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The Influence of Various Biostimulant Formulas Supplemented with Microbes and Their Application Frequency on Corn Productivity in Tidal Swamplands

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Abstract.
Corn is the second most important food commodity in Indonesia. However, its production is lower than the demand. This problem can be overcome using suboptimal land, such as tidal swampland, with its naturally low productivity. The soil quality in such areas can be improved by applying biostimulant. Therefore, this study aims to examine various biostimulant formulas supplemented with microbes and their application frequency on corn productivity in tidal swamplands. It was conducted on a pot experiment using a completely randomized block design with three replications. The treatments were six formulas of biostimulant, each with three application frequencies. The biostimulant materials included rice husk ash, golden apple snail extract, and microbes serving as P-solubilizers, N2-fixers, and IAA phytohormone. The results revealed that biostimulant formulas can increase the production of corn. The B3 formula, followed by the B4 and B5 formula, led to superior grain yields compared to the other treatments. In comparison, the most effective application frequency was observed with four times application (D2) followed by five times application (D3) as the next best option. These biostimulant formulas need to be tested in the field to know their effectiveness in tidal swamplands.

Keywords: corn productivity, biostimulant formulas, tidal swamplands, microbial supplements

1. Introduction

Corn (Zea mays L) currently holds significant national importance as the second most important source of carbohydrates after rice. The demand for corn continues to grow in parallel with population expansion and the need for raw animal feed materials.

The government has implemented measures to boost corn production, including land expansion and intensification. However, due to the diminishing agricultural land due to rapid conversion into non-agricultural purposes and the predominant use of dry land outside of Java for plantations, tidal swamp land is seen as a strategic alternative to increase corn production.

Indonesia possesses approximately 8.92 million hectares of tidal swamp land [1]. Nonetheless, these tidal swamp lands are considered suboptimal due to their naturally low productivity. Corn yield in tidal swamp land stands at 55.97 kg ha^{-1}, which is lower than the national average productivity of 57.09 kg ha^{-1} [2]. Effective management of tidal swamp lands for corn cultivation, considering the biophysical characteristics of the land, socioeconomic and cultural factors, and the utilization of suitable technology to address the unique challenges of these lands, is expected to enhance corn productivity in tidal swamp areas. This approach aims to provide a sustainable source of income for farmers while maintaining environmental safety.

There is a growing trend in the use of biostimulants to improve plant growth, particularly in horticultural and certain food crops [3]. Biostimulants are preferred for their safety, ease of application, and cost-effectiveness [4]. Additionally, biostimulants can be an alternative to excessive use of inorganic fertilizers and enhance mineral absorption [5].

Tidal swamp land is rich in plant and animal resources that can be harnessed for biostimulant production. In tidal swamp areas, agricultural byproducts like rice husks have yet to be fully utilized or reintegrated into the land. Rice husks are particularly noteworthy for their high silica (Si) content. In Indonesia, the untapped potential of rice husk waste is considerable. These husks are a natural silica (SiO_2) source with a wide range of benefits and significant economic value. Silicon (Si) has exhibited advantageous effects not only in corn but also in other crops such as durum wheat, sorghum, cotton, tobacco, rice, cucumber, and coffee, as evidenced by various studies [6–12].

Moreover, tidal swampland often hosts abundant golden apple snails (Pomaceae canaliculate), which can disrupt plant growth and result in significant losses. However, both the flesh and shells of golden apple snails contain essential macro-nutrients such as nitrogen, phosphorus, potassium and micro-nutrients like calcium. Furthermore, it contains hormones that are beneficial for plant growth [13].

Additionally, tidal swamp land boasts a high biodiversity of beneficial microorganisms. Most soil microorganisms benefit plants by aiding in nutrient provision or availability, facilitating optimal plant growth. Several types of microorganisms in Indonesian tidal swamp areas are known for their high capabilities in decomposing organic matter, fixing N_2, and solubilizing phosphate from Ca-P and Al-P complexes in the soil. They also exhibit tolerance to high soil acidity [14].

