CORN GROWTH ON GOLD-MINE TAILINGS INOCULATED WITH NITROGEN-FIXING AND PHOSPHATE-SOLUBILIZING BACTERIA

PERTUMBUHAN JAGUNG PADA TAILING TAMBANG EMAS YANG DIINOKULASI BAKTERI PENAMBAT NITROGEN DAN PELARUT FOSFAT

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ABSTRACT

Gold-mine tailings, challenging environment for plant growth, was our study focus. Introducing nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) provides nutrients and phytohormones for plant growth. A pot experiment was designed to assess the corn growth on tailing inoculated with NFB and PSB. The research, conducted in a completely randomized block design, was replicated seven times; the treatments were : without inoculation (control), single inoculation of Azo-7.2, single inoculation of BPF-9, a mixture of Azo-7.2 and BPF-9. The results revealed that inoculation of NFB and PSB significantly increased plant height, stem diameter, leaf number, and P-uptake but did not affect leaf area, chlorophyll content, root length, S/R ratio, N-uptake, and plant biomass, and NFB and PSB count in the rhizosphere. Single inoculants of BPF-9 and mixed inoculants increased plant height by 1.2% to 7%, stem diameter, leaves number, and S/R ratio; only mixed inoculation increased N-uptake, however, Azo-7.2 potential to enhance leaf area, chlorophyll content, and corn biomass. The population of NFB and PSB in the rhizosphere of all treated and control plants was slightly lower than the initial population. The research, in particular, verified that the corn growth on tailings inoculated with NFB and PSB was better than that of uninoculated.

Keywords: Corn, Nitrogen-fixing bacteria, Phosphate-solubilizing bacteria, Tailing

ABSTRAK

Tailing tambang emas yang merupakan tantangan untuk pertumbuhan tanaman, menjadi fokus penelitian ini. Inokulasi bakteri pengikat nitrogen (BPN) dan bakteri pelarut fosfat (BPF) menyediakan nutrisi dan fitohormon yang penting untuk pertumbuhan tanaman. Percobaan pot dirancang untuk mengevaluasi kinerja pertumbuhan jagung (*Zea mays* L.) pada tailing yang diinokulasi dengan BPN dan BPF. Penelitian dilakukan dengan rancangan acak kelompok yang diulang sebanyak tujuh kali; perlakuan percobaan adalah tanpa inokulasi (kontrol) dan dengan inokulasi tunggal BPN Azo-7.2 dan BPF-9 serta campuran Azo-7.2 dan BPF-9. Hasil penelitian menunjukkan bahwa inokulasi BPN dan BPF dengan nyata meningkatkan tinggi tanaman, diameter batang, dan jumlah daun tetapi tidak mempengaruhi luas daun, kandungan klorofil,

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panjang akar, biomassa tanaman, serta jumlah BPN dan BPF di rizosfer. Inokulan tunggal BPF-9 dan inokulan campuran campuran meningkatkan tinggi tanaman 1,2% sampai 7%, diameter batang, jumlah daun, dan rasio S/R secara signifikan. Namun Azo-7.2 berpotensi untuk meningkatkan luas daun, kandungan klorofil, dan biomassa jagung. Populasi BPN dan BPF di rizosfer seluruh tanaman yang diberi perlakuan dan kontrol sedikit lebih rendah dibandingkan populasi awal sebelum percobaan. Penelitian ini, secara khusus, memastikan bahwa performansi pertumbuhan jagung pada tailing yang diinokulasi dengan BPN dan BPF lebih baik dibandingkan dengan tanaman di tailing tanpa inokulasi.

Kata kunci: Bakteri penambat nitrogen, Bakteri pelarut fosfat, Jagung, Tailing

INTRODUCTION

Small-scale gold mining Cineam of Tasikmalaya Regency produces poorly managed tailings, causing a decline in the soil's physical, chemical, and biological properties around the mine. Tailings piled on productive land take a long time to become a good planting medium. The quality of tailings for plant growth may be increased by bioaugmentation of beneficial microbes that increase nutrient availability and stimulate plant growth (Widawati, 2019; (Newsome & Falagán, 2021). Nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) are classified as plant growth and promoting rhizobacteria (PGPR) which colonize the plant rhizosphere or plant tissue as endophytes (Wang et al., 2023; Perez-Cordero et al., 2014). Both groups of bacteria also produce phytohormones that particularly regulate the cell and tissue development (Jnawali et al., 2015; Widawati & Suliasih, 2019; Wang et al., 2023).

