CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

Enhancing maize yield and fertilizer efficiency in tidal swamp lands by employing biostimulants

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Received: 4 July 2024; Accepted: 12 September 2024, doi:10.4067/S0718-58392025000200170

ABSTRACT

Tidal swamp lands in Indonesia, despite their low productivity, hold significant potential for improving maize (*Zea mays* L.) cultivation and food security. Biostimulants derived from plants, animals, and plant growth-promoting rhizobacteria (PGPR) can enhance productivity and maize yields. Notably, rice husk ash (RHA), rich in silica, and golden apple snail (GAS; *Pomacea canaliculata*, Lamarck, 1822), containing amino acids, offer growth-promoting benefits. This study examines the impact of liquid RHA, GAS, and microbial inoculant (PGPR) on maize yield in acid sulphate soils from Barambai District, South Kalimantan, Indonesia. A randomized complete block design was used to test nine treatments, including a control (no NPK or biostimulants), full NPK, 75% NPK, and six biostimulant formulations (B1 to B6) combining RHA, GAS, and PGPR with 75% NPK. Biostimulants with more than 50% GAS extract significantly improved maize growth and yield while reducing NPK fertilizer use by 25%. Specifically, biostimulant formulations B1 (10% RHA, 80% GAS, 10% PGPR), B2 (20% RHA, 70% GAS, 10% PGPR), and B3 (30% RHA, 60% GAS, 10% PGPR), combined with 75% NPK, produced 126.0, 125.0, and 111.0 g plant^{-1}, respectively, compared to 97.67 g plant^{-1} with full NPK. This suggests that biostimulants can reduce fertilizer use without sacrificing yield. The results highlight the role of amino acids in GAS extract for enhancing maize productivity. Biostimulants, particularly those rich in GAS extract, show promise in increasing maize grain yield and reducing reliance on inorganic fertilizers. Further field testing in real-world tidal swamps is needed to validate these findings.

Key words: Biostimulants, golden apple snail, grain yield, PGPR, *Pomacea canaliculata*, rice husk ash, *Zea mays*.

INTRODUCTION

Tidal swamp lands in Indonesia hold potential for maize (*Zea mays* L.) cultivation, thereby contributing to food security. The total area of tidal swamp lands in Indonesia is approximately 8.92 million hectares, with the majority located in Kalimantan and Papua (Sulaiman et al., 2019). Tidal swamp lands are often deemed suboptimal for productivity due to their naturally low fertility, often caused by high soil acidity. High soil acidity can result in a lack of essential nutrients, which, in turn, affects plant growth. This issue can be particularly pronounced in tidal swamp lands, where acid sulphate soils are common, and can have significant impacts if not properly managed (Khamidah and Saputra, 2020; Alwi et al., 2021). According to data from the Central Statistics Agency, the average maize grain yield (in tidal swamp lands) is 5597 t ha^{-1}. This yield is lower than the national average productivity of 5709 t ha^{-1} (BPS – Statistics Indonesia, 2022).

Efforts can be made to improve growth conditions and enhance plant stress tolerance in tidal swamp lands. One approach is using biostimulants, which can positively influence the morphology and physiology of maize plants (Ma et al., 2022). Biostimulants can be categorized into non-microbial and microbial types, containing amino acids, peptides, and vitamins (Rouphael and Colla, 2020). Biostimulants can be considered fertilizer additives supporting nutrient uptake, enhancing plant health and productivity, and reducing reliance on harmful chemicals. They can originate from various sources and serve as fertilizer additives to improve nutrient utilization efficiency and enhance plant growth (Rathore et al., 2009).

Biostimulants can enhance nutrient utilization efficiency, allowing plants to absorb more nutrients from the soil (Halpern et al., 2015; Rouphael and Colla, 2020). They can also help plants withstand various stresses such as drought, excessive heat, or diseases (Rouphael and Colla, 2020; Baltazar et al., 2021). By promoting plant health and reducing the reliance on traditional fertilizers, pesticides, or herbicides, biostimulants can contribute to organic programs and benefit the environment. Utilizing biostimulants can reduce fertilizer requirements in agriculture, aid in reducing water pollution, and address chemical fertilizer usage limitations (Rouphael and Colla, 2020).

Tidal swamp lands host a variety of plant growth-promoting rhizobacteria (PGPR) that play a crucial role in optimal plant development. These microbes exhibit remarkable abilities in decomposing organic matter, fixing N, and solubilizing phosphates from calcium-phosphate and aluminium-phosphate complexes in the soil. They also demonstrate resilience to high soil acidity (Bowen et al., 2009). Microbial species in Indonesian tidal swamp areas have been shown to exhibit these beneficial properties, contributing to the overall health and productivity of the ecosystem. The PGPR can provide several benefits in maize cultivation in tidal swamp lands, such as enhancing N fixation, phosphate solubilization, and siderophore production, aiding in Fe uptake by plants in Fe-deficient conditions. Additionally, PGPR can assist plants in tolerating abiotic stressors, such as drought and nutrient deficiencies, by enhancing their ability to maintain membrane stability and chlorophyll content. The use of PGPR in biofertilizers can improve plant growth and yield by adding nutrients to the soil and enhancing its fertility, which is particularly important in tidal swamp lands where the soil fertility is often low due to the high rainfall and acidic conditions (dos Santos et al., 2020; Yuliatin et al., 2023).