Nonetheless, information regarding suitable biostimulant formulas and their effectiveness for tidal swamp land conditions is limited. As a product that enhances plant growth and production, finding the right and effective biostimulant formula is crucial for increasing land and corn productivity in tidal swamp land. Therefore, this study aims to examine various biostimulant formulas based on golden apple snail and rice husk ash extract, supplemented with microbes, and their application frequency on corn productivity in tidal swamp lands.

2. Material and Methods

2.1. Bacterial culture and biostimulant

The bacterial strains used were Bacillus marisflavi U7, Bacillus aerius U21, Bacillus aryabhattai U32, Bacillus subtilis P183, and Azotobacter vinelandii 1CM. All the bacterial strains were checked previously for their plant growth-promoting rhizobacteria properties, such as N_2-fixer, P-solubilizer, and IAA phytohormones. These activities demonstrated the potential of these bacteria to be used as biofertilizers. The biostimulant materials used included rice husk ash extract as a source of silica and potassium, golden apple snail extract as a source of amino acids.

2.2. Site description and experimental design

The study was conducted as a pot experiment from April to August 2023 in Banjarbaru Regency, South Kalimantan. Soil analysis before the experiment was conducted for pH, organic carbon (C-organic), total nitrogen (N-total), extractable phosphorus (P-extractable), exchangeable potassium (K-exchangeable), exchangeable calcium (Ca-exchangeable), exchangeable magnesium (Mg-exchangeable), and exchangeable sodium (Na-exchangeable) at the Soil Laboratory of the Faculty of Agriculture, Lambung Mangkurat University.

The experimental design was a randomized complete block design (RCBD) with three replications. The treatments were as follows:

Control (no biostimulant)

Formula B1 (rice husk ash 44%, golden apple snail 44%, microbe inoculant 12%)

Formula B2 (rice husk ash 59%, golden apple snail 29%, microbe inoculant 12%)

Formula B3 (rice husk ash 22%, golden apple snail 66%, microbe inoculant 12%)

Formula B4 (rice husk ash 30%, golden apple snail 58%, microbe inoculant 12%)

Formula B5 (rice husk ash 13%, golden apple snail 75%, microbe inoculant 12%)

Formula B6 (rice husk ash 67%, golden apple snail 33%).

The application frequency treatments were carried out as follows: a) Three times (15, 40, and 65 days after planting - DAP), b) Four times (15, 35, 55, and 75 DAP), c) Five times (15, 30, 45, 60, and 75 DAP).

The acid sulphate soil was collected from tidal swamp areas in Barambai Kolam Kiri village, Barambai District, Batola Regency, South Kalimantan Province. This soil was placed into experimental pots at a rate of 5 kg per pot. Before applying any treatments, soil analysis revealed that the soil was highly acidic and nutrient-deficient (see Table 1). The pH in H_2O was 3.40, and the pH in KCl was 3.30, indicating high soil acidity. The total nitrogen (N) content was 0.30%, considered low. Exchangeable potassium (K) was at 2.24 cmol(+) kg^{-1}, classifying it as very low. Exchangeable calcium (Ca) was also very low at 1.87 cmol(+) kg^{-1}. Exchangeable sodium (Na) stood at 1.01 cmol(+) kg^{-1}, which is a moderate level, and magnesium (Mg) was at 1.95 cmol(+) kg^{-1}, indicating a low concentration. Considering these soil characteristics, it is necessary to apply chemical, organic, and biofertilizers to enhance corn and soil productivity sustainably.

2.3. Application of bacterial inocula, biostimulant and NPK fertilizer

Bacterial inocula were prepared by growing pure bacterial cultures in Nutrient Broth. The inoculated broth was placed on a rotary shaker at 100 rpm and 30°C for 24 to 48 h. All inoculants are mixed as a consortium before being applied to seeds.

Biostimulant formulas were applied by spraying onto the soil and plants according to the treatment plan. The inorganic NPK fertilizer was applied at 150 kg of urea per hectare and 225 kg of compound NPK per hectare. Inorganic NPK fertilizer was applied

TABLE 1: The initial soil characteristics of tidal swamp lands.

