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Research Paper

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CHARACTERISTICS OF GOLDEN APPLE SNAIL BIOSTIMULANTS AND AGRONOMIC EFFECTIVENESS TESTS ON THE COFFEE SEEDLINGS

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ARTICLE HIGLIGHTS

- Golden apple snail extract was successfully developed into a biostimulant containing NPK nutrients, 12 amino acids, and indole-3-acetic acid (IAA).
- The biostimulant significantly enhanced coffee seedling growth, including plant height, root length, leaf area, chlorophyll content, and biomass.
- Golden apple snail biostimulant achieved the highest Relative Agronomic Effectiveness (RAE) of 104%, outperforming conventional NPK fertilization.
- Formulation without EM4 retained higher concentrations of macronutrients and amino acids, whereas EM4 supplementation increased IAA production.
- Utilizing an invasive golden apple snail as a plant biostimulant provides a sustainable alternative to inorganic fertilizers for coffee nurseries.

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ABSTRACT

The use of Golden Apple Snail (GAS), an invasive species, as a potential biostimulant source represents an innovation, particularly in sustainable agriculture and waste management. Therefore, this study aimed to determine the biochemical characteristics of GAS and assess its effectiveness on coffee seedling growth. The first test, detecting biochemical characteristics, was conducted using two formulations, i.e., 1) Formulation 1 = 1,000 g of fresh whole GAS extract + 500 g of sugar and 2) Formulation 2 = 750 g of fresh whole GAS extract + 500 g of sugar + 125 mL of EM 4. The results of the 14-day fermentation showed the presence of nutrients N, P, K, 12 amino acids, and phytohormones. Formulation 1 had a total amount of N-Total (1.76%), P_2O_5 (0.18%) and K_2O (0.29%) and a higher amino acid content than those in Formulation 2. In phytohormone detection, IAA was present in both formulations. The results of this test were used for further tests on the growth of Robusta coffee seedlings. The second test of agronomic effectiveness was carried out using a Randomized Block Design, with 6 types of fertilization treatments: 1) Control (Without GAS and NPK Biostimulants); 2) GAS Biostimulant 50 mL/L; 3) GAS Biostimulant 50 mL/L + ½ dose of NPK; 4) 0.5 g Urea + 1 g TSP + 1 g KCL; 5) GAS Biostimulant 50 mL/L + Full NPK Dose; 6) 1 g Urea + 2 g TSP + 2 g KCL. Each treatment was divided into 3 groups having two plants in each group. Observation data at the age of 4 months were tested using ANOVA and showed that there were significant differences in all morphological observation variables, including plant height, root length, number of leaves, chlorophyll content, leaf area, fresh weight, and dry weight or biomass. Based on the dry weight data, a Relative Agronomic Effectiveness (RAE) test was also conducted, which showed that the GAS Biostimulant treatment produced the highest RAE value, namely 104%, compared to the use of NPK fertilizer, showing agronomic effectiveness of GAS Biostimulant as an alternative to inorganic fertilizers in coffee nurseries.

Keywords: amino acid, biostimulant, golden apple snail, *Pomacea canaliculata* L., relative agronomic effectiveness

INTRODUCTION

Golden Apple Snails (GAS) (*Pomacea canaliculata* L.) are considered invasive pests that can cause major damage to rice plants, especially seedlings. The presence of this pest has a major impact on reducing rice yields (Naylor 1996). However, with proper handling, the GAS pest can be used as an environmentally friendly alternative biopesticide and also as an alternative source of nutrients and amino acids that can fertilize the plant itself (Ranamukhaarachchi &Wickramasinghe 2006).

GAS is known as a source of protein and amino acids for animal feed (Pertiwi & Saputri 2020). Therefore, further studies are needed regarding the use of the GAS pest as a source of biostimulants for plants.

According to Du Jardin (2015), biostimulants come from organic and inorganic materials and can increase plant growth, health, and resistance to biotic and abiotic stress. Several important roles of biostimulants for plants include growth stimulation. Biostimulants can stimulate root and shoot development, promoting the formation of strong plant structures and optimal vegetative growth (Yakhin *et al.* 2017). This provides impacts on increasing crop yields and production quality. In addition, the use of biostimulants can reduce dependence on inorganic fertilizers, thereby contributing to more sustainable and environmentally friendly agricultural management.

The use of robusta coffee as a research material for fertilization with GAS Biostimulants is also important because this commodity has high economic value and is relatively sensitive to changes in environmental conditions, especially during the seedling and early growth phases. Previous research has shown that the application of biostimulants made from GAS extract to robusta coffee seedlings can increase vegetative growth on drought stress condition (Asmono *et al.* 2025).