Nitrogen (N) is a major essential nutrient for plant growth and development; it is part of all living cells and an important component of amino acids and chlorophyll (Hadayanto, Mudarisna, & Fikri, 2017). The N_2 gas molecules in the atmosphere are not available for plants and must be converted into ammonium (NH_4+) and nitrate (NO_3^-) before being uptake by roots. The free-living NFB, including Azotobacter, convert the N_2 to ammonia (NH_3) through an enzymatic nitrogen fixation catalyzed by nitrogenase (Mahanty et al., 2017). The NH_3 in soil is hydrolyzed in the presence of water to NH_4^+ and further converted enzymatically to NO_3^- nitrification bacteria (Ayiti & Babalola, 2022).

Phosphorus (P) is the second most critical major nutrient after N for storing and transferring energy in the form of ATP and ADP and as a component of nucleic acids, phospholipids, and coenzymes (Handayanto et al., 2017). The potential P levels in tropical soil are quite high, but the P available for plants is limited (Handayanto et al., 2017). Phosphate solubilizing bacteria play an important role in converting poorly soluble P into easily soluble P (Singh, et al., 2017) by producing organic acids and phosphatase (Fujita et al., 2017); (Wang et al., 2023).

Gold tailings elsewhere are usually poor in organic carbon (C), total and available N, and high in C-to-N ratio even though the acidity of tailing is diverse from very acidic to very alkaline (Purwantari, 2007). Enriching tailings with NFB and PSB is expected to increase the N and P content and further plant growth, but organic matter amendment is needed to serve the energy, and organic-C for heterotrophs. Organic matter significant improve soil properties mainly soil porosity (Robinson et al., 2022). Revegetation attempts can be initiated by short-term observation of corn (*Zea mays* L.) growth improvement after exogenous NFB and PSB inoculation. The corn (*Zea mays* L.) is utilized in this experiment due to its significant growth response in the various soil fertility statuses and geographical conditions (Kurniadinata et al., 2017). Moreover, corn is an important global commodity and an important cash crop for Indonesian farmers.

Inoculation of Nitrogen-fixer Azotobacter combined with organic matter enhances the vegetative growth of corn (Ikhsani et al., 2018; Hindersah et al., 2021), however, research about using mixed of NFB and PSB to promote the growth of food crops on tailings in Indonesia has rarely been done. Insignificant effect of NFB and PSB on legume cover crops (non-food crops) grown in Cineam Tailing was observed (Hindersah et al., 2021). Therefore, this pot experiment was conducted to assess the vegetative growth performance of corn on tailings inoculated with single and mixed NFB and PSB inoculants compared to the plant growth without those beneficial microbes.

MATERIALS AND METHODS

Nitrogen Fixing Bacteria (NFB) and Phosphate Solubilizing Bacteria (PSB)

The NFB Azo-7.2 was isolated from the rhizosphere of *Colocasia esculenta*, which was grown in gold mine tailings, while PSB-encoded BPF-9 was obtained from the rhizosphere of *Paspalum conjugatum* grass growing on the overburden of the same mining area. Liquid inoculants of Azo 7.2 and BPF 9 were prepared in N-free Ashby media and nutrient broth, respectively, at the Soil Biology Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran. The Azo-7.2 isolate produced IAA of 1.27 mg L^{-1} while BPF-9 produced IAA of 0.71 mg L^{-1} and phosphatase of 0.36 mg L^{-1}.

Tailing and Goat Manure Properties

The pot experiment was carried out in the greenhouse of the Faculty of Agriculture, Universitas Siliwangi University, Tasikmalaya, Mugarsari campus, Tasikmalaya, from September 2020 to January 2021. The research location was at an altitude of 367 m above sea level. Tailings were taken from artisanal gold mining in Citambal, in Karanglayung Village, Karangjaya District, Tasikmalaya Regency (Figure 1). The tailings contained 2% organic C; 0.02% total-N; 5.03 mg100 g^{-1} P_2O_5 available; 34.67 mg 100 g^{-1} total P_2O_5; 40.33 mg 100 g^{-1} total K_2O; the tailing acidity (pH) was 7.41 with a silty clay texture. The goat manure contained 23.21% organic C; 1.28% total-N; 2.26 % total P_2O_5; 1.76 % total K_2O; the goat manure acidity (pH) was 7.98.

