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The effect of humic acid and inorganic fertilizer application on the growth and yield of *Ipomoea reptans* Poir. (kangkong)

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Abstract

Humic acid (HA), a plant growth stimulator, has not yet established its credibility despite being utilized for several decades in agriculture. Therefore, this study determined the effect of HA and its combined effect with inorganic fertilizer on the growth and yield of *I. reptans*. In each of the two sites, four plots replicated thrice were constructed with three treatment groups - T0B (HA only), T1 (inorganic fertilizer only), and T2 (HA + inorganic fertilizer) - and one control group (T0A, soil only). The plant height and stem diameter of ten randomly chosen plants were measured every 7 days for 21 days while the crop yield was determined 35 days after sowing. From the analysis, significant differences were found in only one of the two sites. Results showed that the treatment with HA only was significantly higher in plant height and crop yield. Data from this study contributes to the current knowledge of crop production for local farmers and the agricultural community.

Introduction. - Agriculture is an essential part of the Philippine economy as it involves 22.9% of Filipino workers and contributes about 9.2% of the gross domestic product as of 2019. However, there is a decrease in crop production of 0.7% in 2018 and 1.0% in 2019 compared to the years preceding them [1]. To address this, researchers have studied the many factors affecting plant growth and productivity, mainly the effects of nutrient composition [2]. Different fertilizers with varying concentrations of nutrients, mainly nitrogen, phosphorus, and potassium, supply plants with the nutrients necessary for their growth. In addition, the increasing amounts of fertilizer increase plant growth, and there are optimal concentrations of these nutrients for optimizing the growth of certain plants [3]. Aside from these nutrients, plant growth simulators such as humic substances have been studied on their positive effects on plant growth and yield.

Humic substances (HSs) are the brown to black, fully decomposed remains of plant or animal organic matter that compose about 80% of organic matter in dark soils [4]. These substances arise from the physical, chemical, and microbiological transformation of biomolecules, and can be divided into three components: fulvic acids (FAs), humic acids (HAs), and humin [5]. Due to their molecular structure, HSs provide numerous benefits to crop production as they help break up clay and compacted soils, assist in transferring micronutrients from the soil to the plant, enhance water retention, increase seed germination rates and penetration, and stimulate the development of microflora populations in soils [6].

Humic substances have been established to also facilitate plant growth and yield as they increase nutrient availability and improve the physical structure of the soil [7, 8]. These substances also have a role in plant metabolism as they contain growth-triggering hormones such as auxin and gibberellins [9]. These hormones promote cell elongation which mainly affects plant height and the development of plant roots [10]. Furthermore, HA has been shown to increase macronutrient and micronutrient uptake of plants [11].

In the Philippines, one of the commonly-used agricultural plants is upland kangkong, also known as *Ipomoea reptans*, which is one of the most cultivated leafy vegetables in Southeast Asia [12]. Although being primarily used for human consumption, *I. reptans* is also considered a common medicinal plant in some Southeast Asian countries, having purgative, anti-inflammatory, hypolipidemic, antidiabetic, diuretic, antiepileptic, and antimicrobial properties [13]. However, in the Philippines, the growth and yield potential of *I. reptans* has not yet been fully exploited due to the inadequate use of inputs and lack of information on its production [14], which includes the utilization of humic substances among others. Given these conditions, it is necessary to utilize HA in fertilizers and determine whether it improves the growth of *I. reptans*. It is hypothesized that combining HA with fertilizers will improve *I. reptans* growth and yield as HA increases nutrient uptake and photosynthesis and respiration rates of plants [8].

This study focused on the effect of HA and its combined effect with inorganic fertilizer on the

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growth and yield of *I. reptans*. Data from this research can be used to establish the credibility of HA utilization in agriculture, and contribute to the knowledge of the production of *I. reptans* species for local farmers.

This research aims to determine the effect of HA and additionally its combined effect with inorganic fertilizer on the growth and yield of *I. reptans*. Specifically, it aimed to:

(i) measure the plant height (cm), stem diameter (cm), and yield (kg/ha) of *I. reptans*. treated without HA (control; T0A), with HA alone (T0B), without HA but with inorganic fertilizer (T1), and treated with combined HA and inorganic fertilizer (T2);

(ii) determine whether using HA improves growth and yield of *I. reptans* by comparing the means of each parameter per treatment group using One-Way ANOVA;

(iii) determine which among the treatment groups is best used for *I. reptans*. production.