Therefore, to determine the character and effectiveness of GAS biostimulant on plant growth, it is necessary to have an appropriate formulation including the use of a concentration of GAS extract and also the addition of EM 4 (Effective Microorganism 4) bioactivator. The results of the formulation were tested on the Nitrogen, Phosphorus, Potassium, Phytohormone, and Amino Acid content produced. Testing of several concentrations of GAS biostimulant was carried out to test its effectiveness compared to inorganic fertilizer in increasing growth of the robusta coffee seedlings.

MATERIALS AND METHODS

Experimental Design

This research was carried out at the Bioscience Laboratory Politeknik Negeri Jember and carried out from July 2023 to September 2024. The main ingredient was the extract of the whole Golden Apple Snail (GAS) including the shell, with an average weight of 11.5 g, obtained from the rice fields of Tegal Gede Village, Sumbersari District, Jember Regency (8o09'01"S 113o43'48"E) at an altitude of 110 masl.

Fermentation solvent used were aquadest, white sugar, and EM4. Apart from that, 72 seedling materials employed were hybrid robusta coffee (for 6 treatments, 6 blocks with 2 seedlings per block), topsoil media, sand, cow manure, water, urea fertilizer, TSP, and KCL. Fermentation equipment included a meat grinder, measuring bottle, SPAD-502 (Soil Plant Analysis Development); digital scales, stationery, and thermometers.

GAS Biostimulant Character Test Method in Several Extract Formulations

In the biostimulant character test, the two formulations were compared, namely: 1) Formulation 1 = 95% whole fresh GAS extract + 5% sugar; 2) Formulation 2 = 75% whole fresh GAS extract + 5% sugar + 5% EM 4 + 15% distilled water. Technically, each formulation was made for 1,000 mL. Therefore, Formulation 1 used 950 mL of whole fresh GAS extract + 5 g of sugar, while Formulation 2 used 750 mL of whole fresh GAS extract + 5 g of sugar + 5 mL of EM4 + 15 mL of aquadest. EM4 is a mixture of several types of microorganisms and one of them is proteolytic bacteria.

Fermentation was carried out for 14 days under anaerobic conditions. The observation data was analyzed quantitatively and descriptively. After fermenting for 14 days, the solution was tested for its biochemical content, including amino acid and phytohormone content using the Liquid Chromatography-Mass Spectrometry (LCMS) method, N-Total using Kjeldahl; P_2O_5 and K_2O use SNI 7763:2018.

Test Method for the Agronomic Effectiveness of GAS Biostimulants and NPK Fertilizer on the Growth of Coffee Seedlings

This test used 3-month-old robusta coffee seedlings with an average initial height of 8.2 cm which only had 2 cotyledons leaves and 2 core leaves, and an average initial leaf length of 2.5 cm. Polybags used had a size of 20 cm x 30 cm with the planting media consisted of sand, topsoil, and manure with a ratio of 2:1:1. Seeding was carried out with a reduction in light intensity under 50% paranet.

The method was designed using a Randomized Block Design by testing 6 fertilizer formulations of GAS biostimulant from Formulation 2 (previous test) and NPK fertilizer according to the technical standards Direktorat Jenderal Perkebunan (2014) as follows.

1. **C:** Control (without GAS biostimulant and NPK);
2. **BGS:** GAS biostimulant 50 mL/L;
3. **BGS+ ½ NPK:** GAS biostimulant 50 mL/L + ½ NPK dose;
4. **½ NPK:** 0.5 g urea + 1 g TSP + 1 g KCL;
5. **BGS+ Full NPK:** GAS biostimulant 50 mL/L + Full NPK Dose;
6. **Full NPK:** 1 g urea + 2 g TSP + 2 g KCL.

Each treatment was divided into 6 blocks, each block consisted of 2 seedlings, making a total of 72 experimental units. As much as 200 mL of biostimulant was applied per plant. Biostimulants and inorganic fertilizers were applied every 1 month until the seedlings reached 4 months old or 120 Days After Planting (DAP).

The parameters observed include: 1) Seedling height: measured from the base of the stem to the growing point using a ruler; 2) The number of leaves: recorded on leaves that have grown to a minimum length of 0.5 cm; 3) Leaf area: measured using the measurement method with millimeter blocks of paper and carried out at the end of the research (seedlings aged 120 DAP); 4) Root length: measured from the base of the root to the tip of the longest root using a ruler; data were recorded at the end of the study (seedlings aged 120 DAP); 5) Fresh weight: measured by weighing the whole plant including stems, leaves, and roots in fresh condition; 6) Dry weight: carried out by oven- drying the whole plant at 65 ^{o}C for 48 hours (until the weight remains constant); 7) Chlorophyll content: measured using a chlorophyll meter/ SPAD-502 (Minolta Japan); data were recorded every month. Criteria for chlorophyll value SPAD-502 value were: < 50 = Low; 50 – 53 = Medium; > 53 = High (Prabowo *et al.* 2018); 8) Relative Agronomic Effectiveness (RAE).