Soil characteristics	Value
pH H_2O	3.40
pH KCl	3,20
C-org (%)	4,31
N-total (%)	0.30
C/N ratio	14,36
P-total (%)	39.16
K-total (%)	16.30
P-extractable (ppm)	73,80
K-exchangeable ($cmol_+ kg^{-1}$)	2,24
Ca-exchangeable ($cmol_+ kg^{-1}$)	1,87
Mg-exchangeable ($cmol_+ kg^{-1}$)	1,95
Na-exchangeable ($cmol_+ kg^{-1}$)	1,01
CEC ($cmol\ kg^{-1}$)	29,48

in two stages: one-third of the dose was administered at 10 DAP, and the remaining two-thirds were applied at 30 DAP. Pest and disease control were carried out intensively using insecticides and fungicides. The corn variety used was BISI 329.

2.4. Data processing and statistical analysis

The parameters observed included plant height, the number of leaves, stem diameter at 60 DAP, and plant dry weight and yield. The data obtained were analyzed using the SAS package (SAS Institute Inc.) for analysis of variance (ANOVA), and significant differences between treatments were assessed using Duncan's multiple range test ($p < 0.05$).

3. Results and Discussion

3.1. Characteristics of rice husk ash and golden apple snail extract

The silica in rice husk ash can be extracted by dissolving it in an alkaline solution, resulting in a potassium silicate solution. Laboratory analysis reveals that the rice husk ash extract contains high levels of silica and potassium, as detailed in Table 2. The extract contains 11.63% silica and 10.20% potassium. Additionally, it contains 0.04%

calcium, 0.05% magnesium, 0.13% phosphorus, 0.02% iron, and 3,902.214 mg kg^{-1} of sodium.

Silica (Si) and potassium (K) are essential for plants, including corn. Silica enhances root oxidation, increases enzyme activity in photosynthesis, and thickens cell walls to protect against pests. Meanwhile, potassium plays a crucial role in carbohydrate formation and transportation, acts as a catalyst in protein synthesis, raises carbohydrate and sugar levels in fruits, and improves fruit quality in shape, content, and color compared to those without potassium supplementation [15].

Table 2: Characteristics of rice husk ash extract.

Parameters	Nutrients contain
Silica oxide (SiO2)	11.63 %
Calcium oxide (CaO)	0.04 %
Magnesium oxide (MgO)	0.05 %
Phosphorus pentoxide (P2O5).	0.13 %
Potassium oxide (K2O).	10.20 %
Iron oxide (Fe2O3).	0.02 %
Sodium oxide (Na2O).	3,902.214 $mgkg^{-1}$

Laboratory analysis revealed that the golden apple snail extract contains 16 types of amino acids, with nine classified as essential amino acids (see Table 3). The essential amino acids in the extract include arginine, histidine, isoleucine, leucine, lysine, valine, phenylalanine, threonine, and tryptophan. Tryptophan, in particular, serves as an initiator compound for the formation of auxin and can be utilized as a growth regulator substance. Amino acids offer various benefits to plants, including enhancing photosynthesis, increasing resistance to stressors such as high temperatures, low humidity, drought, pest and disease attacks, and promoting the metabolism of plant growth.

3.2. Corn growth and productivity

The analysis results for plant height, the number of leaves, and stem diameter at 60 DAP are presented in Figures 1, 2, and 3. The results demonstrate that the biostimulant formulas and their application frequency significantly improved plant height, the number of leaves, and stem diameter compared to the control treatment. Among the biostimulant formulas and their application frequency treatments, a significant increase in plant height was observed for the B4, B5, and B3 formulas. However, no significant differences were found among the biostimulant formulas and their application frequency treatments for the number of leaves and stem diameter. While there were no significant differences in application frequency, the highest values for plant height, the number of leaves, and stem diameter were observed in the four-times treatment (D2), followed by the five-times treatment (D3).

TABLE 3: Characteristics of golden apple snail extract.