One potential biostimulant is the golden apple snail (*Pomacea canaliculata*) (Lamarck, 1822), often found in rice fields as a pest. Although the golden apple snail is a pest in rice fields, it has significant potential as a source of amino acids to replace urea. Several studies have shown that the golden apple snail contains 17 amino acids, including eight essential amino acids, which can be utilized as nutrition for plants and other organisms. One of these amino acids, tryptophan, acts as an inducer in auxin formation and can function as a growth regulator (Nurshanti et al., 2021). The golden apple snail may have applications in plant growth regulation or as a source of bioactive compounds for various purposes.

Golden apple snail extract is utilized in maize cultivation primarily as a biostimulant to enhance plant growth and development. The extract can be combined with inorganic fertilizers, such as NPK fertilizers, and applied to the soil during land preparation or after planting. The dosage of biostimulant extract varies, with concentrations ranging from 10 to 30 mL L^{-1} water-based golden apple snail extract. This application is typically carried out daily for 7 d after planting, and the soil is irrigated with the extract using a measuring cup. Golden apple snail extract can increase plant height, leaf number, leaf width, and melon branch number (Nurshanti et al., 2021). Additionally, the extract can contribute to improving soil quality by increasing pH, preventing soil degradation, and enriching nutrients (Wang et al., 2020).

In addition to the golden apple snail as a pest of rice plants, agricultural waste such as rice husks contains high levels of silica, which is beneficial for maize plants. Rice husk ash can be ameliorated in maize cultivation in tidal swamp lands due to its ability to improve soil properties and enhance plant productivity. It contains high levels of silica, which plays a role in neutralizing soil acidity and maintaining soil pH relatively stable. It can be particularly beneficial in acid sulphate soil of tidal swamp lands, where the soil can be highly acidic and may require improvement to support plant growth. Furthermore, rice husk ash can help reduce the uptake of toxic elements, such as Al and Fe, which can harm plants (Severo et al., 2020). By incorporating rice husk ash into the soil, maize plants can experience increased grain yields and better health, leading to enhanced crop production in tidal swamp lands (Maftu'ah et al., 2023).

The combination of biostimulants, comprising a mixture of golden apple snail and rice husk extract, along with PGPR, makes this innovation highly beneficial as an alternative to inorganic NPK fertilizers, aiming to enhance maize productivity in tidal swamp lands. This biostimulant combination is hoped to help address inorganic fertilizer scarcity. This study aimed to develop a biostimulant formula using ingredients derived from microbes, plant extracts, and animal extracts to optimize inorganic fertilizers and enhance maize productivity in tidal swamp lands.

MATERIALS AND METHODS

Site description

A controlled pot experiment was conducted in a greenhouse from April to August 2023 utilizing soil samples sourced from an acid sulphate site within Barambai Kolam Kiri Village (2°55'11.334" S, 114°42'14.4" E), Barambai District, Batola Regency, South Kalimantan Province, Indonesia. Following preparation, these soils were transferred into experimental pots with a weight of 5 kg each.

Before conducting the experiment, soil analysis included measurements for pH, organic C using the Walkley-Black method, total N, and P through HCl 25%, as well as available P according to Bray-1 method, exchangeable cations such as K, Na, Ca, and Mg via ammonium acetate solution with a concentration of 1 N and adjusted pH to 7. The samples were analysed in the Soil Laboratory of the Faculty of Agriculture at Lambung Mangkurat University in Banjarbaru, South Kalimantan.

Preparation of bacterial culture

The bacterial consortium utilized in this study comprised *Bacillus marisflavi* U7, *Bacillus aerius* U21, *Bacillus aryabhattai* U32, *Bacillus subtilis* P183, and *Azotobacter vinelandii* 1CM. *Bacillus marisflavi* U7, *B. aerius* U21, and *B. aryabhattai* U32, they were isolated from rice (*Oryza sativa* L.) root samples and mangrove/mangrove mud sediments in Batang Regency and Pekalongan Regency (Central Java) (Badrudin et al., 2024), while *A. vinelandii* 1CM and *B. subtilis* P183 were isolated from the rhizosphere of rice plants in Bogor Regency, West Java (Pratiwi et al., 2016). These bacterial strains have been previously evaluated for their N-fixing ability, phosphate solubilization capacity, and indole-3-acetic acid (IAA) phytohormone and organic acid production (Pratiwi et al., 2016; Badrudin et al., 2024).

Each bacterium was grown individually in nutrient broth liquid media and incubated on an orbital shaker for 72 h at room temperature. After reaching the stationary phase, the cell density of each strain was determined spectrophotometrically by comparing the optical density at 600 nm obtained with the growth calibration curve. All inoculants were then combined to achieve a final density of 10^9 cfu mL^{-1}.

Extraction of rice husks

The rice husks were ground until smooth using a grinding machine or manual grinder, then heated in a large pan over medium heat until the husks turn greyish ash. This heating process took several hours to ensure the ash forms evenly. Once cool, the ashes were filtered to separate the coarse parts. The filtered husk ash can be used as an additive in various applications, such as organic fertilizer.

Husk ash extract as a silicate source was made by dissolving 3 kg KOH in 10 L water and heating it over medium heat until it reaches a temperature of 80 °C. Next, the husk ash was added to the alkaline solution with a volume ratio 1:1 and stirred for approximately 1 h, with the temperature maintained at around 80 °C. The solution was removed, cooled, and filtered using a filter cloth. The husk ash extract obtained had a pH of around 13.2.