The RAE value was determined using the following formula (Mackay *et al.* 1984; Putra & Pamungkas 2022):

$$\text{RAE} = (Pp - Pk)/(Pa - Pk) \text{ x } 100\%$$

where:

Pp = results obtained from the fertilizer or biostimulant being tested

Pk = results obtained from controls without fertilization or biostimulants

Pa = results obtained from Full Dose NPK treatment (1 g urea + 2 g TSP + 2 g KCL)

RESULTS AND DISCUSSION

Biochemical Characters (Nitrogen, Phosphorus, Potassium, Amino Acid, Phytohormone)

The laboratory test results indicated that each Golden Apple Snail (GAS) Extract formulation contained the NPK nutritional content and 12 types of amino acids. Only IAA phytohormones were detected, Cytokinins and Gibberellins were not detected. However, there were differences in the concentration of NPK and amino acids, and the hormone IAA (Fig. 1).

Formulation 1 contained more NPK than formulation 2 (Fig. 1), which may be atrributed to the different amount of GAS extract added to each formulation. The decrease in NPK levels in formulations containing EM4 during the fermentation process may have been caused by the activity of microorganisms contained in EM4 that requires Nitrogen for their life processes (Kieliszek *et al.* 2021). The analysis results also showed 12 types of amino acids in the GAS extract (Fig. 2).

The two formulations tested produced different results. On average, Formulation 1 contained higher amino acid concentration than that of Formulation 2. The low concentration in the second formulation may have been caused by the amount of GAS extract added, and also the addition of the EM4 bioactivator.

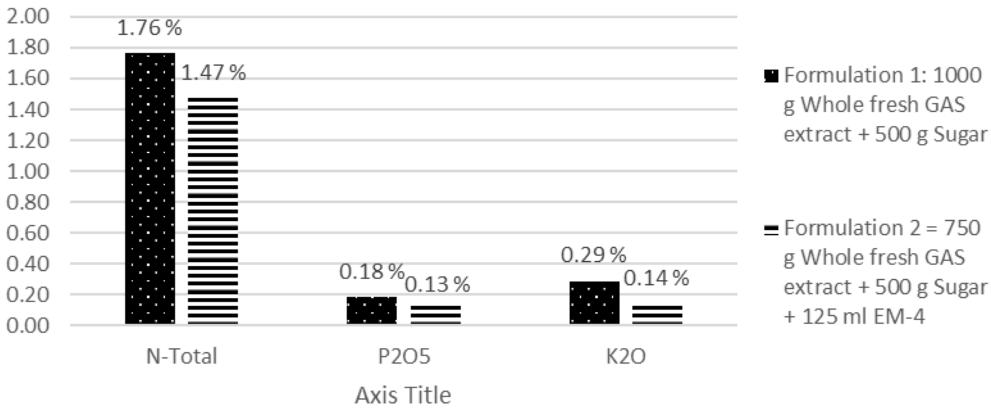

Figure 1 Differences in NPK concentration resulting from fermentation of GAS extract in different formulations

