstrating that combining biopesticides and bionutrients yields more optimal growth responses and productivity than single-input applications.

From a practical perspective, the study provides an environmentally friendly technological alternative that can be readily adopted by horticultural farmers, particularly in broccoli cultivation. The basil leaf extract–Bionutrient S-367B composite was shown to improve vegetative growth and yield while simultaneously reducing pest and disease damage. This natural-based formulation is relatively easy to apply, utilizes locally available resources, and has strong potential to reduce dependence on synthetic chemical pesticides. Consequently, the findings serve as a valuable reference for developing safer and more sustainable agricultural practices that benefit the environment, farmers, and consumers alike.

Finally, at the policy level, these findings offer a scientific foundation for promoting the adoption of bio-based agricultural inputs within sustainable agricultural development programs. Policymakers can use this evidence to formulate supportive regulations, incentives, and farmer mentoring initiatives related to environmentally friendly biopesticides and bionutrients. Integrating biopesticide–bionutrient technologies into agricultural policy frameworks can contribute to reducing pesticide residues, enhancing food safety, and protecting environmental health. With appropriate institutional support, this innovation holds strong potential for

large-scale development as part of a national strategy toward a competitive, resilient, and sustainable agricultural system.

4. Conclusion

This study demonstrates the value of integrating chemical characterization and biological evaluation to investigate the potential of plant-based biopesticide systems. Phytochemical screening, total phenolic content analysis, and FTIR characterization confirmed the presence of bioactive compounds in basil (*Ocimum basilicum* L.) leaf extract, providing a chemical basis for its application in sustainable crop management. Although no statistically significant differences were observed among treatments, the composite formulation of basil extract and Bionutrient S-367B showed favorable trends in broccoli growth and biomass production, with the 50% concentration at 7.5 mL/L exhibiting the most promising response. These findings suggest the potential of combining plant-derived biopesticides with nutrient supplementation as an environmentally friendly alternative to conventional agricultural inputs.

This study is limited by its implementation within a single cultivation cycle and specific agroecological conditions, which may restrict the broader applicability of the findings. Furthermore, the evaluation focused primarily on plant growth responses, while quantitative assessments of pest suppression and detailed identification of active compounds were not conducted. Future studies should incorporate multi-location trials, comprehensive pest population analyses, and advanced analytical techniques such as GC–MS or LC–MS to identify bioactive constituents and clarify their mechanisms of action. Such investigations would strengthen the scientific basis and practical application of plant-based biopesticide systems in sustainable agriculture.

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