Extraction of golden apple snail

Golden apple snails (*Pomacea canaliculata*) (Lamarck, 1822) were collected from rice fields in Barambai District, Batola Regency, South Kalimantan Province. The snails were soaked in water for 24 h to remove mud and dirt. Subsequently, 10 kg snail meat (without shells) was boiled in 10 L water for 1 h. The boiled snails were blended and mixed with ripe pineapple in a 1:1 ratio (1 kg snail, 1 kg pineapple) and left to ferment for 40 d. After fermentation, the mixture was filtered to extract the biostimulant liquid. The resulting snail extract had a pH of approximately 4.0. An amino acid content analysis was conducted at Saraswanti Indo Genetech in Bogor, West Java. Conversely, nutrient content analysis, including N, P, K, and pH levels, was performed in the Soil Laboratory of the Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru, South Kalimantan.

Characteristics of rice husk ash

Rice husk ash extract contains high levels of SiO_2 and K_2O, at 11.63% and 10.20%, respectively (Table 1). It also contains smaller amounts of CaO, MgO, P_2O_5, and F_2O_3, each less than 0.20%. Silicon and K are essential for plant growth. Silicon promotes root oxidation, increases enzyme activity in photosynthesis, and thickens cell walls to protect against pests. Meanwhile, K plays an important role in the formation and transport of carbohydrates, acts as a catalyst in protein synthesis, increases carbohydrate and sugar levels in fruits, and improves fruit quality in shape, content, and colour (Sumadi and Muljani, 2016).

Table 1. Chemical content of rice husk ash extract.

Compound	Amount (%)
Silicon dioxide (SiO_2)	11.63
Calcium oxide (CaO)	0.04
Magnesium oxide (MgO)	0.05
Phosphorus pentoxide (P_2O_5)	0.13
Potassium oxide (K_2O)	10.20
Ferric oxide (Fe_2O_3)	0.02

Characteristics of microbial inoculants

The bacterial consortium consists of *Bacillus marisflavi* U7, *B. aerius* U21, *B. aryabhattai* U32, *B. subtilis* P183, and *Azotobacter vinelandii* 1CM.

Bacillus marisflavi U7, *B. aerius* U21, and *B. aryabhattai* U32 were isolated from rice root samples and mangrove/mangrove mud sediments in Batang Regency and Pekalongan Regency (Central Java) (Badrudin et al., 2024), while *A. vinelandii* 1CM and *B. subtilis* P183 were isolated from the rhizosphere of rice plants in Bogor Regency, West Java (Pratiwi et al., 2016). These bacterial strains have been previously evaluated for their N_2-fixing ability, phosphate solubilization capacity, and indole-3-acetic acid (IAA) phytohormone and organic acid production (Pratiwi et al., 2016; Badrudin et al., 2024).

Application of biostimulant

The biostimulant formulation was applied to the soil and sprayed on plants according to the designated treatment plan. It consisted of two primary components: Rice husk ash (RHA) extract, rich in silica and K, and golden apple snail (GAS) extract, which provides essential amino acids. Additionally, microbial inoculants or PGPRs were included to introduce beneficial microorganisms functioning as biofertilizers. Six variations of liquid biostimulant formulations were tested, each differing in the proportions of RHA, GAS extracts, and PGPR: Formula B1: 10% RHA, 80% GAS, 10% PGPR; Formula B2: 20% RHA, 70% GAS, 10% PGPR; Formula B3: 30% RHA, 60% GAS, 10% PGPR; Formula B4: 45% RHA, 45% GAS, 10% PGPR; Formula B5: 60% RHA, 30% GAS, 10% PGPR; Formula B6: 70% RHA, 20% GAS, 10% PGPR.

The liquid biostimulant application on maize (*Zea mays* L.) plants in the greenhouse uses a mixture of RHA extract, GAS extract, and PGPR with a concentration of 10 mL L^{-1} water. Each application was done manually using a hand sprayer, applying 50 mL $plant^{-1}$. The first application was performed 15 d after planting (DAP), spraying evenly on the soil and the plants. Subsequent applications at 30, 45, and 60 DAP were sprayed only on the plants. Each spray ensures that 50 mL well-mixed biostimulant is consistently applied to each plant. The maize was harvested at 115 DAP.

Application of NPK fertilizer

Inorganic NPK fertilizers (16:16:16) were administered in a two-stage process: One-third of the dosage was applied 10 DAP, while the remaining two-thirds were applied 30 DAP. Intensive pest and disease management was conducted utilizing insecticides and fungicides. The maize 'BISI 329' was used, with one seed per pot.

Experimental design

The experimental design was a randomized complete block design (RCBD) with three replicates. The treatments consisted of a control (without NPK or biostimulants), F1 (100% NPK: 300 kg ha^{-1} NPK + 150 kg ha^{-1} urea), F2 (75% NPK), and six biostimulant combinations with 75% NPK: B1F2 (Formula B1 + 75% NPK), B2F2 (Formula B2 + 75% NPK), B3F2 (Formula B3 + 75% NPK), B4F2 (Formula B4 + 75% NPK), B5F2 (Formula B5 + 75% NPK), and B6F2 (Formula B6 + 75% NPK).