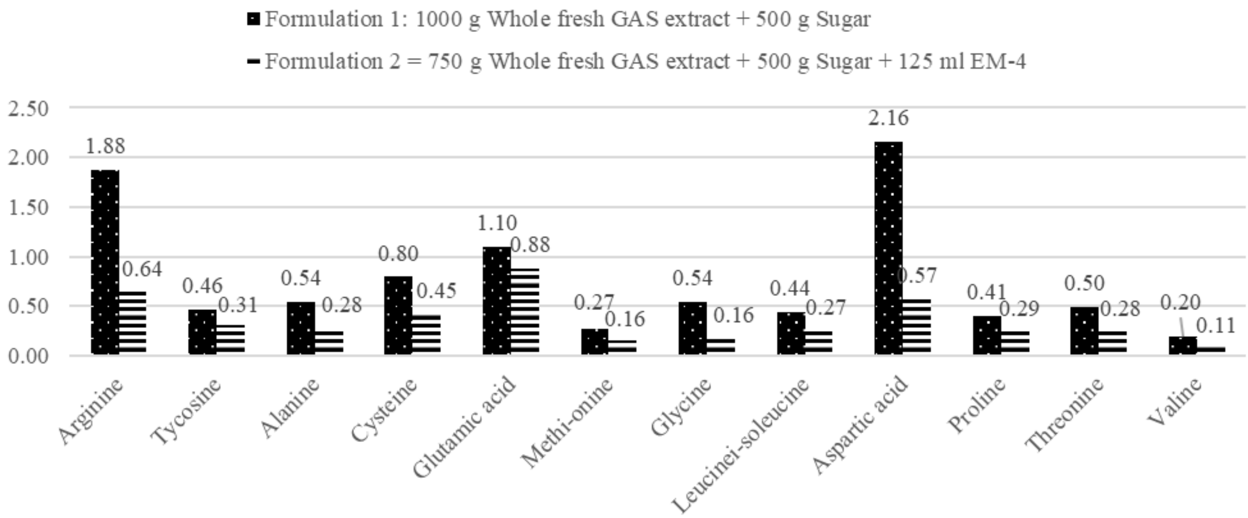

Figure 2 Types and concentrations of amino acids in different formulations

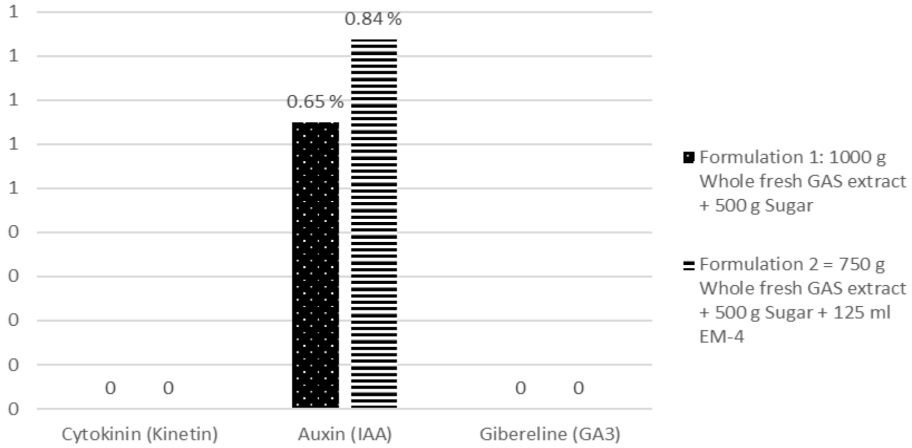

Figure 3 Detection results of the three types of phytohormones in different GAS extract formulations

Notes: Cytokinin (Kinetin) and Gibberellin (GA3) were not detected by the LC-MS test.

The proteolytic bacteria break down proteins and dissolve phosphates and amino acids so that they have a lower concentration compared to the condition without EM4. The purpose of EM4 addition was to help break down GAS protein into amino acids through proteolytic bacteria. Proteolytic bacteria are bacteria that produce extracellular protease enzymes that break down proteins produced in the cell and to be released from the cell. Protease can catalyze the breaking of peptide bonds in proteins into oligopeptides and amino acids (Ariole & Ilega 2013). In general, proteolytic bacteria are bacteria from the genus *Bacillus*, *Pseudomonas*, *Proteus Streptobacillus*, *Staphylococcus*, and *Streptococcus* (Puspitasari *et al.* 2012).

The fermented samples were tested to detect the content of the hormones cytokinin, auxin, and gibberellin, which results only detected the presence of Auxin-IAA.

The results of the samples tested showed that the formulation with the addition of EM4 (Effective Microorganism-4) produced a higher amount of the phytohormone IAA compared to the formulation that only contain GAS extract. The presence of the IAA hormone in GAS extract has not shown particular benefit. It has not been known whether GAS are capable of producing phytohormones or whether they come from microorganisms having a symbiotic relationship with GAS. However, research of Rusmini (2016) and Meliani *et al.* (2017) reported the presence of *Pseudomonas fluorescens* and *Bacillus* bacteria in the intestines of GAS, which can dissolve phosphate and can produce IAA.

The difference in hormone concentration may been caused by the addition of EM4. *Bacillus* such as those found in EM4 and GAS can produce the phytohormone IAA (Tsotetsi *et al.* 2022; Wagi & Ahmed 2019). Furthermore, *Bacillus* mixed with amino acids without the addition of an energy source is capable of producing IAA (Sobarzo *et al.* 2013). The research of Istiqomah *et al.* (2017) also proved that *Bacillus subtilis* and *Pseudomonas fluorescens* can produce IAA, which increased tomato plant growth by increasing shoot and root biomass and chlorophyll content.

Test Results of the Agronomic Effectiveness of GAS Biostimulant on the Morphology of Coffee Seedlings

Plant Height and Root Length

The results of the ANOVA analysis showed that the control treatment and the two formulations had very significantly different results in seedling height and root length. For the plant height parameter, the calculated F value was 15.56 and the root length parameter had a calculated F value of 5.71 with an F table of 5% 2.53 and 1% 3.70. Therefore, further testing was carried out using Duncan 5% analysis, as shown in Figure 4.

