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Assay of IAA Activity in Biofertilizer Formulations with Vermicompost Tea Adition on Maize (*Zea mays* L.) Growth

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Abstract

A biofertilizer formulated by PT Great Giant Pineapple and enriched with vermicompost tea has the potential to produce the plant growth hormone *indole-3-acetic acid* (IAA), which is essential for supporting plant growth and development. This study evaluated changes in IAA activity in biofertilizer formulations during a four-week storage period and examined their effects on the vegetative growth of maize (*Zea mays* L.). The experiment was conducted using a completely randomized design (CRD) with three treatments consisting of vermicompost tea to water ratios of 1:3, 1:5, and 1:10, each aerated for 96 hours. IAA activity was measured using UV–Vis spectrophotometry based on absorbance values and quantified using a standard curve. Plant growth data were analyzed using analysis of variance (ANOVA), followed by the Honest Significant Difference (HSD) test at a 5% significance level. Observed growth parameters included plant height, number of leaves, stem diameter, fresh weight, and dry weight, with five replications per treatment. The results showed that IAA activity in vermicompost tea-based biofertilizers increased progressively during storage. In addition, biofertilizer application significantly enhanced maize vegetative growth, with the 1:10 formulation producing the most optimal results by promoting higher IAA activity, leaf development, and biomass accumulation in both fresh and dry weights.

Keywords: Maize; Vermicompost tea; *Indole Acetic Acid* (IAA); Biofertilizer; Vegetative phase

1. Introduction

Maize (*Zea mays* L.) is a strategic food crop with high economic value in Indonesia. Data from the Indonesian Central Bureau of Statistics indicate that national maize production increased from 19.61 million tons in 2017 to 23.00 million tons in 2021 [1], highlighting the importance of sustainably enhancing maize productivity to support national food security. Crop productivity is strongly influenced by sustainable soil fertility management, defined as the soil's capacity to supply essential nutrients that support plant growth [2]. In this context, biofertilizers serve as environmentally friendly alternatives, as they enhance nutrient availability through the activity of soil microorganisms, including nitrogen fixation and the production of plant growth–promoting compounds [3].

PT Great Giant Pineapple has undertaken efforts to improve the quality of Liquid Organic Biofertilizer (LOB) by incorporating vermicompost tea as a source of organic matter and functional microorganisms. Vermicompost tea is known to contain nutrients, beneficial microbes, and bioactive compounds that promote plant growth and potentially enhance resistance to biotic stress [4]. Previous studies have reported that the application of vermicompost and vermicompost tea, either individually or in combination, can improve maize growth, enhance soil nutrient status, and suppress pest infestation through the improvement of plant physiological parameters, thereby offering a sustainable alternative to synthetic inputs in organic farming systems [5].

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Nevertheless, previous studies have primarily focused on agronomic responses, physiological traits, and pest management, with limited attention given to the role of vermicompost tea in enhancing plant hormone activity. Vermicompost tea contains microorganisms capable of producing *Indole-3-Acetic Acid* (IAA), such as *Bacillus* spp. and *Pseudomonas* spp., as well as organic compounds that serve as precursors for IAA biosynthesis, which play a crucial role in stimulating root development and supporting vegetative growth [6]. Therefore, this study aims to evaluate IAA activity in biofertilizer formulations enriched with vermicompost tea and to examine its relationship with the vegetative growth of maize.

2. Material and methods Heading

2.1. Sampling

The vermicompost used in this study was obtained and facilitated by PT Great Giant Pineapple, processed into vermicompost tea, and subsequently formulated with Liquid Organic Biofertilizer (LOB) produced by PT Great Giant Pineapple according to the designated treatments.