No.	Parameter	Amino acid contain
1	L-Alanine (mg kg^{-1})	1164.49
2	L-Arginine (mg kg^{-1})	1070.92
3	L-Aspartic Acid (mg kg^{-1})	1607.03
4	Glycine (mg kg^{-1})	1536.27
5	L-Glutamic Acid (mg kg^{-1})	2557.71
6	L-Histidine (mg kg^{-1})	374.04
7	L-Isoleucine (mg kg^{-1})	775.48
8	L-Leucine (mg kg^{-1})	1486.06
9	L-Lysine (mg kg^{-1})	891.04
10	L-Valine (mg kg^{-1})	949.07
11	L-Phenylalanine (mg kg^{-1})	847.42
12	L-Proline (mg kg^{-1})	950.33
13	L-Serine (mg kg^{-1})	854.76
14	L-Threonine (mg kg^{-1})	996.18
15	L-Tyrosine (mg kg^{-1})	496.73
16	L-Tryptophan (mg kg^{-1})	133.28

Plant dry weight reflects the accumulation of assimilates resulting from plant growth activity. The results showed a significant increase in treatments involving biostimulant formulas and their application frequency compared to the control (see Table 4). The highest plant dry weight was observed in the B5 formula treatment, followed by the B4 and B3 treatments. The most effective application frequency was four times (D2), followed by five times (D3).

Remarks: B1: 44% rice husk ash, 44% golden apple snail; 12% microbes, B2: 59% rice husk ash, 29% golden apple snail; 12% microbes, B3: 12% rice husk ash, 66% golden apple snail; 12% microbes, B4: 30% rice husk ash, 58% golden apple snail; 12% microbes, B5: 13% rice husk ash, 75% golden apple snail, 12% microbes, and B6: 33% rice husk ash, 67% golden apple snail). D1: three times application; D2: four times application;

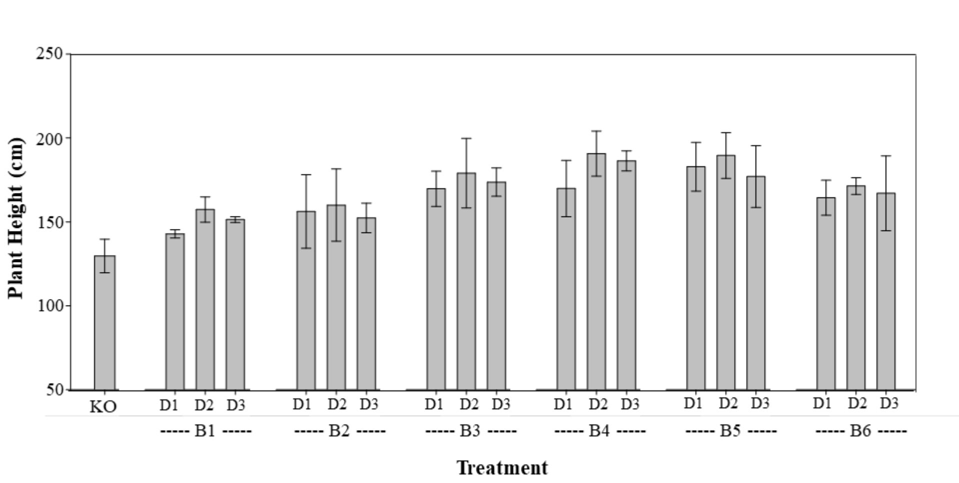

Figure 1: Effect of biostimulant formulas and application frequency on corn plant height at 60 DAP. B1: 44% rice husk ash, 44% golden apple snail; 12% microbes, B2: 59% rice husk ash, 29% golden apple snail; 12% microbes, B3: 12% rice husk ash, 66% golden apple snail; 12% microbes, B4: 30% rice husk ash, 58% golden apple snail; 12% microbes, B5: 13% rice husk ash, 75% golden apple snail, 12% microbes, and B6: 33% rice husk ash, 67% golden apple snail). D1: three times application; D2: four times application; and D3: five times application.