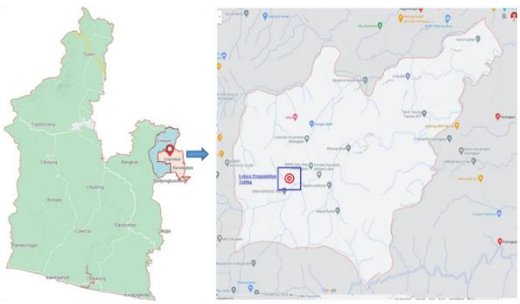


Figure 1. Artisanal gold mine in Karangjaya District, Tasikmalaya Regency

The experiment was designed as a completely randomized block design consisting of four treatments and seven replications. The experimental treatment was without inoculation, with Azo-7.2, BPF-9, and a mix of Azo-7.2 and BPF-9. Each experimental replication consisted of four polybags.

The tailings were air-dried and then sieved using a 2-mm opening sieve. Polybags with 25 cm width x 35 cm height were filled with 3 (three) kg of media composed of tailings mixed with 5% (w/w) goat manure. Each pot was planted with 3 BISI-2 corn seeds at a distance of 3 cm but was not treated with chemical fertilizer. Bacterial inoculation was carried out at planting by pouring 10 mL of liquid inoculant with a cell density of 10^9 CFU mL^{-1}. The volume composition of the mixed inoculant of Azo-7.2 and BPF-9 was equal. Plants were maintained for 42 days in the greenhouse; the water content of the planting medium was maintained at field capacity conditions.

Plant height and number of leaves were measured every two weeks from 14 to 42 days after sowing (DAS), while stem diameter was measured at 28 and 42 DAS. Leaf area, root length, shoot-to-root ratio (S/R), and microbial population in the rhizosphere were analyzed at the end of the experiment. Leaf area was measured using a CID-bioscience leaf area meter, and leaf chlorophyll content was measured using a DUALEX chlorophyll meter. Determination of the bacterial population was performed using the serial dilution plate method using free-N Ashby's mannitol agar for NFB and Nutrient agar for PSB. Data were subjected to analysis of variance with Fisher's F-test of $p < 0.5$ and tested further with the Least Significant Difference Test (LSD) of $p < 0.5$. All statistical analysis was conducted using Sigmastat 3.1 Software.

RESULTS AND DISCUSSION

The NFB and PSB count in tailing and goat manure before the experiment were analyzed (Table 1).

Table 1. The population of total bacteria and fungi, NFB, and PSB in tailing and manure before the experiment

Samples	Microbial count ($CFUg^{-1}$)			
	Total bacteria	Total fungi	NFB	PSB
Tailing	8.4×10^8	5.4×10^4	6.2×10^8	5.9×10^8
Manure	8.1×10^8	1.0×10^4	6.9×10^8	4.5×10^8

Nutrient Deficiency

Without any chemical fertilization, corn plants demonstrated the symptoms of N deficiency, which were characterized by yellow coloration on old leaves at the tips of the leaves and spreading to the middle of the leaf veins (Figure 2a). The plants also exhibited signs of a magnesium deficiency, indicated by the white coloration on both sides of the leaf veins from the bottom to the top of the leaf (Figure 2b).

Nitrogen and Magnesium (Mg) are critical for plant development and growth. Nitrogen is a key component of amino acids, proteins, and chlorophyll biosynthesis (Hadayanto et al., 2017) while Mg is a fundamental part or structure of the chlorophyll molecule (Ishfaq et al., 2022). The tailing is deficient in N and Mg; the ratio of Mg to N for optimal corn growth is 1:6, which provides the best influence on the growth of corn plants (Damanhuri et al., 2022).

Figure 2. Nitrogen (a) and Magnesium (b) deficiency symptoms of corns grown in tailings

Plant growth traits

Inoculation of beneficial microbes significantly influenced plant height, stem thickness, and leaf number. At the ages of 28 and 42 DAS, plants inoculated with NFB and PSB were 1.2% to 7.0% higher than the control (Table 2). However, up to 42 DAS, the height of plants inoculated with a mixture of Azo-7.2 and BPN-9 was similar to that of the plants treated by single inoculation, particularly BPF-9.