2.2. Vermicompost Tea Production

Vermicompost tea (VCT) was prepared by mixing solid vermicompost with water at different ratios, namely 1:3 (P1), 1:5 (P2), and 1:10 (P3). For the P1 treatment, 1,240 g of vermicompost was combined with 3,720 mL of water; for P2, 828 g of vermicompost was mixed with 4,132 mL of water; and for P3, 451 g of vermicompost was mixed with 4,509 mL of water. Each mixture was subjected to continuous aeration for 96 h to produce vermicompost tea. The resulting vermicompost tea was then formulated with a liquid organic biofertilizer (LOB) at a proportion of 80% VCT and 20% LOB. For each treatment, 4,960 mL of vermicompost tea was mixed with 1,240 mL of LOB, resulting in a total formulation volume of 6,200 mL.

2.3. Preparation of Salkowski Reagent

The Salkowski reagent was prepared according to method [7] with minor modifications to a final volume of 80 mL. The reagent consisted of 30 mL of concentrated H_2SO_4, 50 mL of distilled water, and 1 mL of 0.5 M $FeCl_3{\cdot}6H_2O$. Concentrated sulfuric acid was slowly added to distilled water, followed by the addition of $FeCl_3{\cdot}6H_2O$ with continuous stirring until a homogeneous solution was obtained. The reagent was stored in a dark bottle and used to detect IAA production based on color development.

2.4. *Indole-3-Acetic Acid* (IAA) Activity Assay

IAA activity was determined following method [8]. A 1.5 mL vermicompost tea sample was centrifuged for 5 min, and the supernatant was reacted with 4 mL of Salkowski reagent. The mixture was homogenized and incubated in the dark for 15 min, and absorbance was measured at 530 nm using a UV–Vis spectrophotometer. IAA activity was monitored weekly for four weeks.

2.5. Observation of maize (*Zea mays* L.) plants

2.5.1. Maize planting

Soil was evenly placed into polybags measuring 45 × 45 cm. Seeds previously soaked for 30 minutes were planted at a depth of 3 cm and covered with soil until completely buried [9].

2.5.2. Maize maintenance

Plant maintenance followed method [9], including daily watering in the morning, replanting two weeks after planting if plants failed to grow or died, and manual weed control.

2.5.3. Application of formulations

The formulations were applied once every 7 days for a total of 28 days during the maintenance period, with a volume of 60 mL per polybag [10].

2.5.4. Observation of maize growth (Zea mays L.)

The growth parameters of maize (*Zea mays* L.) included plant height, number of leaves, stem diameter, root length, fresh weight, and dry weight. Plant height was measured from the soil surface to the shoot tip, stem diameter at the basal stem above the soil surface, and root length from the base to the tip of the longest root. Fresh weight was measured using a digital balance, while dry weight was determined after oven-drying at 50 °C until constant weight. Measurements were expressed in centimeters (cm) and grams (g) [12].

2.6. Data Analysis

2.6.1. IAA Activity Data Analysis

Qualitatively detected IAA production was further analyzed quantitatively using spectrophotometric analysis at a wavelength of 530 nm to determine IAA activity in each formulation. Absorbance values were calculated based on an IAA standard calibration curve.

2.6.2. Plant Growth Data Analysis

Maize growth data were analyzed using analysis of variance (ANOVA) to determine differences among treatments. Prior to analysis, data were tested for normality and homogeneity. When significant differences were detected by ANOVA, further analysis was conducted using the Honest Significant Difference (HSD) test at a 5% significance level.

3. Results and Discussion

3.1. *Indole Acetic Acid* (IAA) Test

The IAA standard solution was prepared at concentrations ranging from 0 to 80 ppm, producing absorbance values between 0 and 0.734. The results demonstrated a clear positive linear correlation between IAA concentration and absorbance, consistent with the principles of colorimetric analysis [13]. This relationship is illustrated in Figure 1, which presents the IAA standard curve constructed by plotting absorbance on the y-axis against concentration on the x-axis. The linear regression equation obtained was $y = 0.032x - 0.057$, with a coefficient of determination (R^2 = 0.997), indicating high accuracy and reliability of the model. This equation was subsequently applied to quantify IAA concentrations in the biofertilizer samples. (Figure 1).