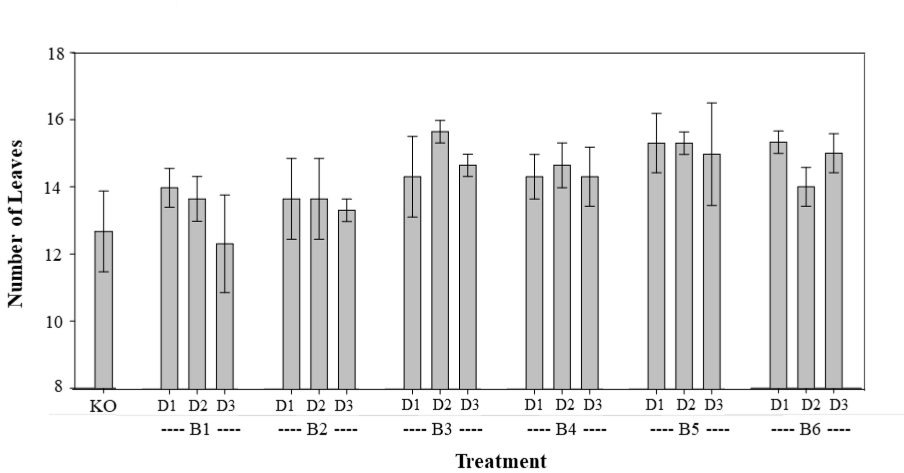

Figure 2: Effect of biostimulant formulas and application frequency on number of corn leaves at 60 DAP. B1: 44% rice husk ash, 44% golden apple snail; 12% microbes, B2: 59% rice husk ash, 29% golden apple snail; 12% microbes, B3: 12% rice husk ash, 66% golden apple snail; 12% microbes, B4: 30% rice husk ash, 58% golden apple snail; 12% microbes, B5: 13% rice husk ash, 75% golden apple snail, 12% microbes, and B6: 33% rice husk ash, 67% golden apple snail). D1: three times application; D2: four times application; and D3: five times application.

and D3: five times application. Values followed by the same letter are not significantly different according to the Duncan's multiple range test ($p < 0.05$).

Remarks: B1: 44% rice husk ash, 44% golden apple snail; 12% microbes, B2: 59% rice husk ash, 29% golden apple snail; 12% microbes, B3: 12% rice husk ash, 66% golden

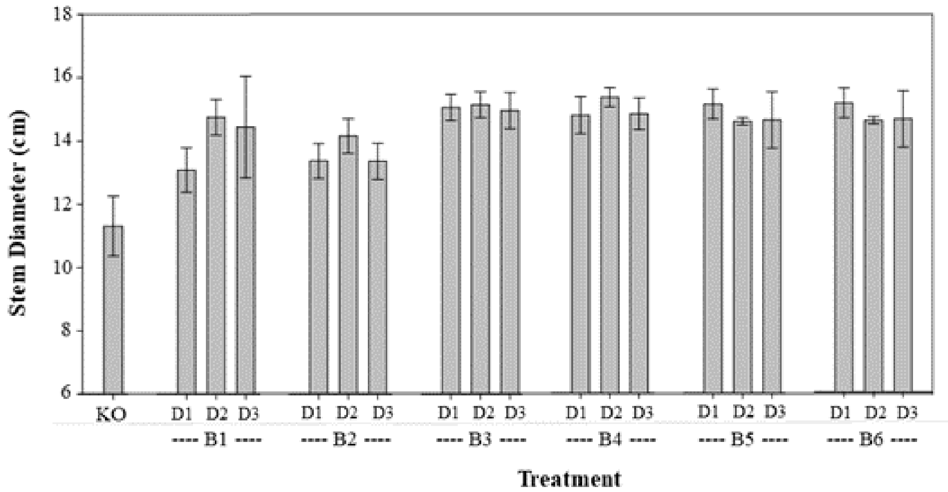

Figure 3: Effect of biostimulant formulas and application frequency on stem diameter at 60 DAP. B1: 44% rice husk ash, 44% golden apple snail; 12% microbes, B2: 59% rice husk ash, 29% golden apple snail; 12% microbes, B3: 12% rice husk ash, 66% golden apple snail; 12% microbes, B4: 30% rice husk ash, 58% golden apple snail; 12% microbes, B5: 13% rice husk ash, 75% golden apple snail, 12% microbes, and B6: 33% rice husk ash, 67% golden apple snail). D1: three times application; D2: four times application; and D3: five times application.

Table 4: Effect of biostimulant formulas and application frequency on plant dry weight (g plant-1) at 60 DAP.