Data processing and statistical analysis

The parameters observed encompassed plant height, number of leaves, stem diameters at 30 and 60 DAP and yield and its constituents. We employed the SAS (SAS Institute, Cary, North Carolina, USA) software package to analyse these data statistically, specifically conducting an ANOVA. Significant variations among treatment groups were determined utilizing the least significant difference (LSD) test, setting the significance threshold to $p < 0.05$.

The data was processed using Microsoft Excel, and the standard error was subsequently utilized to analyse the data variation. The results were then visually represented using SigmaPlot software (Grafiti LLC, Palo Alto, California, USA).

RESULTS

Soil characteristics

Soil chemical analysis indicates that soils are classified as highly acidic with low base saturation (Table 2). It becomes imperative to implement a comprehensive fertilization strategy encompassing chemical, organic, and biofertilizers to promote continual improvement in maize growth and soil fertility.

Table 2. Chemical characteristics of the initial soil of tidal swamp lands, Barambai Kolam Kiri Village, Barambai District, Barito Kuala Regency, South Kalimantan Province. *BPSI Tanah dan Pupuk (2023).

Soil characteristics	Value	Category*
pH H_2O	3.40	Highly acidic
pH KCl	3.20	Highly acidic
Organic C, %	4.31	High
Total N, %	0.30	Moderate
C/N ratio	14.36	Moderate
Total P, mg 100 g^{-1}	39.16	Moderate
Total K, mg 100 g^{-1}	16.30	Low
Available P, mg kg^{-1}	73.80	Very high
Exch. K, $cmol_{(+)}$ kg^{-1}	2.24	Very high
Exchangeable Ca, $cmol_{(+)}$ kg^{-1}	1.87	Very low
Exchangeable Mg, $cmol_{(+)}$ kg^{-1}	1.95	Moderate
Exchangeable Na, $cmol_{(+)}$ kg^{-1}	1.01	Very high
Cation exchange capacity, $cmol_{(+)}$ kg^{-1}	29.48	High
Base saturation, %	23.98	low

Plant growth

The plant height, number of leaves, and stem diameter in the biostimulants + NPK application treatment showed an increase and different compared to the control treatment at 30 and 60 DAP (Figure 1). The treatments of B1F2, B2F2, and B3F2 at 60 DAP always resulted in larger stem diameters, more leaves, and greater plant heights compared to using inorganic NPK fertilizer at recommended doses and control treatment. These findings suggest that biostimulants promote plant development and can lessen reliance on inorganic NPK fertilizers.