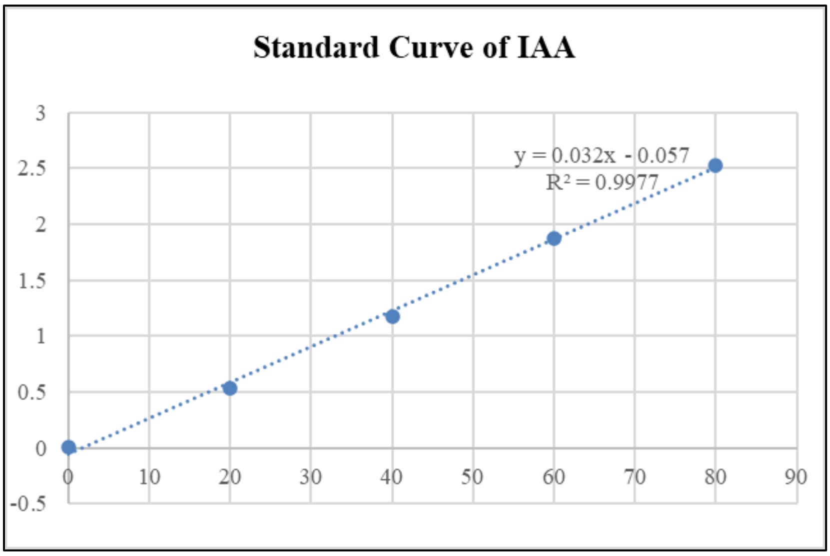

Figure 1 *Indole Acetic Acid* (IAA) Standard Curve

The results presented in (Figure 2) indicate that storage duration had a significant effect ($p < 0.05$) on IAA production across the formulations, with IAA levels generally increasing throughout the fermentation period.

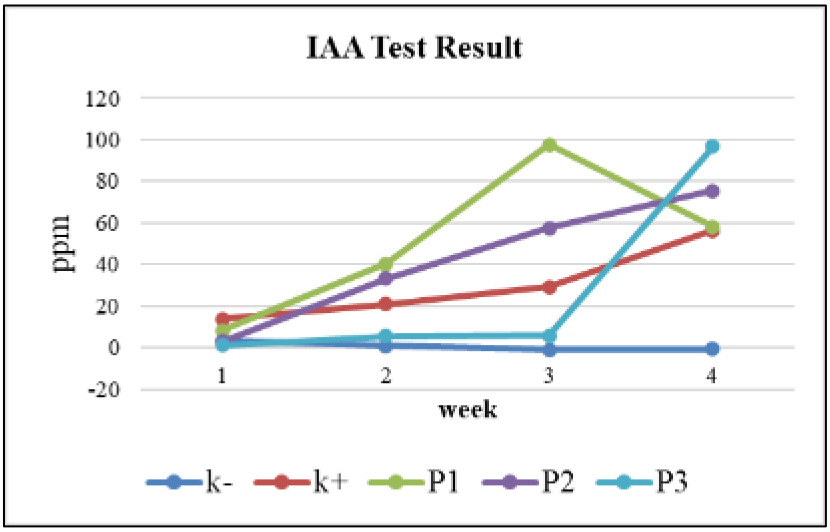

Figure 2 *Indole Acetic Acid* (IAA) Activity Test Data

Treatment P1 exhibited a rapid increase in IAA production up to the third week, followed by a decline in the subsequent week, suggesting that bacterial populations entered the stationary phase earlier and experienced nutrient limitation. Treatment P2 showed a more stable and consistent increase in IAA production, reflecting balanced fermentation conditions that supported sustained bacterial activity. In contrast, treatment P3 displayed a slower increase during the early fermentation stages but a sharp rise in the final week, indicating that the bacterial stationary phase was reached toward the end of the observation period. Overall, these results suggest that storage duration and formulation composition influenced the dynamics of IAA biosynthesis. Maximum IAA production is generally associated with the bacterial stationary phase and is regulated by environmental factors such as pH, nitrogen availability, aerobic conditions, and incubation time [14].