Methods. - The methodology is divided into six (6) parts: plot preparation and seed sowing, randomization, watering and monitoring, treatment application, measuring of parameters, and statistical analysis.

Research sites. The two (2) set-ups were done in Barangay Buray, Oton, Iloilo (10° 42' 12" N 122° 28' 6" E) which has soil classified in the Santa Rita soil series [15] and Barangay Balabag, Malay, Aklan (11° 58' 0" N 121° 55' 39" E) using the same soil series. The locations were chosen due to their proximity to the residences of the researchers for easy monitoring. The data was gathered for 35 days. The research conducted in Oton lasted from January 12, 2021, to February 16, 2021, while the one conducted in Malay lasted from January 19, 2021, to February 25, 2021.

Plot Preparation and Seed Sowing. A plot had dimensions of 30 cm by 40 cm. Before sowing the seeds, the soil was tilled up to 15 cm deep while it was moist. Six (6) planting holes were placed in each plot with three (3) seeds in each planting hole, 1-centimeter deep into the soil [12], which amounted to a total of 18 seeds per unit plot. Commercially available *I. reptans* seeds were used.

Randomization. The replicates and plots were laid out in a randomized complete block design. Each replicate was randomized separately (RCBD) [16, 17]. The 18 plants in each plot were labeled from 1 to 18 respectively. A random number generator from 1 to 18 was used to generate 10 random numbers in each plot to determine which plants were measured. The same selected plants that were measured remained constant throughout the weekly data gathering process.

Watering and Monitoring. The treatment plots were monitored daily. They were watered once at 17:00 with a volume of 500 mL of tap water per plot [18]. The plants were not watered if the surface of the soil is moist to the touch and the soil was also weeded when necessary. A screen was also constructed to protect the plants from any damage from pests.

Treatment Application. Two (2) set-ups were made simultaneously, each having three (3) treatment groups and one (1) control group. The treatments evaluated are as follows — T0B: soil applied with HA; T1: inorganic fertilizer + soil; T2: inorganic fertilizer + HA +soil. On the other hand, the control group that was evaluated was T0A: soil only. For the inorganic fertilizer, 250 kg/ha of urea fertilizer (46% N) was applied 7, 14, 21, and 28 DAS [19]. Five hundred (500) mL of 0.1% (1 g/L) solution of HA was applied in the plot every 14 days [20]. POWHUMUS® WSG 85 was used for the HA, derived as potassium humate, manufactured by HuminTech and contains 68-73% total humic acids [20]. The instructions indicated on the product packaging were the basis for the concentrations used in the study. Ramgo Plant Nutrition: Urea Fertilizer by Ramgo International Corporation was used for the inorganic fertilizer.

Measuring of parameters. The plant height and stem diameter were measured every seven (7) days starting at 14 DAS until 35 DAS and were recorded in centimeters (cm). The plant height was measured in cm from the ground level to the tip of the highest growing point using a ruler with ± 0.1 cm accuracy [21]. On the other hand, the stem diameter was measured in cm at the ground level at the base of the plant using a Vernier caliper with ± 0.05 cm accuracy [21]. The crop yield was determined by measuring the fresh weight in grams (g) using a top-loading balance with an accuracy of ±0.01g. The measured weight was then used in the formula below to determine the crop yield in kilograms per hectare (kg/ha) [22].

$$crop\ yield\ (\frac{kg}{ha}) = \frac{yield/plot\ (in\ g)\ \times\ 10^8 cm^2}{1200\ cm^2\ \times\ 1000}$$

Statistical analysis. The raw data collected was subjected to analysis using One-way Analysis of Variance (ANOVA) at 95% confidence level (α=0.05) using Microsoft Excel 365. It was done on groups of the same time point. The Least Significant Difference post-hoc analysis was then made to determine which groups exist a significant difference.

Safety Procedure. The wearing of proper gardening attire was observed during the conduct of the study. The chemicals used were also stored and sealed properly in a glass bottle and a copy of the chemical's MSDS was also kept at all times.

Results and Discussion. - Means of the gathered data were statistically analyzed using one-way ANOVA, and subjected to the Least Significant Difference (LSD) test as post-hoc analysis.

Kangkong in Oton site vs in Malay site. Significant differences were found in the three parameters—plant height, stem diameter, and crop yield—specifically at 28 and 35 DAS at the Oton site. However, no significant differences ($p \leq 0.05$) among treatments were found in all three parameters within five weeks of cultivation at the Malay site.