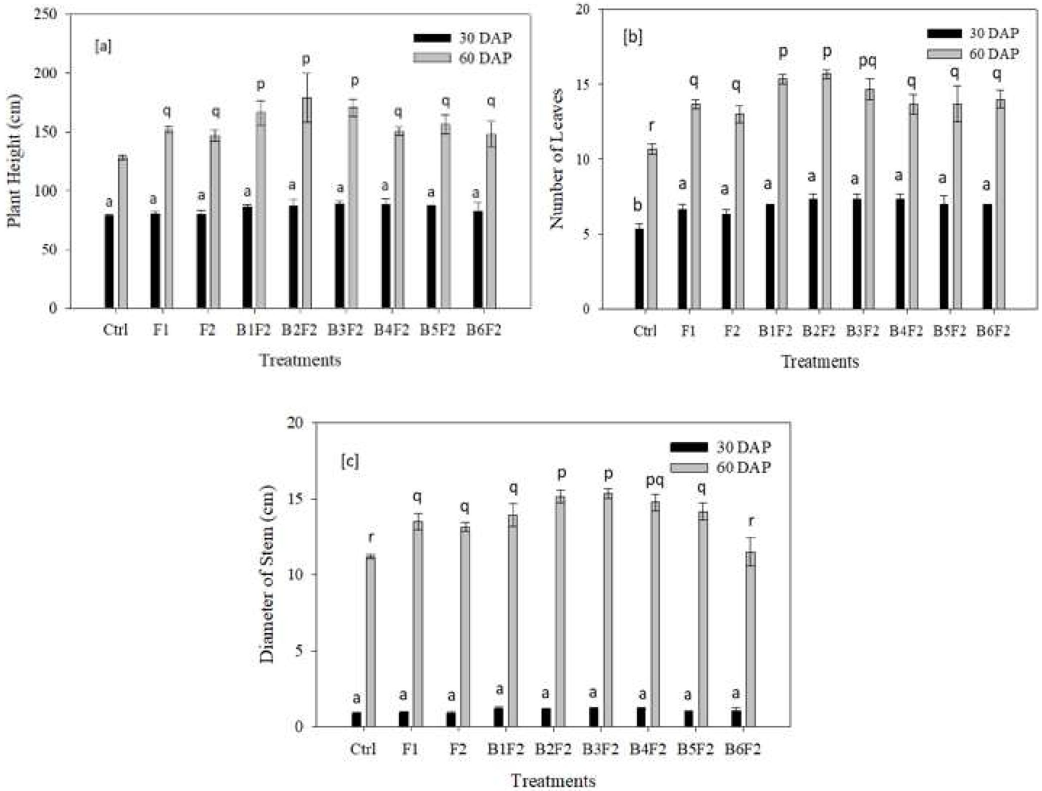

Figure 1. Effect of biostimulant formula and inorganic NPK fertilizer on maize plant height (a), number of leaves (b), and diameter of stem (c) at 30 and 60 d after planting (DAP). Ctrl: Control (without NPK or biostimulants); F1: 100% NPK (300 kg ha^{-1} NPK + 150 kg ha^{-1} urea); F2: 75% NPK; B1F2: 10% rice husk ash (RHA), 80% golden apple snail (GAS), 10% plant growth-promoting rhizobacteria (PGPR) + 75% NPK; B2F2: 20% RHA, 70% GAS, 10% PGPR + 75% NPK; B3F2: 30% RHA, 60% GAS, 10% PGPR + 75% NPK; B4F2: 45% RHA, 45% GAS, 10% PGPR + 75% NPK; B5F2: 60% RHA, 30% GAS, 10% PGPR + 75% NPK; B6F2: 70% RHA, 30% GAS + 75% NPK. Values followed by the same letter are not significantly different based on LSD test ($p < 0.05$).

Yield components

Applying of formulas B1, B2, and B3 combined with 75% inorganic NPK fertilizer showed higher cob weight and cob diameter than the 100% NPK treatment (Table 3). In contrast, the number of seeds per row, dry biomass weight, number of grains per cob, and weight of 100 seeds did not significantly differ from 100% NPK. It is known that formulas B1, B2, and B3 contain 80%, 70%, and 60% golden apple snail (GAS) extract respectively.

The impact of biostimulant formula and dose of inorganic NPK fertilizer on maize yield is illustrated in Figure 2. The application of this biostimulant formulation and inorganic NPK fertilizer resulted in a significant increase in grain yield compared to the control treatment. This increase in yield is closely related to plant growth and can be influenced by improvements in soil chemistry due to the application of biostimulants and inorganic NPK fertilizers. The growth of vegetative organs significantly impacts crop yields, as greater vegetative growth, acting as a source of assimilation, can contribute to better resource utilization, such as water and nutrients, further supporting the development of robust plants with higher yield potential. By optimizing vegetative organ growth, farmers can enhance the productivity and quality of their crops, ultimately maximizing yields and ensuring a successful harvest.

Table 3. Effect of biostimulant formula and inorganic NPK fertilizer on maize yield components. Control (without NPK or biostimulants); F1: 100% NPK (300 kg ha^{-1} NPK + 150 kg ha^{-1} urea); F2: 75% NPK; B1F2: 10% rice husk ash (RHA), 80% golden apple snail (GAS), 10% plant growth-promoting rhizobacteria (PGPR) + 75% NPK; B2F2: 20% RHA, 70% GAS, 10% PGPR + 75% NPK; B3F2: 30% RHA, 60% GAS, 10% PGPR + 75% NPK; B4F2: 45% RHA, 45% GAS, 10% PGPR + 75% NPK; B5F2: 60% RHA, 30% GAS, 10% PGPR + 75% NPK; B6F2: 70% RHA, 30% GAS + 75% NPK. Values followed by the same letter are not significantly different based on LSD test ($p < 0.05$).

Treatments	Cob weight	Rows per cob	Seeds per row	Weight 100 seeds	Cob diameter
	g	Nr cob^{-1}	Nr row^{-1}	g	mm
Control	42.67^{c}	12.00^{a}	14.67^{c}	20.67^{b}	24.41^{c}
F1 100%	99.00^{b}	13.00^{a}	30.00^{a}	27.00^{a}	32.60^{b}
F2 75%	86.00^{b}	12.33^{a}	23.33^{b}	25.67^{a}	30.94^{b}
B1F2	131.33^{a}	13.67^{a}	$32,33^{a}$	26.33^{a}	38.29^{a}
B2F2	104.33^{a}	12.00^{a}	31.67^{a}	27.67^{a}	36.70^{a}
B3F2	123.67^{a}	13.00^{a}	33.33^{a}	29.67^{a}	36.39^{a}
B4F2	92.67^{b}	14.00^{a}	25.67^{b}	25.00^{a}	33.73^{b}
B5F2	91.33^{b}	13.33^{a}	24.67^{b}	27.67^{a}	32.71^{b}
B6F2	91.33^{b}	13.00^{a}	24.00^{b}	24.67^{a}	32.76^{b}

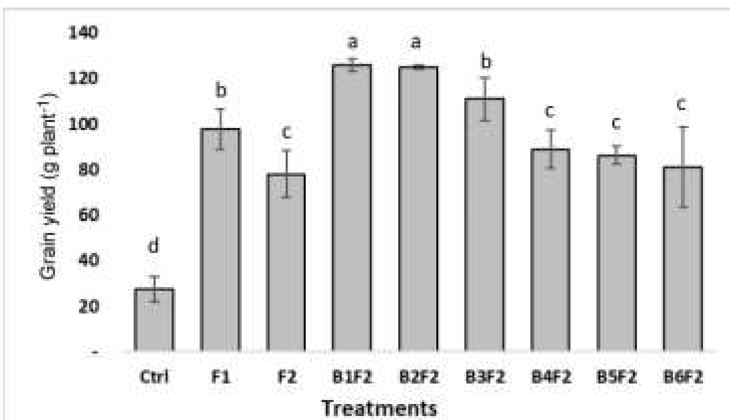

Figure 2. Effect of biostimulant formula and inorganic NPK fertilizer on maize grain yield. Ctrl: Control (without NPK or biostimulants); F1: 100% NPK (300 kg ha^{-1} NPK + 150 kg ha^{-1} urea); F2: 75% NPK; B1F2: 10% rice husk ash (RHA), 80% golden apple snail (GAS), 10% plant growth-promoting rhizobacteria (PGPR) + 75% NPK; B2F2: 20% RHA, 70% GAS, 10% PGPR + 75% NPK; B3F2: 30% RHA, 60% GAS, 10% PGPR + 75% NPK; B4F2: 45% RHA, 45% GAS, 10% PGPR + 75% NPK; B5F2: 60% RHA, 30% GAS, 10% PGPR + 75% NPK; B6F2: 70% RHA, 30% GAS + 75% NPK. Different letters indicate significantly different mean LSD test $p < 0.05$.

