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Yield of mungbean (*Vigna radiata* L.) applied with different biofertilizers and plant biostimulants

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Abstract

Excessive used of synthetic chemicals affects yield and income of farmers. Agricultural intensification through the use of biofertilizers and biostimulants is needed. As support to biofertilizer and biostimulant production, this project aimed to determine the effect of bio-products as microbial inoculant and plant stimulant on growth and yield, and determine which treatment combinations increase the yield of mungbean. The experiment employed a Factorial Completely Randomized Design with three replicates with biofertilizers (A_1-Control+Recommended Rate, A_2-Rhizobium+Recommended Rate and A_3-Azospirillum+Recommended Rate only) as factor A, and biostimulants (B_1-Control, B_2-humic acid, B_3-carrageenan and B_4-AMO organic supplement as factor B. Rhizobium combined with humic acid are influenced the root length, number of pods and weight of seeds or seed yield, growth and development as well as it increases the of yield of mungbean. The strain of rhizobium and humic acid is an appropriate combination supplemented to the recommended rate of inorganic fertilizer, thus increasing the growth and yield parameter of mungbean. Considering the positive effects of rhizobium and humic acid, the findings may provide new insights in the interactions between biofertilizers and biostimulants which may affect its growth and development, hence, government agencies, and private companies mandated to produce biofertilizers and biostimulants to adapt and commercialize.

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Introduction

The continuous use of chemicals and synthetic fertilizers leads plant tissues to frequently absorb and accumulate heavy metals, which consequently decreases the nutritional and grain quality of the crops. Although chemical fertilizers act as beneficial input to get higher crop productivity, however high dosage is associated with reduction of the soil properties and crop yields over time. According to the Philippine Statistics Authority, mungbean production declined during the second quarter of 2023, with an estimated output of 22.74 thousand metric tons from 36.70 thousand hectares, a decrease compared to previous years.

Enhancing agricultural yield necessitates tailored management strategies that consider specific crops and growing conditions. Identifying key growth stages allows for targeted interventions to optimize yield potential. While numerous technologies and information sources exist for crop production, the application of biofertilizers and biostimulants remains understudied. Biofertilizers contain living microorganisms that colonize the rhizosphere or plant roots. These microorganisms promote plant growth by enhancing nutrient availability, stimulating root development, and fostering beneficial symbiotic relationships, thereby improving soil fertility and overall plant productivity without causing pollution due to their biodegradability. Biostimulants, on the other hand, are a diverse group of substances used to improve crop vigor, quality, and yield, as well as enhance tolerance to abiotic stresses such as drought, salinity, and heat. Their mechanisms of action include facilitating nutrient uptake, promoting the development of beneficial soil microorganisms, and stimulating root growth to increase water use efficiency.

The application of beneficial microorganisms and organic amendment is essential in enhancing the microbial diversity and aim sustainable production of crops. Taking these aspects in consideration, it is necessary to find alternative source to sustain crop productivity without deteriorating the soil fertility. With this, it may also be effective to the yield performance of mungbean production with the same geniality and may help reduce the use of pesticides for the sustainable agriculture.

Materials and methods

Securing of seeds and microbial inoculant

The seeds of Kusapo mungbean was procured from Agricultural Training Institute-San Mateo, Isabela. The Rhizobium microbial inoculant to be used in the study was acquired and inoculated in the Department of Agriculture-Cagayan Valley Integrated Agricultural Laboratory, while the *Azospirillum* microbial inoculant was inoculated in the Department of Agriculture–Southern Cagayan Research Center, Iguig, Cagayan.

Location of the experimental area

Mungbean production in Cagayan Valley is a significant industry, with the region being considered as a potential Mungo Capital. Isabela province have the largest mungbean production area among the five provinces having 286,574 hectares. It is the main reason why the experimental area was established in the nursery of the author, Ms. April Ann Calamayan Marquez at Sagat, Cordon, Isabela. It is also adjacent to Quirino and Nueva Vizcaya provinces.