Treatments	**D1**	**D2**	**D3**	**Average**
B1	199,00	214,00	262,00	225,00b
B2	175,67	254,00	251,00	226,89b
B3	216,00	257,33	272,67	248,67a
B4	214,33	314,00	231,00	253,11a
B5	199,67	289,00	274,67	254,45a
B6	208,33	228,00	221,00	219,11b
Average	202,17b	259,39a	252,06a	
Control				132,45c

apple snail; 12% microbes, B4: 30% rice husk ash, 58% golden apple snail; 12% microbes, B5: 13% rice husk ash, 75% golden apple snail, 12% microbes, and B6: 33% rice husk ash, 67% golden apple snail). D1: three times application; D2: four times application; and D3: five times application. Values followed by the same letter are not significantly different according to the Duncan's multiple range test ($p < 0.05$).

In line with the observed pattern in crop growth, the grain yield of corn was significantly influenced by the application of biostimulant formulas and their application frequency (see Table 5). The highest grain yield was achieved with the B3 biostimulant formula, followed closely by B4 and B5. The most effective application frequency was

TABLE 5: Effect of biostimulant formulas and application frequency on corn yield (g plant^{-1}) at 60.

Treatments	D1	D2	D3	Average
B1	60,00	64,00	72,33	65,44b
B2	62,33	69,67	71,33	67,78b
B3	84,00	82,33	79,33	81,89a
B4	71,67	86,67	80,33	79,56a
B5	72,67	77,67	72,00	74,11ab
B6	62,33	65,33	64,67	64,11b
Average	68,83b	74,28a	73,33a	
Control				44,67c

seen with four times application (D2), with five times application (D3) being the next best option. The composition of B3, B4, and B5 formulas primarily consists of golden apple snail extract, making up 66%, 58%, and 75%, respectively. It is evident that the amino acids derived from the golden apple snail extract play a more substantial role in promoting the growth and yield of corn.

This yield increase is closely related to plant growth and may be influenced by the improvement of soil chemical properties due to the application of biostimulants. The growth of vegetative organs significantly impacts crop yields, as greater vegetative growth, acting as a source of assimilation, facilitates the development of reproductive organs (sinks), ultimately leading to higher crop output. Biostimulant formula consists of rice husk ash and golden apple snail extracts supplemented with beneficial microbes. Rice husk ash extract contains mostly silica (Si) and potassium (K) that are essential for plant growth, benefiting root oxidation, enzyme activity in photosynthesis, and cell wall reinforcement to deter pests. Potassium contributes to carbohydrate formation, protein synthesis, and enhances fruit quality. Silica application influences the growth and production of rice plants in terms of plant height during the vegetative phase, grain filling percentage, and the number of empty grains [16]. The utilization of Si increased chlorophyll in leaves, root volume, fresh and dry biomass, and the number of tillers in rice plants [17]. The golden apple snail extract contains nine essential amino acids for plant growth. Amino acids play diverse beneficial roles in plant development owing to their structure as protein units, which play a crucial function in glutamine biosynthesis and as plant growth regulators [18,19]. Kucira [20] state that the frequency and concentration of amino acid biostimulant applications significantly altered both the yield and quality of crops, as well as the nutraceutical and antioxidative potential of soybeans.

Similarly, the microbial inoculum in this biostimulant comprises a consortium of nitrogen-fixing microbes, phosphorus-solubilizing microbes, and IAA phytohormones known to adapt well to acidic soil conditions in wetlands. The application of biofertilizer containing decomposers, nitrogen-fixing bacteria, and phosphorus-solubilizing bacteria, combined with NPK fertilizer, can improve soil fertility in acid sulphate of tidal swamplands and enhance the growth of sweet corn and soybean [14,21]. These materials are more efficient, cost-effective, readily available, and environmentally friendly.

4. Conclusion

The biostimulant formula based on rice husk ash and golden apple snail extract, supplemented with beneficial microbes, can potentially enhance corn growth and yield in tidal swamplands. The highest corn yield (81.89 g plant^{-1}) was achieved with the B3 formula, with no significant difference compared to the B4 (79.56 g plant^{-1}) and B5 (74.11 g plant^{-1}) formulas. The most effective application frequency was observed with four times application (D2), followed by five times application (D3) as the next best option.

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