The varied set of data gathered from the Malay site may be attributed to the different microclimate parameters present in the areas during the duration of the experiment [23]. Considering that the two sites belong to two different climate types classified by PAGASA, this seems to be the case.

With this, the results that will be presented in this study are purely from the Oton site.

Plant height. Results showed that the plant height means of the treatments at 28 and 35 days after sowing (DAS) are significantly different ($p \leq 0.05$).

As seen in Figure 1, at 28 DAS, the mean plant height of the *I. reptans* treated with 0.1% HA (T0B = 23.40 cm) was significantly higher than those of other treatments – T0A (19.82 cm), T1 (19.69 cm), and T2 (18.38 cm). At 35 DAS, the mean plant height of the *I. reptans* treated with 0.1% HA (T0B = 38.24 cm) was also significantly higher than those of other treatments – T0A (30.73 cm), T1 (28.94 cm), and T2 (31.28 cm).

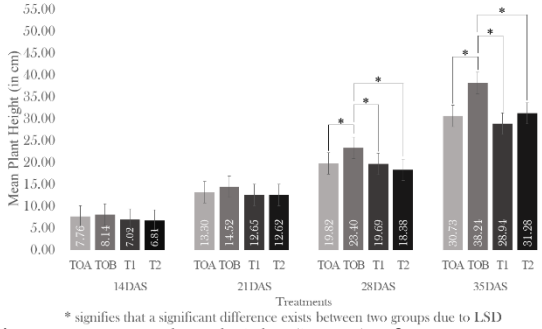

Figure 1. Mean plant height (in cm) of *I. reptans* under different treatments in the Oton site.

This indicates that there was a rise in nitrogen uptake [24]. Nitrogen boosts the growth of plants by stimulating height growth [25]. This may also be attributed to the hormones found in humic substances like auxins and gibberellins, as well as compounds such as amino acids, indole acetic acid, etc. [9][26]. The activation of auxin caused by the HA can induce cell elongation [10][27]. Cell elongation is an obligatory component of plant growth as it refers to the irreversible, rapid, and manifold increase in cell size and volume. It occurs in axial organs, such as stems and roots, wherein cells are elongated predominantly by cell wall growth on the cell lateral sides, resulting in the increase in plant height as plant morphogenesis depends on it [28].

Stem diameter. The One-way ANOVA showed that the stem diameter means of the different treatments were not significantly different ($p \leq 0.05$) as seen in Figure 2.

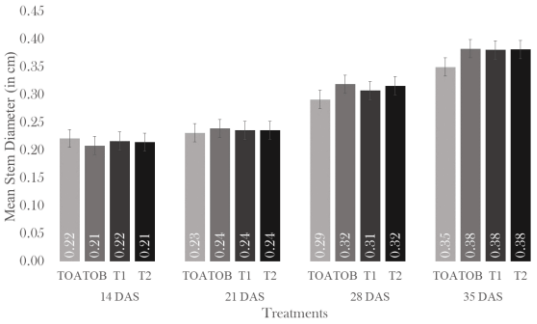

Figure 2. Mean stem diameter (in cm) of *I. reptans* under different treatments in the Oton site.

It can be speculated that there may be an insufficient concentration of Ca^{2+} ions in the soil that HA can keep in a dissolved state [29] and can be absorbed by the plant. This is because Ca^{2+} plays an important role in strengthening plant stems by forming bonds with pectin compounds in plants which are important for plant tissue rigidity and integrity resulting in thicker and stronger stems [30].

Another reason could be because of how cell elongation works which is facilitated by HA. Cell elongation is defined as cells expanding in one dimension to elongate cells and organs [31]. Since the auxins activated by HA induce cell elongation [10] rather than expansion (in all three dimensions), the stem diameter of the plants cannot significantly increase.

Crop yield. One-way ANOVA showed that the crop yield means (in kg/ha) of the different treatments are significantly different ($p \leq 0.05$).

The mean crop yield (kg/ha) of the *I. reptans* treated with 0.1% HA (T0B = 6738.90) was significantly higher ($p \leq 0.05$) than those of other treatments – T0A (4872.20 kg/ha), T1 (4711.10 kg/ha), and T2 (3616.67 kg/ha) (Figure 3). This indicates that the treatment with 0.1% HA (T0B) improves crop yield the most among other treatments.