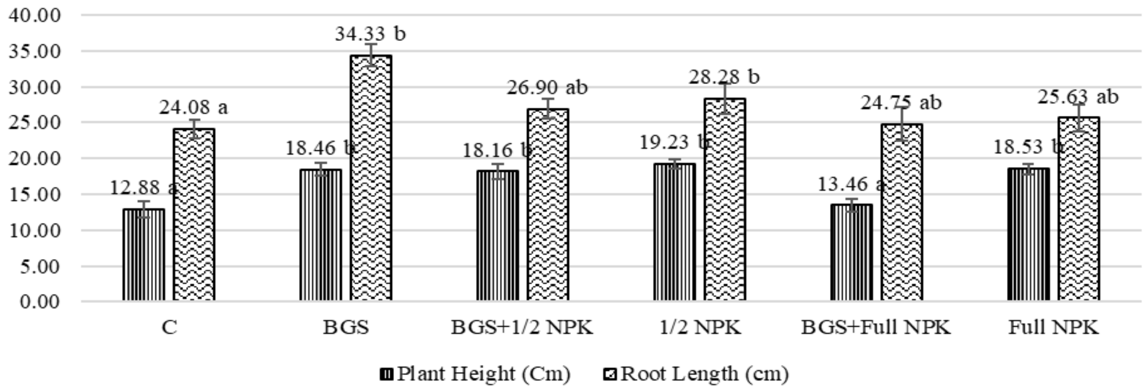

Figure 4 Average plant height and root length of coffee seedlings aged 120 DAP from several fertilizer formulations and GAS biostimulant

Notes: Numbers followed by the same letter in the same parameter indicate non-significant difference at P = 5% using Duncan test.

The average plant height from GAS Biostimulant (BGS) (18.46 cm) was not significantly different from the plant height from full NPK fertilizer (18.53 cm). In addition, it can also be seen that the average plant height data from the ½ NPK fertilization treatment was higher (19.23 cm) compared to the average plant height observed in the full NPK and also the BGS + ½ NPK combination fertilizer. This indicates that in the seedling phase, the coffee plants has not required the application of a full dose of NPK. Excessive amount of NPK in the seedling phase can cause nutrient imbalance and reduce growth efficiency. Fertilizer application at the optimum dose is actually more sufficient to support vegetative growth, including stem elongation, compared to doses exceeding the dose required by the plant. These results are consistent with research of Sari *et al.* (2019) which reported that an NPK dose of 1.5 g/polybag provided the best growth on robusta coffee seedlings and that the response of coffee seedlings tends to be optimal at medium doses, rather than higher doses.

However, the control treatment showed the shortest plant height compared to the other treatments, namely an average of 12.88 cm. These results showed that the single application of GAS biostimulant can increase the height of coffee seedlings. Biostimulants are known for their ability to increase plant growth and development, including increasing plant height (Chaski & Petropoulos 2022). Based on the results of previous analysis, GAS biostimulant contains important nutrients such as Nitrogen, P_2O_5, K_2O, Auxin IAA hormone, and other beneficial amino acid compounds.

Root length also showed very significantly different from the control treatment and the results of the two formulations. The longest roots were seen in seedlings that were only given GAS biostimulant (an average of 34.33 cm), very significantly different from the results from full doses of inorganic NPK fertilizer (an average of 25.63 cm) (Fig. 5).

The combination of GAS biostimulant and NPK fertilizer also produced an average root length of 25 cm. The shortest roots were found in the control treatment with an average of 24.08 cm. GAS biostimulant can lengthen roots or stems, allegedly because of the hormones and nutrients in it, especially the Auxin IAA hormone which stimulates cell elongation and division, which ultimately produces taller roots and plants (Parrado *et al.* 2008; Colla *et al.* 2014). Good root growth from the GAS biostimulant treatment can also be caused by amino acids and the role of symbiosis and microorganisms to optimize nitrogen fixation and increase nutrient absorption by roots (Hildebrandt *et al.* 2015; Weiland *et al.* 2015).