Table 2. Plant height and root length of corn grown in tailing after NFB and PSB inoculation 42 DAS

Bacterial Treatment	Plant height (cm)			Root length (cm) at 42 DAS
	14 DAS	28 DAS	42 DAS	
Control	36.63 ± 2.49 a	77.67 ± 3.30 a	118.10 ± 6.66 a	61.84 ± 14.96 a
Azo-7.2	37.10 ± 4.00 a	78.59 ± 5.17 ab	119.67 ± 9.42 ab	68.39 ± 15.28 a
BPF-9	40.00 ± 3.21 a	81.71 ± 2.93 b	126.34 ± 7.22 b	73.99 ± 8.67 a
Azo-7.2 + BPF-9	37.82 ± 3.00 a	81.92 ± 2.27 b	125.93 ± 3.54 b	72.51 ± 8.23 a

Numbers in a column followed by the same letter were not significantly different based on (LSD) of $p < 0.5$

At the end of the experiment, inoculated plants had longer roots than the control, although insignificant (Table 2). Inoculation with PSB-9 and mixed of Azo-7.2 and PSB-9 had a potency to increase root length by 19.6% and 17.2%, respectively. The BPF-9 inoculation caused higher root lengths than the Azo-7.2 inoculation and mixed isolates. The phytohormone IAA produced by both isolates plays a role in increasing root growth (Bunsangiam et al., 2021). Moreover, NFB and PSB inoculation increased the availability of soil N and P through N fixation and P solubilization, respectively; the two nutrients play a role in root elongation. The N availability affects the levels of endogenous hormones, including auxin, cytokinin, and abscisic acid, which will influence root growth and morphology (Armita, 2019). The effect of P on root growth architecture has been discussed (Liu, 2021); therefore, the PSB inoculation in this research has an essential function in root growth.

The increase in shoot height of plants inoculated with NFB and PSB may be caused by the moderate supply of IAA (auxin) by both beneficial bacteria. Moderate concentrations of IAA stimulate root cell division and elongation (Gao et al., 2022), further increasing plant height.

At the age of 42 DAS, the increase in leaf number ranged from 4.5 to 10.7% (Table 3). Mixed inoculation of NFB and PSB resulted in more leaf number increments than single and without inoculation. The NFB and PSB inoculation increased the availability of essential nutrients N and P needed for leaf formation. This result agrees with the role of various species of NFB and PSB inoculation combined with chemical fertilizer in increasing the growth and yield of caysim in soil (Hindersah et al., 2021).

Table 3. Leaf number of Corn grown in tailing after NFB and PSB inoculation at 14 to 42 DAS

Bacterial Treatment	Leaf number		
	14 DAS	28 DAS	42 DAS
Control	3.28 ± 0.20 a	5.76 ± 0.17 a	6.39 ± 0.35 a
Azo-7.2	3.29 ± 0.24 a	5.65 ± 0.27 a	6.68 ± 0.27 ab
BPF-9	3.39 ± 0.22 a	5.79 ± 0.51 a	7.00 ± 0.36 b
Azo-7.2 + BPF-9	3.37 ± 0.24 a	6.37 ± 0.46 b	7.04 ± 0.46 b

Numbers in a column followed by the same letter were not significantly different based on (LSD) of $p < 0.5$.

Application of Azo-7.2 slightly reduced the stem diameter at 28 DAS, while another treatment enhanced the stem diameter. The stem diameter was increased after inoculation of BPF-9 and a mixture of Azo-7.2 and BPF-9; it was 5.5% and 6.6% higher than the control, respectively (Table 4).

The activity of NFB and PSB increased the availability of N and P nutrients. It changed the hormonal balance in plants, thereby affecting stem diameter, which agrees with the increase in tomato and sorghum stem thickness after PSB *Bacillus pullus* (Martínez-Cano et al., 2022; Amora-Lazcano et al., 2021).