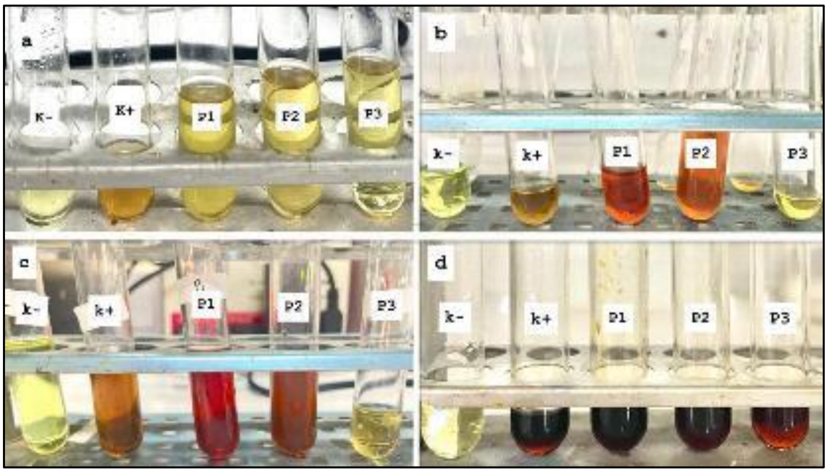

Figure 3 Final Color Change of the Formulation After Administration of Salkowski Reagent (a) First Week (b) Second Week (c) Third Week (d) Fourth Week

(Figure 3) shows the Salkowski color changes during four weeks of fermentation. In week 1, all treatments were pale yellow, indicating low IAA. By week 2, P1 and P2 turned orange–red, reflecting increased IAA, while the negative control remained pale. Week 3 showed stronger red intensity in P1 and P2. In week 4, the darkest red appeared in P2 and P3, indicating the highest IAA levels, whereas P1 decreased in intensity. The progressive color change from yellow to pink and dark red reflects increasing IAA production during fermentation, with color intensity directly proportional to IAA concentration [15]. The Salkowski method enables qualitative detection of IAA production based on color development and is commonly used to assess the plant growth–promoting potential of microorganisms [16].

3.2. Maize Growth Results

3.2.1. Plant height of maize (Zea mays L.*)*

The HSD test at 5% showed no significant differences in maize height among treatments during the first week. Differences began to appear in week two, with P2 and P3 exhibiting significantly higher plant height compared to other treatments. In week three, P2 showed greater height, and by week four, P2 had the tallest plants, significantly different from the negative control, while K+, P1, and P3 did not differ significantly. The superior performance of P2 is attributed to the role of the auxin hormone *Indole-3-Acetic Acid* (IAA), which promotes cell elongation and expansion in stem tissues, directly contributing to plant height increase [17]. IAA enhances cell wall plasticity, enabling optimal cell elongation [18], and in addition to endogenous production by plants, it is also synthesized by plant growth–promoting microorganisms, stimulating vegetative growth through stem elongation and enhanced physiological activity [19] (Figure 4).