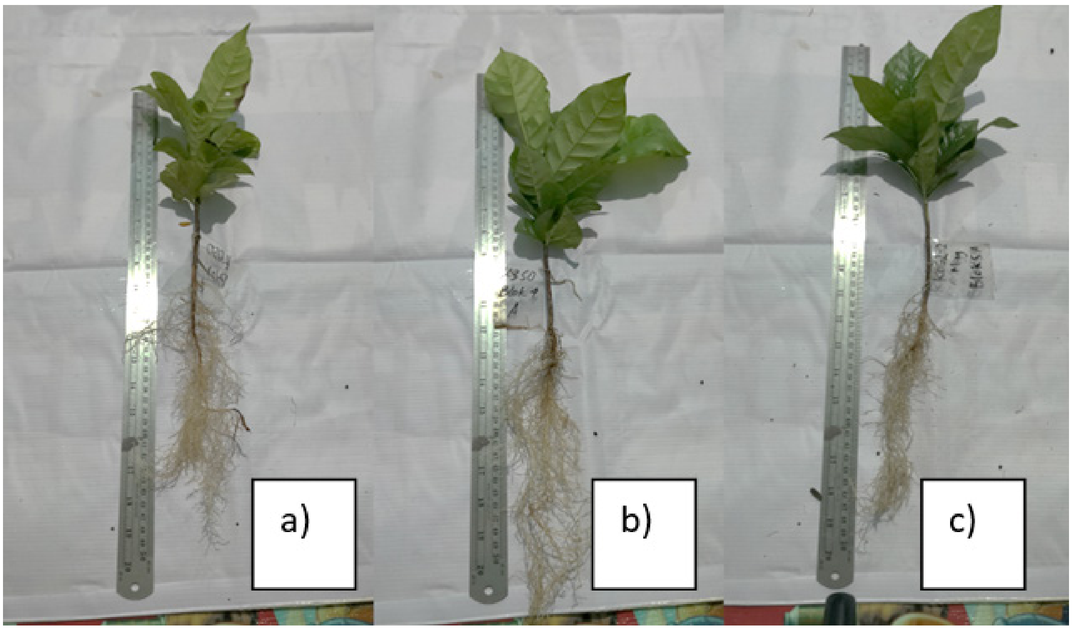

Figure 5 Growth results of seedlings aged 140 DAP
Notes: a) without fertilizer; b) GAS Biostimulant; c) NPK fertilizer

Number, Area, and Chlorophyll of Leaves

The effect of single application of GAS biostimulant or in combination with inorganic NPK fertilizer on leaf growth parameters is presented in Table 1.

On the number of leaves parameter of seedlings aged 120 DAP, there was no significant effect between biostimulant and inorganic NPK Fertilizer. However, a very significant difference was shown in the treatment with GAS biostimulant and the control. The average number of leaves in the control was only 10.92 leaves, while in the ½ dose NPK treatment, the average was 13.50 leaves and was not significantly different from the effect of the GAS biostimulant with an average of 13.33 leaves. From the number of leaves, it is known that biostimulants also have a function in physiological and enzymatic activities and also stimulate the performance of hormones in the meristem so that they influence the increase in the number of plant leaves (Tahapary *et al.* 2020).

The results of the analysis of leaf area data also showed the same trend, the average leaf area was the highest in the full dose of NPK treatment (an average of 114.18 mm2) and was not significantly different from ½ dose of NPK or GAS Biostimulant alone (an average of 113.66 mm2 and 106.83 mm2, respectively). A very significant result can be seen from the smallest average leaf area (31.25 mm2) in the control treatment.

The role of nutrients in leaf formation and expansion of leaf area is very important in the process of cell division, chlorophyll formation, and photosynthesis processes in leaves (Fathi 2022). The GAS biostimulant was also able to balance the amount of Nitrogen, Phosphorus, and Potassium from Urea, SP36, and KCL fertilizers which were tested to stimulate the formation and expansion of the leaf area of coffee seedlings. Moreover, GAS biostimulant contains amino acids which can have a good impact on nitrogen efficiency under certain conditions. Research by Khan *et al.* (2019), proved that amino acids increase the efficiency of nitrogen use in the photosynthesis process in plants under environmental stress conditions.

The results of the chlorophyll values measured using the SPAD-502 (Soil Plant Analysis Development) tool showed that the control treatment with an average of 35.53 had low category chlorophyll levels, and in the GAS Biostimulant treatment and inorganic NPK fertilizer, the results of statistical analysis showed non-significant differences in the medium category of chlorophyll levels with an average of less than 50 (Prabowo *et al.* 2018).

Several previous studies have also proven that the application of biostimulants can increase chlorophyll levels in leaves (Khandaker *et al.* 2017; Dalorima *et al.* 2018; Ellyzatul *et al.* 2018; Sowmya *et al.* 2023). The GAS biostimulant that was tested also contained the nutrient potassium (see Fig. 1) which was able to optimize the work of stomata and chlorophyll for the photosynthesis process. In addition, one of the amino acids from the GAS extract detected in this study was Cysteine (Fig. 2) which is also a synthetic precursor of Chlorophyll (Baqer *et al.* 2019). This amino acid is presumably capable in optimizing the leaf chlorophyll value in this study.