Table 4. Stem diameter of corn grown in tailing after NFB and PSB inoculation at 28 and 42 DAS

Bacterial treatment	Stem diameter (mm) at	
	28 DAS	42 DAS
Control	8.03 ± 0.63 ab	10.52 ± 0.59 a
Azo-7.2	7.97 ± 0.66 a	10.62 ± 0.69 ab
BPF-9	8.47 ± 0.55 bc	10.99 ± 0.55 b
Azo-7.2 + BPF-9	8.51 ± 0.56 c	11.21 ± 0.66 b

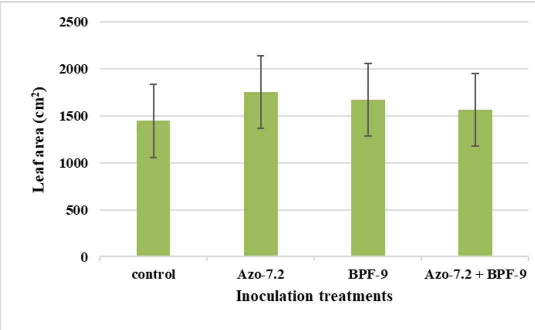
Numbers in a column followed by the same letter were not significantly different based on (LSD) of $p < 0.5$.

Physiological traits

Based on the variance analysis, bacterial inoculation did not affect leaf area and chlorophyll content (Figures 3a and 3b). However, introducing beneficial microbes had the potential to increase all growth parameters. The leaf area of corn inoculated with Azo-7.2 and BPF-9 was 21.3% and 15.6% higher than control, although the difference was insignificant. Regardless of statistical analysis. Applying Azo-7.2 and mixed isolates showed the potency to increase the chlorophyll content by 4.2% and 6.1%, respectively. The chlorophyll content is a vital plant characteristic because it is closely related to the rate of photosynthesis. The synthesis of chlorophyll is greatly influenced by the N and other nutrients, including Mg and micronutrients (Hadayanto et al., 2017; Ishfaq et al., 2022; Roosta et al., 2018).

Plant biomass

The bacterial inoculation did not influence the biomass. However, regardless of statistical analysis, plant biomass with Azo-7.2 was higher than other treatments. Inoculation of beneficial microbes increased intact plants' fresh and dry weight even though the difference was insignificant compared to control plants. Inoculation of Azo-7.2, BPF-9, and their mixture increased the fresh weight by 19.11%, 8.2%, and 12.8%, respectively. Meanwhile, inoculation of the Azo-7.2 increased dry weight up to 24.1%. The leaf area and chlorophyll content of plants with Azo-7.2 were slightly higher, although not significantly different from control plants and other inoculant treatments (Figure 3b). This combination might increase the photosynthesis process and further plant biomass formation.



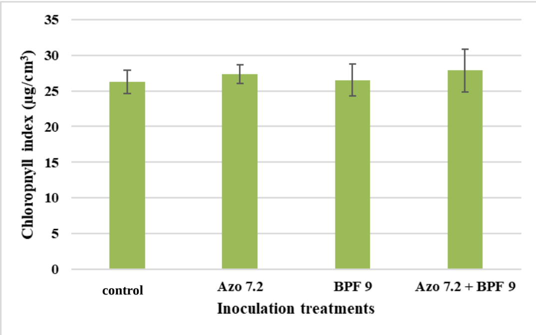


Figure 3. Leaf area (a) and chlorophyll content (b) of corn after NFB and PSB inoculation at 42 DAS

The S/R ratio of plants with different treatments was greater than one, indicating that the assimilate was more distributed to the shoot than to the roots. This ratio is influenced by the roots' ability to uptake nutrients, and photosynthesis takes place in the leaf chlorophyll; therefore, it is an essential trait for estimating plant production (Bláha, 2019). After BPF-9 and mixed inoculation, the S/R ratio slightly increased, but the difference was insignificant.

Table 5. Nitrogen and Phosphor uptake of intact corn plants grown in tailing after NFB and PSB inoculation at 42 DAS

Bacterial Treatment	Nutrient uptake (mg plant^{-1})	
	Nitrogen	Phosphor
Control	20.25 a	10.19 a
Azo-7.2	23.28 a	10.57 a
BPF-9	25.19 a	12.96 a
Azo-7.2 + BPF-9	25.77 a	14.46 b

Numbers in a column followed by the same letter were not significantly different based on (LSD) of $p < 0.5$.