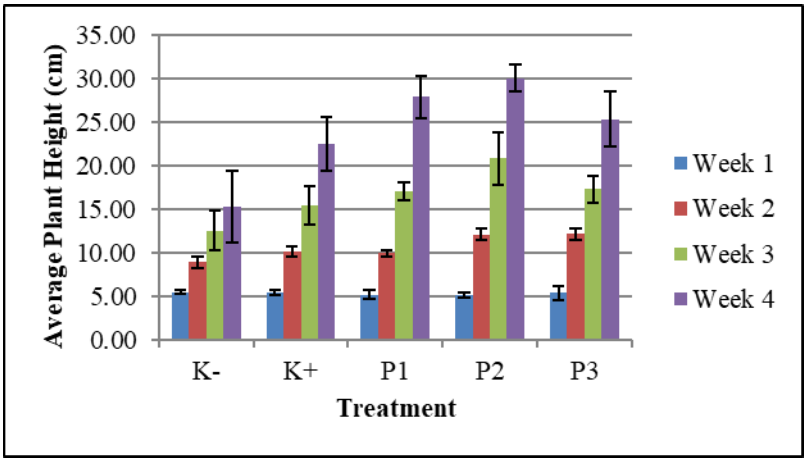

Figure 4 Growth Chart of Maize (*Zea mays* L.) Plant Height Over Four Weeks

3.2.2. Leaf Number of Maize (Zea mays L.*)*

The number of leaves showed no significant differences among treatments from week 1 to week 3, indicating that the application of LOB and vermicompost tea had not yet affected early vegetative growth. Significant differences appeared in week 4, with P3 exhibiting the highest leaf number, while K+, P1, and P2 did not differ significantly. The superior performance of P3 is attributed to the role of the auxin hormone *Indole-3-Acetic Acid* (IAA), which regulates cell division and differentiation in shoot apical meristems, promoting the formation of new leaves [20]. IAA also stimulates leaf primordia initiation, contributing to increased leaf number during the vegetative phase [21]. In addition to endogenous production by the plant, IAA produced by plant growth–promoting microorganisms enhances vegetative growth and provides a more optimal supply of growth hormones, further supporting leaf development [18] (Figure 5).

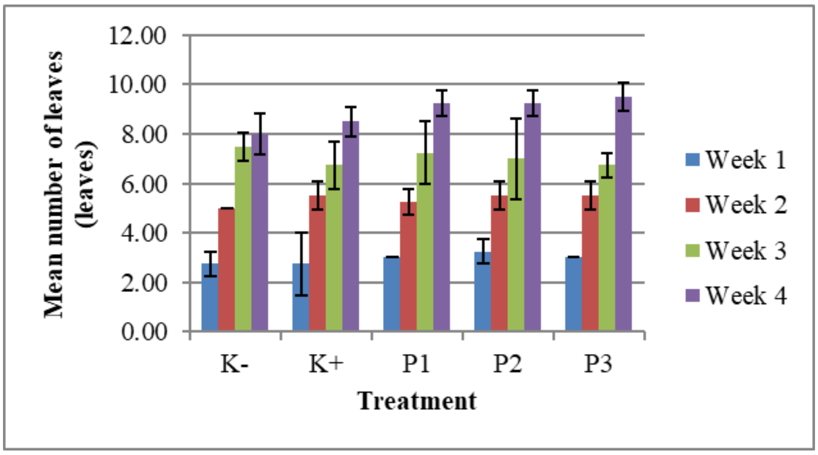

Figure 5 Leaf Number of Maize (*Zea mays* L.) Over Four Weeks

3.2.3. Stem Diameter of Maize (Zea mays L.*)*

During the first week, no significant differences in stem diameter were observed among treatments. In weeks 2 and 3, P1 exhibited significantly greater stem diameter compared to the control treatments. By week 4, P1 maintained the highest diameter, significantly different from K− and K+, demonstrating consistent positive effects throughout the observation period. This increase is associated with the role of *Indole-3-Acetic Acid* (IAA) in regulating cambial activity, which promotes the formation of secondary vascular tissues [22]. Higher IAA levels stimulate cambial cell division, resulting in stem thickening during the vegetative phase [23]. Additionally, IAA produced by plant growth–promoting rhizobacteria enhances growth hormone availability, supporting stem development and improving nutrient absorption efficiency, thereby contributing to increased stem diameter [24] (Figure 6).