Table 1 Average number, area, and chlorophyll of leaves of coffee seedlings aged 120 DAP from several formulations of GAS biostimulant and inorganic NPK fertilizer

Treatment	Leaves number		Leaf Area (mm2)		Chlorophyll	
C	10.92a	± 1.21	31.25a	± 1.75	35.33a	± 0.15
BGS	13.33b	± 0.98	106.83cd	± 3.05	51.68b	± 1.80
BGS+1/2 NPK	13.00b	± 1.04	73.12bc	± 2.86	52.20b	± 1.07
1/2 NPK	13.50b	± 0.86	113.66d	± 2.56	48.79b	± 0.54
BGS+Full NPK	11.50a	± 0.73	50.85ab	± 2.40	49.44b	± 1.35
Full NPK	13.33b	± 0.97	114.18d	± 4.90	50.58b	± 2.82

Note: Numbers followed by the same letter in the same parameter indicate a non-significant difference at P = 5% in Duncan test.

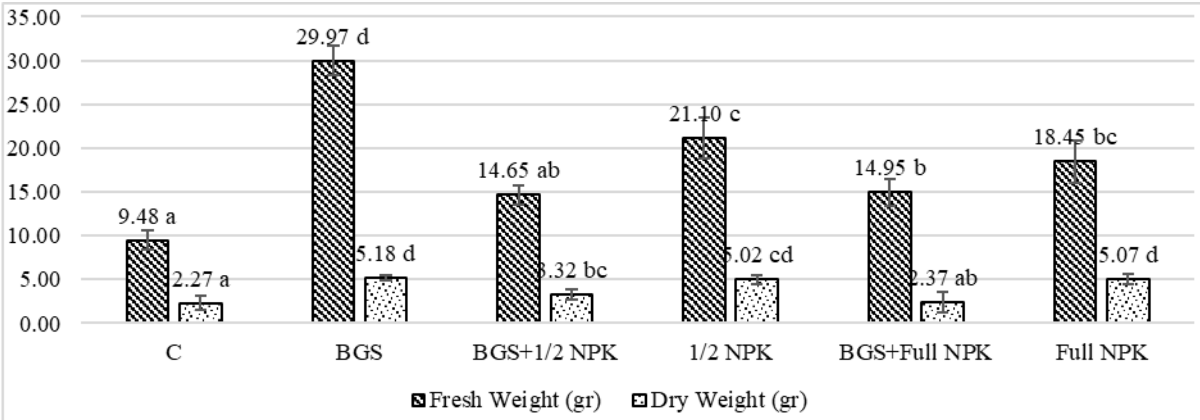

Figure 6 Average fresh weight and dry weight of coffee seedlings aged 120 DAP from several fertilizer formulations and GAS Biostimulant

Notes: Numbers followed by the same letter in the same parameter indicate non-significant differences at P = 5% using Duncan test.

Fresh and Dry Weight

The results of the ANOVA analysis showed that the treatment of several biostimulant formulations and inorganic NPK fertilizer had a very significant effect on the parameters of fresh weight and dry weight of coffee seedlings at the age of 120 DAP. In the fresh weight and dry weight parameters, the calculated F values were 17.86 and 23.2,4 respectively with 5% F table of 2.53 and 1% F table of 3.70. The data from the analysis were further tested using Duncan at the P = 5% level (Fig. 6).

The seedlings with the highest fresh weight value were produced from the GAS biostimulant treatment only with a weight of 29.97 g and were significantly different from other treatments that used full inorganic NPK fertilizer (an average fresh weight of 18.45 g). The control treatment had a fresh weight of 9.48 g. The fresh weight of plants is the result of a process of cell growth and differentiation and a representation of biomass accumulation and efficiency in nutrient absorption and utilization. The nutritional components, hormones, and amino acids in GAS Biostimulant may have been optimal enough for cell formation, cell division, and cell differentiation and are accumulated as fresh weight of the seedlings. These results is aligned with previous studies reporting that amino acids can significantly increase the fresh weight of plants (Abdelkader *et al.* 2023; Canellas *et al.* 2024).

In terms of dry weight, the treatment of GAS biostimulant and inorganic NPK fertilizer and their combination produced a dry weight that was not significantly different (an average weight of 5 g). Meanwhile, the dry weight in the control treatment was only 2.27 g. According to Abdelkader *et al.* (2023), dry weight refers to all absorbed substances, biomass, and assimilate formed by plant tissue except water. Plant dry weight describes the balance between photosynthate utilization and respiration. If the essential elements N, P, and K can be fulfilled, then plants can photosynthesize well and plant growth will also be better, so that the dry weight produced will also be higher. Therefore, the use of GAS biostimulant has a good effect on the growth of coffee seedlings because it produces a high dry weight and is relatively the same when compared to the use of inorganic NPK.