Nutrient uptake

The data depicted in Table 5 confirm that the N uptake did not depend on bacterial treatment, but the P uptake did. The mixed inoculation of Azo-7.2 and BPF-9 resulted in a significant enhancement of P uptake by up to 40%.

The insignificant effect of both beneficial microbes on N uptake might be due to the microbes' incapability to provide sufficient N in the substrate, which contained extremely low N (0.02%). Low N in soil induces N fixation, but available N supplied by bacteria is low compared to chemical fertilizer. Increased P uptake in intact corn plants was only shown by mixed inoculation and their availability in soil, although the P content in soil was not analyzed. The PSB in mixed inoculant was possibly effective in increasing P uptake only with the presence of NFB Azo-7.2. This is a novel finding related to the nutrient uptake of corn grown in tailing after mixed inoculation of NFB and PSB. Nonetheless, the interaction of the N-fixer and P-solubilizer bacterial group was also found to promote the P uptake by field-grown chickpeas in soil (Wani et al., 2007; Yu et al., 2012).

The abundance of NFB and PSB in the rhizosphere

The abundance of NFB and PSB in the corn rhizosphere at the end of the experiment was not determined by microbial inoculation (Table 6), indicating that indigenous bacteria were able to compete with exogenous bacteria to maintain the population. However, the abundance of NFB and PSB in the corn rhizosphere decreased slightly, although not by one decimal. This decrease indicated that there might be competition between exogenous bacteria and indigenous bacteria that were more adaptable to tailings properties. The reduction in bacterial population showed limited nutrients for their proliferation. In this case, the bacteria competed with plants for nutrients, mainly essential N and P.

Table 6. The abundance of NFB dan PSB in the rhizosphere of 42-day-old corn grown in tailing

Bacterial Treatment	Population of bacteria (10^8 CFU g^{-1})	
	NFB	PSB
Control	3.89 a	3.83 a
Azo-7.2	4.40 a	3.03 a
BPF-9	4.06 a	3.40 a
Azo-7.2 + BPF-9	4.76 a	3.41 a

Numbers in a column followed by the same letter were not significantly different based on (LSD) of $p < 0.5$.

This experiment provides the information that both bacterial groups survived and reproduced in the corn rhizosphere. The presence of root exudates in the rhizosphere supplies the source of carbon and nutrients for soil heterotrophic microorganisms (Pascale et al., 2020). Tailings contained low N and P, which supported N fixation by Azo-7.2 and P solubilization by BPF-9. The limitation of this experiment was that the content of available N and P in soil was not measured, but the results verified the increase in soil P uptake after the experiment, and plant growth. In addition, the plants were not treated with chemical fertilizers to better understand the direct effect of microbes on plant growth. Applying chemical fertilizers such as NPK compound fertilizer would explain the function of microbes in tailings with higher availability of N and P nutrients to reduce the nutrient competition between bacteria and plants.

This experiment showed that single or mixed inoculation of NFB and PSB only significantly influenced plant height, stem diameter, and number of leaves. However, plants with NFB and PSB looked better than plants without inoculation. In general, the cause of insignificant effect of treatments on leaf area, chlorophyll content, root lengths, S/R, and biomass. The reason was insufficient organic manure for bacterial proliferation and limited N and P since the plants did not receive chemical fertilizer. The nutrients in this vegetative phase were only for plant growth in terms of increasing the plant height, stem diameter, and number of leaves.

CONCLUSION

1. The vegetative growth performance of corn plants in tailings was improved due to NFB and PSB inoculation. This experiment showed that bacterial inoculation of NFB and PSB significantly influenced plant height, stem diameter, leaf number, and P uptake but did not change leaf area, chlorophyll content, N uptake, root lengths, S/R, and biomass.
2. Nonetheless, bacterial treatments had a potency to increase those parameters. The treatments did not affect the population of NFB and PSB in the rhizosphere; unfortunately, both bacterial populations in the rhizosphere of treated and control plants were slightly lower than the population before the experiment.
3. Single inoculants of BPF-9 and mixed inoculants of The Azo-7.2 and BPF-9 mixture significantly increased in plant height, stem diameter, number of leaves, and S/R ratio. However, the Azo-7.2 had the potency to enhance leaf area, chlorophyll content, and corn biomass.

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