The higher grain yield was attained through the combination of biostimulant formulas B1 (10% RHA, 80% GAS, 10% PGPR), B2 (20% RHA, 70% GAS, 10% PGPR), and B3 (30% RHA, 60% GAS, 10% PGPR) with 75% of the recommended dose of inorganic NPK fertilizer (treatments B1F2, B2F2, and B3F2), reaching 126.0, 125.0, and 111.0 g $plant^{-1}$, respectively (Figure 2). Conversely, the maize yield in the recommended inorganic NPK fertilizer treatment (F1) reached only 97.67 g $plant^{-1}$. It means that the biostimulant produced can reduce the use of inorganic NPK fertilizer by 25%. Grain yield produced by applying formulas B4, B5, and B6 containing less than 50% GAS extract was less than 90 g $plant^{-1}$. Amino acids derived from GAS extract play a significant role in enhancing maize growth and yield. The higher proportion of amino acids from snail extract appears to be more significant in enhancing maize growth and yield.

Amino acid content of golden apple snail extract

The GAS extract contains 16 amino acids in relatively high concentrations, nine of which are essential (Table 4): L-Alanine, L-arginine, L-aspartic acid, glycine, L-glutamic acid, L-histidine, L-isoleucine, L-leucine, L-lysine, L-valine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-tryptophan. Among these, L-glutamic acid is the most abundant non-essential amino acid, with a concentration of 2557 mg kg^{-1}, as reported by Ernawati and Rosida (2022). Generally, the high amino acid content in the extract serves as an active biostimulant, positively impacting plant growth. Amino acids such as L-alanine, L-arginine, L-aspartic acid, glycine, and L-leucine exceed 1000 mg kg^{-1}, while the others are below 1000 mg kg^{-1}. L-Tryptophan was detected in the GAS extract, though it is the smallest amount at 133.28 mg kg^{-1}.

Table 4. Amino acid content of golden apple snail extract.

Amino acid	Total (mg kg^{-1})
L-Alanin	1164.49
L-Arginine	1070.92
L-Aspartic acid	1607.03
Glycine	1536.27
L-Glutamic acid	2557.71
L-Histidine	374.04
L-Isoleucine	775.48
L-Leucine	1486.06
L-Lysine	891.04
L-Valine	949.07
L-Phenylalanine	847.42
L-Proline	950.33
L-Serine	854.76
L-Threonine	996.18
L-Tyrosine	496.73
L-Tryptophan	133.28

Amino acids play integral roles in maize growth, development, and stress response. Each amino acid has specific functions that contribute to various physiological processes within the plant. L-Alanine is involved in N metabolism and serves as a building block for other amino acids and proteins essential for maize growth (Heinemann and Hildebrandt, 2021). Amino acid L-arginine plays a crucial role in synthesizing nitric oxide, which regulates stress responses and pathogen defence in maize (Miller, 2010).

Glycine is important for synthesizing glutathione, an antioxidant that helps maize cope with oxidative stress; L-histidine is essential for synthesizing histamine, which aids in regulating stress responses and defence mechanisms in maize plants (Miller, 2010).

The branched-chain amino acids (L-isoleucine, L-leucine, and L-valine) are crucial for protein synthesis and have roles in regulating maize growth and development. They help in maintaining the balance of N and energy metabolism, thus influencing stress tolerance and adaptation (Miller, 2010).

L-Proline acts as an osmoprotectant, aiding in stress tolerance under conditions of dehydration and salinity by stabilizing proteins and cellular structures; L-threonine is vital for protein synthesis and enzyme function, influencing maize growth and stress responses (Miller, 2010). L-Tryptophan, in particular, is a precursor for the synthesis of indole-3-acetic acid (IAA), a crucial plant hormone regulating growth and development by influencing cell elongation, division, and differentiation (Miller, 2010).

DISCUSSION

The biostimulant used in this research consists of three components: Rice husk ash (RHA), golden apple snail (GAS) extract, and plant growth-promoting rhizobacteria (PGPR). Combining RHA, GAS extract, PGPR, and a 25% reduction in NPK fertilizer significantly enhanced maize growth and production. It indicates that biostimulants provide an effective strategy to reduce reliance on inorganic fertilizers without compromising crop productivity. The biostimulant formula used in this experiment showed a significant increase in plant height, leaf number, stem diameter, and grain yield compared to applying NPK fertilizers at recommended doses and control treatments.

The study found that RHA primarily contains 11.63% SiO_2 and 10.20% K_2O. According to França et al. (2017), Si and K are crucial for plant growth, root health, and stress resistance. Silicon (strengthens plant defence mechanisms against pathogens and reinforces cell walls, reducing pest damage, while K enhances carbohydrate synthesis, protein production, and overall plant quality. Research by Saranya et al. (2018) confirms that RHA promotes maize growth and yield by raising soil pH and improving nutrient availability and hydrophysical properties.