Relative Agronomic Effectiveness/RAE

The results of calculating the RAE value for using GAS Biostimulant and inorganic NPK fertilizer are taken from the dry weight value of 120 DAP age seedlings (Table 3).

Relative Agronomic Effectiveness (RAE) is the level of effectiveness of organic fertilizer or in this case the results of the GAS biostimulant compared to the results of standard fertilizer. In this test, the data used is the dry weight of the entire plant, including stems, leaves, and roots (Putra & Pamungkas 2022). Plant dry weight is a parameter of nutrient status, plant biomass accumulation, and a representation of photosynthetic activity and assimilate concentration in plants (Stanghellini *et al.* 2019). Dry weight is also one of the best parameters to indicate good plant growth quality (Mañas *et al.* 2009). The GAS biostimulant treatment showed the highest RAE value (104%), the ½ NPK concentration had a value of 98% and was lower than the full dose of NPK fertilizer.

Table 2 Effect of several formulations of GAS Biostimulant and inorganic NPK on RAE values

Treatment	RAE (%)
C	0
BGS	104
BGS+1/2 NPK	38
1/2 NPK	98
BGS+Full NPK	4
Full NPK	100

Table 3 Comparison of NPK nutrition in GAS biostimulant with compound fertilizer

Nutrition	Golden Snail Biostimulant (BGS) Dosage 200 mL per plant	NPK compound fertilizer Dosage per plant (1 g urea, 2 g TSP, 2 g KCl)
N	2.94 g	0.46 g
P	0.26 g	0.40 g
K	0.28 g	1.20 g

This test also demonstrated that the combination of the GAS biostimulant and full inorganic NPK fertilizer had a low RAE value. The dry weight RAE value for the BGS + full NPK fertilizer treatment only reached 4%, indicating that the addition of the Golden Snail GAS biostimulant (BGS) at a full NPK dose actually reduced the agronomic effectiveness of seedling growth compared to full NPK fertilizer alone (RAE 100%). Based on the analysis, the NPK content of BGS in Formulation 2 was N = 1.47%; P = 0.13%; K_2O = 0.14%, compared to the compound NPK fertilizer (Table 4).

From these data, the combination of BGS + NPK Full may have exceeded the optimal total nutrient requirements of plants in the seedling phase, so that nutrients are not optimally absorbed due to excessive osmotic pressure and salinity in the media (Nurshanti *et al.* 2021).

Thus, the use of GAS biostimulant can be an alternative to inorganic NPK fertilizer. According to Wozniak *et al.* (2020), biostimulants have been proven to increase shoot growth, which is measured based on plant height, shoot fresh weight, shoot dry matter, leaf area, leaf elongation, etc. Apart from that, the application of liquid amino acid fertilizer through the leaves has also been proven to significantly increase the yield of Cowpea plants compared to chemical fertilizer treatment. This shows that amino acids can be used as a more environmentally friendly alternative to inorganic fertilizers (Wang *et al.* 2019).Viewed from an economic aspect, the use of biostimulants is also very beneficial in saving production costs for farmers (Kocira *et al.* 2020).

Based on all the results, the GAS biostimulant showed macronutrient content (N-Total, P_2O_5, K_2O) and amino acids, and detected the presence of IAA as a phytohormone. From the biochemical content, the agronomic effectiveness test of GAS Biostimulant was carried out by comparing it with NPK inorganic fertilizer on coffee seedlings. The results also revealed significant differences in all morphological variables. GAS biostimulant was proven to achieve the highest Relative Agronomic Effectiveness (RAE) of 104% compared to NPK fertilizer. These results prove that GAS biostimulant is an effective sustainable alternative to inorganic fertilizers in coffee seedling propagation. This research contributes to invasive waste management and environmentally friendly agriculture in the coffee sector.

CONCLUSION

Formulation 1 (GAS extract without EM4) with a 14-day fermentation produced N-Total (1.76%), P_2O_5 (0.18%) and K_2O (0.29%), while Formulation 2 (GAS extract with the addition of EM4) produced higher IAA phytohormones (0.84%). There were 12 types of amino acids in the GAS extract. On average, Formulation 1 has a higher concentration of amino acids than Formulation 2. The morphological observation variables, including plant height, root length, number of leaves, chlorophyll content, leaf area, fresh weight and dry weight or biomass showed very significant differences from the 6 fertilization treatments given, with the best average results in the use of GAS biostimulant. The results of the Relative Agronomic Effectiveness (RAE) test taken from the total dry weight of the plant showed that the GAS biostimulant from Formulation 2 showed the highest RAE value, namely 104%. The ½ NPK concentration had a value of 98% and was lower than the full dose of NPK fertilizer with a value of 100%. The overall results showed that the use of GAS biostimulant can be an alternative to inorganic fertilizers in coffee nurseries.

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