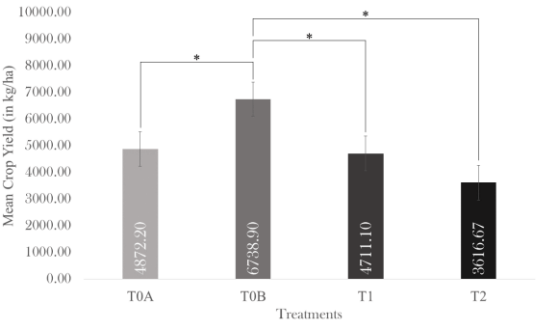

Figure 3. Mean crop yield (in kg/ha) of *I. reptans* under different treatments in the Oton site.

This increase in crop yield is attributed to the aforementioned growth-promoting hormones, auxins and gibberellins, which also play a role in the development of plant roots [32]. Increased vegetative growth and productivity are mainly due to the hormone-like activities of HA as it is involved in cell respiration, oxidative phosphorylation, protein synthesis, photosynthesis, antioxidants, and various enzymatic reactions [28]. The influence of HA is also

found in the development of plant roots, thus increasing plant production [24].

Optimal treatment for I. reptans growth and production. Among the four treatments, T0B (soil applied with 0.1% HA) was the best treatment for *I. reptans* growth and production. Despite not having a notable impact on stem diameter, the treatment yielded positive significant effects on plant height and crop yield. Given that these parameters indicate the quality of plant growth and production, it can be deduced that the sole application of HA is the best treatment for *I. reptans*.

This is in contrast however to the initial hypothesis based on the studies conducted by Sangeetha et al. [33] and Zhang et al. [9]. The positive effects of HA and the positive effects of urea separately may improve plant growth and yield when both substances are used in combination. To summarize, HA may contain auxin- and gibberellin-like substances or induce the activity of these to promote cell elongation of axial organs such as stems and roots. Urea provides nitrogen for the plant, as nitrogen boosts the growth of plants by stimulating height growth [34].

It is suspected that plant toxicity by NH_4^+ has occurred due to an increase in soil pH, which generally has adverse effects on higher plants physiologically [35]. The toxicity of NH_4^+ depends upon the substrate-solution concentration of NH_3, the un-ionized form of NH_4^+. Since NH_3 increases as pH increases, this makes NH_4^+ more toxic as the soil becomes more acidic]. Furthermore, a decrease in phosphorus (P) uptake and utilization as an effect of an increase in soil pH due to urea fertilization can also be the case. This has caused plants to use urea inefficiently. Phosphorus (P) deficiency reduces plant growth which is attributed to either decrease in photosynthesis or an increase in energy investment and negatively impacts crop yield and quality [35].

Limitations. The data gathering was conducted within 35 days in two sites with varying climate types and weather conditions, which affected the plant samples even with significant efforts to control the effect of these factors. Although *I. reptans* can already be harvested at this time, the difference in plant growth and yield among treatments can be seen more clearly within a longer timespan. Furthermore, due to the unavailability of laboratory equipment, other parameters such as nutrient uptake, soil moisture, and other soil parameters were not measured.

Despite this, the findings of this study may aid future research in improving the growth and production methods of agricultural crops and in establishing the credibility of humic substances in agriculture.

Conclusion. - Significant differences were found between groups in only one site at 95% confidence level in mean plant height both at 28 and 35 DAS, and mean crop yield. Meanwhile, no significant differences were found in stem diameter means at all seven-day intervals. Post-hoc analysis using Fisher's LSD test showed significant differences between T0A and T0B, T1 and T0B, and T2 and T0B but none in other pairs of treatments in terms of plant height and crop yield. Thus, the sole application of HA produced *I. reptans* with the tallest plant height and largest crop yield among the treatments used.

Recommendations. - Results in this study can be improved by replicating in an enclosed space more suitable for plant growth to avoid the effects of weather conditions and to prevent infiltration and infestation of pests. Other treatments can be utilized such as other types and levels of fertilizers and HA, and the use of other humic substances such as naturally extracted HA or substances. It is also recommended to conduct the study with a larger plant population and sample size for better representation. Certain parameters such as the number of leaves and branches, nutrient uptake, and soil parameters can be evaluated and measured. These parameters help determine their overall effect on plant growth and measure the growth of the plant itself. Replication of these types of studies on other plant species may be done. The use of HA in agriculture is recommended as it has positive results on crop growth and yield.

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