In addition, RHA enhances soil structure, aeration, and water-holding capacity, further boosting maize growth and productivity. It contains vital elements such as Si, Ca, Mg, P, K, and Fe, which contribute to improving soil quality and plant nutrition. Combined with inorganic NPK fertilizers, it helps maintain nutrient balance in the soil, promoting better maize growth. Studies have shown that rice husk biochar application can increase maize grain yield by 60% to 80% at higher doses. Furthermore, rice husk application is linked to improvements in stem diameter, cob length, and leaf area index, with the high Si content facilitating nutrient uptake and enhancing photosynthesis, disease resistance, and stress tolerance (Islam et al., 2018; Karam et al., 2022).

The GAS extract contains 16 amino acids, including essential ones that benefit plant growth through various mechanisms. For instance, L-tryptophan is a precursor to indole acetic acid (IAA), a hormone that promotes lateral root development (Naveed et al., 2015). In this study, glycine, the most abundant amino acid in the extract at 1536.27 mg kg^{-1}, enhances maize growth by improving nutrient uptake, photosynthesis, stress resistance, and overall metabolism (Miller, 2010). Its application is handy for optimizing crop production in challenging environments, such as the acidic soils of tidal swamp lands.

The microbial inoculum in this biostimulant consists of a consortium of N_2-fixing bacteria, P-solubilizing microbes, and IAA-producing bacteria, all well-adapted to acidic soil conditions in wetlands. The combination of GAS extract and PGPR promotes maize growth by enhancing nutrient uptake, stimulating development, and protecting plants from environmental stressors (Kumari et al., 2023). Similarly, Mukhlis and Lestari (2013) found that biofertilizers containing decomposers, N_2-fixing bacteria, and P-solubilizing bacteria, when combined with conventional NPK fertilizers, improved sweet corn growth and soil fertility in acid-sulphate soils of tidal swamplands

CONCLUSIONS

The combination of rice husk ash (RHA), golden apple snail (GAS) extract, and beneficial microbes with traditional inorganic NPK fertilizers has shown promise in enhancing maize growth and yield in tidal swamp lands. The biostimulant formulas containing over 50% GAS extract exhibit higher maize growth and grain yield while concurrently reducing inorganic NPK fertilizer usage by up to 25%. Remarkably, the use of biostimulant formula 10% RHA, 80% GAS, 10% plant growth-promoting rhizobacteria (PGPR); 20% RHA, 70% GAS, 10% PGPR; and 30% RHA, 60% GAS, 10% PGPR, in combination with 75% of the recommended NPK dosage, led to superior maize growth and yields compared to other treatments.

Author contributions

Conceptualization: M.M., E.P., S.N. Methodology: M.M., E.P., E.M. Software: N.W., S.N. Validation: E.M., M.M., A.P. Formal analysis: E.P., E.M., M.M. Investigation: M.M., E.P., S.N., E.M, Y.L., S.A., Y.L., R.A., Resources: E.P., S.N., A.P. Data curation: S.N., N.W. Writing-original draft: M.M., E.P, S.N. Writing-review & editing: M.M., E.P., E.M., S.N., Y.L., S.A. Visualization: N.W., A.P. Supervision: M.M., E.P. Project administration: R.A., S.N. Funding acquisition M.M., E.M. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgement

This work was funded by National Research and Innovation Agency, Republic of Indonesia (BRIN).

References

Alwi, M., Wahida, A.Y., Fahmi, A. 2021. Improving productivity of rice yield on tidal swampland using soil amendment. IOP Conference Series: Earth and Environmental Science 648:012141.

Badrudin, U., Ghulamahdi, M., Purwoko, B.S., Pratiwi, E. 2024. Optimization of saline inundated soil for rice production using microbes (Optimasi Tanah Salin Tergenang untuk Produksi Padi Sawah dengan Mikroba). Dissertation. Bogor Agricultural University, Bogor, Indonesia.

Baltazar, M., Correia, S., Guinan, K.J., Sujeeth, N. 2021. Recent advances in the molecular effects of biostimulants in plants: An overview. Biomolecules 11(8):1096. doi:10.3390/biom11081096.

Bowen, J.L., Crump, B.C., Deegan, L.A., Hobbie, J.E. 2009. Salt marsh sediment bacteria: Their distribution and response to external nutrient inputs. The ISME Journal 3(8):924-934. doi:10.1038/ismej.2009.44.

BPS – Statistics Indonesia. 2022. The 2021 Analysis of maize and soybean productivity in Indonesia. BPS – Statistics Indonesia, Jakarta, Indonesia.

BPSI Tanah dan Pupuk. 2023. Chemical analysis of soil, plants, water and fertilizer (Analisis Kimia Tanah, Tanaman, air, dan Pupuk). Technical Manual Edition. Kementerian Pertanian Republik Indonesia. Available at https://tanahpupuk.bsip.pertanian.go.id.

dos Santos, R.M., Diaz, P.A.E., Lobo, L.L.B., Rigobelo, E.C. 2020. Use of plant growth-promoting rhizobacteria in maize and sugarcane: Characteristics and applications. Frontiers in Sustainable Food Systems 4:136. doi:10.3389/fsufs.2020.00136.