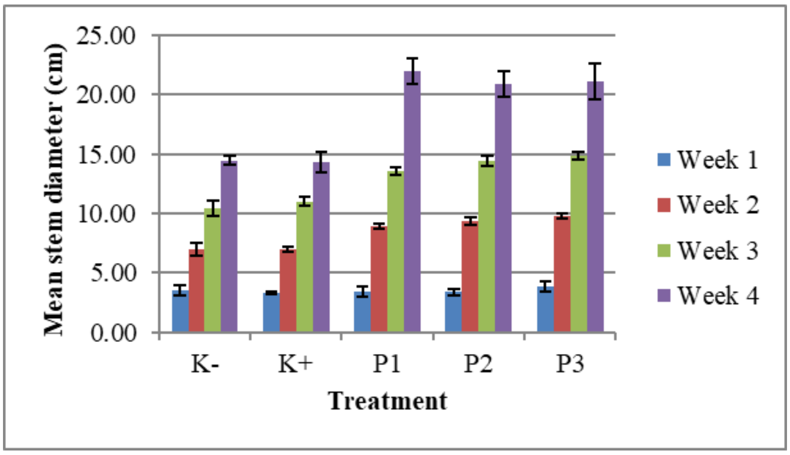

Figure 6 Stem Diameter of Maize (*Zea mays* L.) Over Four Weeks

3.2.4. Root Length of Maize (Zea mays L.)

At the 5% significance level, treatments P1 and P2 showed superior effects on root growth parameters compared with the other treatments. Auxin is a crucial regulator of maize root development, primarily by controlling cell division and cell elongation within root meristematic tissues [25]. This hormone promotes root initiation and elongation through the regulation of genes associated with cell wall modification and tissue differentiation, thereby facilitating the formation of a more efficient root system for water and nutrient absorption [26]. Elevated auxin activity enhances lateral root development and primary root elongation, which contributes to improved nutrient uptake during the early vegetative stage [27]. However, root growth responses to auxin are strongly concentration-dependent; low auxin concentrations generally exert optimal stimulatory effects by promoting root elongation and lateral root formation without disturbing hormonal equilibrium, whereas excessive IAA levels may suppress root growth by causing hormonal imbalance, restricting cell elongation, and inducing physiological stress in root tissues [28] (Figure 7).

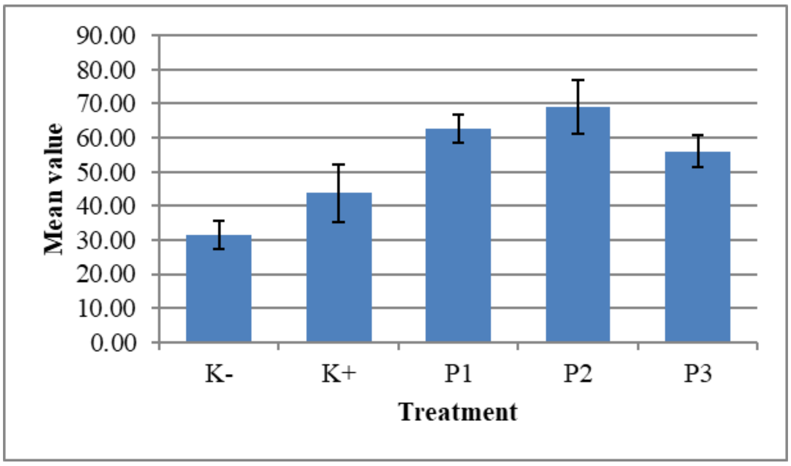

Figure 7 *Root Length of* Maize (*Zea mays* L.) Over Four Weeks

3.2.5. Fresh Weight of Maize (Zea mays L.)

The results of the study showed that the best formulation for maize plants was treatment P3, which is consistent with the findings of [29], reporting that the application of *indole-3-acetic acid* (IAA) significantly increased the fresh weight of maize plants through its role as an auxin hormone that stimulates cell division and cell elongation. Foliar application of IAA promoted tissue expansion in both shoots and roots, leading to greater biomass accumulation, as indicated by higher fresh weight values compared to untreated control plants. Maize plants treated with IAA, either alone or in combination with boron, exhibited more intensive vegetative growth due to enhanced physiological processes, including increased water uptake, cell enlargement, and the formation of new tissues. The increase in fresh weight indicates that IAA plays an important role in accelerating plant tissue growth and enhancing the plant's capacity to store water and photosynthetic assimilates during the vegetative phase (Figure 8).