Ernawati, D., Rosida, D.F. 2022. The physicochemical properties of flavor enhancer made from different types of snail protein hydrolysates. International Journal on Food, Agriculture and Natural Resources 3(1):1-7.

França, A.A., Schultz, J., Borges, R., Wypych, F. 2017. Rice husk ash as raw material for the synthesis of silicon and potassium slow-release fertilizer. Journal of the Brazilian Chemical Society 28:2211-2217.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D. 2015. The use of biostimulants for enhancing nutrient uptake. Advances in Agronomy 130:141-174.

Heinemann, B., Hildebrandt, T.M., 2021. The role of amino acid metabolism in signaling and metabolic adaptation to stress-induced energy deficiency in plants. Journal of Experimental Botany 72(13):4634-4645.

Islam, S.M.J., Mannan, M.A., Khaliq, Q.A., Rahman, M.M. 2018. Growth and yield response of maize to rice husk biochar. Australian Journal of Crop Science 12(12):1813-1819.

Karam, D.S., Nagabovanalli, P., Rajoo, K.S., Ishak, C.F. 2022. An overview on the preparation of rice husk biochar, factors affecting its properties, and its agriculture application. Journal of the Saudi Society of Agricultural Sciences 21(3):149-159.

Khamidah, N., Saputra, R.A. 2020. Soil acidity mapping of a swampland planted with rice in Ampukung Village, Kelua District, Tabalong Regency. Tropical Wetland Journal 6(2):50-54.

Kumari, M., Swarupa, P., Kesari, K.K., Kumar, A. 2023. Microbial inoculants as plant biostimulants: A review on risk status. Life 13(1):12. doi:10.3390/life13010012.

Ma, Y., Freitas, H., Dias, M.C. 2022. Strategies and prospects for biostimulants to alleviate abiotic stress in plants. Frontiers in Plant Science 13:1024243.

Maftu'ah, E., Nurzakiah, S., Sulaeman, Y., Lestari, Y. 2023. Use of humic and silica materials as soil ameliorant to improve the chemical properties of acid sulphate soil. IOP Conference Series: Earth and Environmental Science 1162:012002.

Miller, A. 2010. Plant nitrogen nutrition and transport. Encyclopedia of Life Sciences, John Wiley & Sons, Chichester, UK. doi:10.1002/9780470015902.a0021257.

Mukhlis, Lestari, Y. 2013. Effects of biofertilizer "M-STAR" on land productivity and growth of sweet corn in acid sulphate soil of swampland. Journal AGRIVITA 35(3):242-248.

Naveed, M., Qureshi, M.A., Zahir, Z.A., Hussain, M.B. 2015. L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by Burkholderia phytofirmans PsJN. Annals of Microbiology 65:1381-1389. doi:10.1007/s13213-014-0976-y.

Nurshanti, D.F., Defrian, D., Novriani, N. 2021. Growth and yield of okra using bio-stimulant of golden apple snails extracts and fertilizer on Ultisol. Jurnal Lahan Suboptimal: Journal of Suboptimal Lands 10(1):37-45.

Pratiwi, E., Saraswati, R., Nursyamsi, D. 2016. The current status and development of biofertilizers in Indonesia. International Conference on Biofertilizers and Biopesticides 3-5:31-39.

Rathore, S.S., Chaudhary, D.R., Boricha, G.N., Ghosh, A. 2009. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (Glycine max) under rainfed conditions. South African Journal of Botany 75(2):351-355. doi:10.1016/j.sajb.2008.10.009.

Rouphael, Y., Colla, G. 2020. Biostimulants in agriculture. Frontiers in Plant Science 11:40. doi:10.3389/fpls.2020.00040.

Saranya, P., Sri Gayathiri, C.M., Sellamuthu, K.M. 2018. Potential use of rice husk ash for enhancing growth of maize (*Zea mays*). International Journal of Current Microbiology and Applied Sciences 7(3):899-906. doi:10.20546/ijcmas.2018.703.105.

Severo, F.F., da Silva, L.S., Moscôso, J.S.C., S., Qamar, Rodrigues Júnior, L.F., Lopes, A.F., et al. 2020. Chemical and physical characterization of rice husk biochar and ashes and their iron adsorption capacity. SN Applied Sciences 2:1286. doi:10.1007/s42452-020-3088-2.

Sulaiman, A.A., Sulaeman, Y., Minasny, B. 2019. A framework for the development of wetland for agricultural use in Indonesia. Resources 8(1):34. doi:10.3390/resources8010034.

Sumadi, K., Muljani, S. 2016. Pupuk Kalium Silika ($K_2O.SiO_2$) berbahan baku geothermal sludge dengan metode gelling. Seminar Nasional Teknik Kimia Soebardjo Brotohardjono XII, Surabaya, 1 Juni 2016.

Wang, J., Lu, X., Zhang, J., Ouyang, Y. 2020. Using golden apple snail to mitigate its invasion and improve soil quality: a biocontrol approach. Environmental Science and Pollution Research 27:14903-14914. doi:10.1007/s11356-020-07998-9.

Yuliatin, E., Rosadi, I., Hariani, N., Oktavianingsih, L. 2023. The ecological significance of plant growth promoting rhizobacteria in tropical soil Kalimantan: A narrative review. Journal of Tropical Life Science 13(20):407-420. doi:10.11594/jtls.13.02.20.