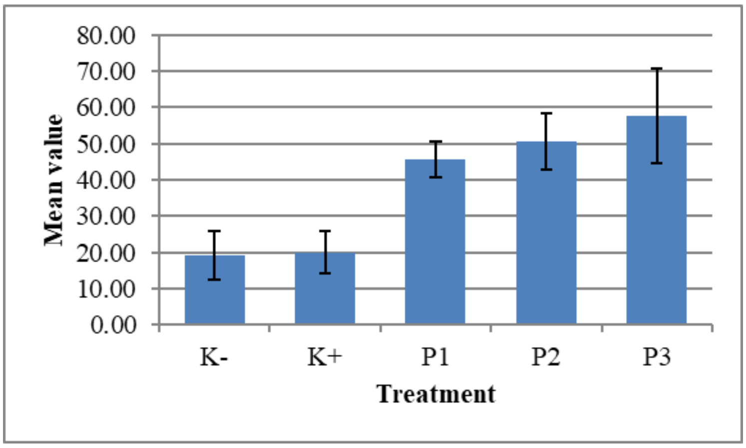

Figure 8 *Fresh Weight* of Maize (*Zea mays* L.) Over Four Weeks

3.2.6. Dry Weight of Maize (Zea mays L.)

The best formulation for dry weight was observed in treatment P3, which is consistent with the findings of [29], reporting that *indole-3-acetic acid* (IAA) contributes to increased dry weight of maize plants through the stimulation of growth and enhanced plant vigor compared to control treatments. Foliar application of IAA significantly increased dry weight in both shoots and roots of maize plants, along with improvements in other growth-related parameters. The increase in dry weight indicates that IAA promotes greater biomass accumulation through enhanced physiological processes, including nutrient assimilation, tissue development, and improved photosynthetic efficiency, which collectively contribute to dry matter production beyond water content alone. These findings confirm the important role of IAA in supporting biomass accumulation and vegetative growth in maize (Figure 9).

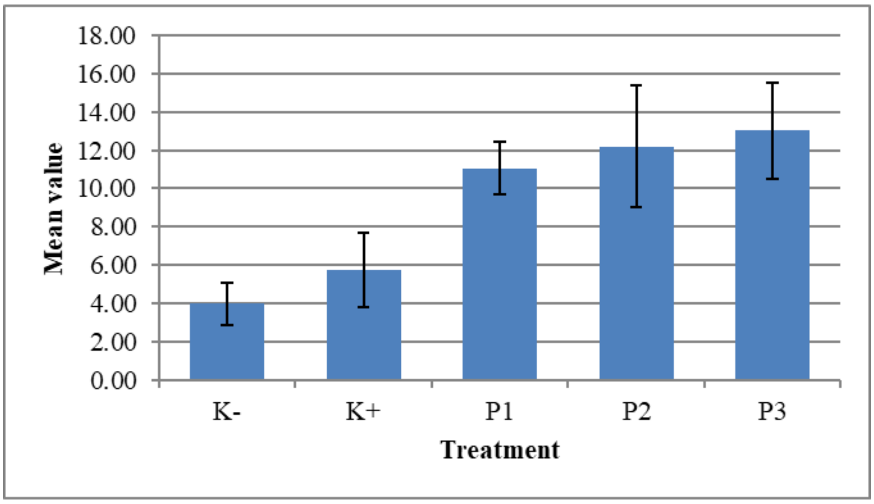

Figure 9 *Dry Weight* of Maize (*Zea mays* L.) Over Four Weeks

4. Conclusion

The addition of vermicompost tea increased IAA activity during storage and enhanced maize vegetative growth. Among the tested formulations, the 1:10 formulations showed the highest effectiveness by consistently increasing IAA levels and significantly improving leaf development as well as fresh and dry biomass accumulation. This formulation demonstrated the greatest potential for supporting IAA production and promoting vegetative growth in maize.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflict of interest.

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