Soil analysis

Soil sampling was done before potting the soil prior to the study. One kilogram of soil was air dried and foreign materials was eliminated and brought to the Cagayan Valley Research Center (CVRC) San Felipe, City of Ilagan, Isabela for analysis.

Potting of soil

One Hundred Eighty (180) black polyethylene bags filled with soil with a dimension of 10 × 10 × 16 inches were prepared. Holes were provided at the bottom end to provide proper aeration and proper drainage after irrigation.

Lay-out of the experiment and experimental design

The experiment was set- up under the nursery. A total of 180 pots were used in the study following the

Completely Randomized Design in factorial with three replications.

Seed inoculation and fertilizer application

The seeds were inoculated with *Rhizobium* and *Azospirillum*. Afterwhich, the seeds of mungbean were moistened with tap water following the slurry method. The recommended rate of inorganic fertilizer at a rate of 30-10-20 kg NPK ha^{-1} was applied.

Experimental design

The study was conducted in a Two-factorial Completely Randomized Design with the following treatments to be employed:

The different biofertilizer as factor A are the following:

A_1- 30-10-20 kg NPK ha^{-1} (Control)

A_2-*Rhizobium* + 30-10-20 kg NPK ha^{-1}

A_3-*Azospirillum* + 30-10-20 kg NPK ha^{-1}

Factor B: Biostimulants

B_1-Without biostimulants

B_2-Humic Acid

B_3-Carrageenan

B4-AMO organic supplement

Planting

Each potting medium was planted with five seeds per pot, which were gently pressed into place. One week after planting, replanting was conducted for any missing hills. Ten days after planting, thinning was performed to leave three healthy plants per pot.

Care of the plants

Cultivation was done three weeks after planting to support the base of the plants, improve soil aeration and eradicate weeds growth. In addition, hand weeding was applied to minimize weeds growth. The plants were watered as need arises. Irrigation was done to support the moisture requirement of the plants.

Data gathering

The gathered data were analyzed to determine the effects of different treatment combinations on growth and yield of mungbean. The data gathered were plant height, number of nodules, root length, pod yield, number of seeds per pod, weight of seeds, and computed yield per hectare. Height of the plants were measured using meter stick from the base of the plants starting at 30 and 60 DAS until its third priming. The number of nodules of the representative plants per treatment count manually at 30 and 60 DAS-nodule. Destructive sampling was done to measure the root length using meter stick. The roots of mungbean were washed in running water to remove the soil coated in the roots of the plants. Data on the pod yield were gathered at harvesting time at minimum of three priming. Number of seeds per pod of the representative plants was calculated and recorded one by one. The weight of seeds of the representative were weighted using a digital weighing scale. The computed yield per hectare was calculated by multiplying the yield from the study area 10,000 m^2 (1 hectare).

Statistical analysis

The data were analyzed using the analysis of variance (ANOVA). The significant means were compared using Honestly Significant Difference (HSD) using the Statistical Tool for Agricultural Research program.

Results and Discussion

The result of the study shows that the growth of plants at 30 and 60 days after owing (DAS) were influenced by application of rhizobium and humic either as single factor of the interaction of both factors. It also increases the number of nodules. The root length, number of pods as well as the weight of mungbean pods and weight of seeds obtained significant differences as influenced by the said combination. The following Tables (1-7) were the Analysis of Variance (ANOVA) on plant height, number of nodules, root length, number of pods, weight of pods, weight of seeds and computed yield per hectare.

Plant height

The effect of biofertilizers as inoculant on Mungbean responded significantly on the height of the plants at 30 days after sowing. As a single factor, the addition of *Rhizobium* (A_2) produces the tallest plants with mean of 54.42 cm followed by the plants in A_3 with 51.39 cm.

The uninoculated plants were the smallest with mean of 49.39 cm. Humic acid (B_2) as biostimulants, likewise, produced the tallest plants with mean of 54.30 cm however similar to AMO organic supplement (B_4) with 52.40 centimeters which consistently followed by the plants with Carrageenan (B_3) with 51.03 centimeters while 49.19 centimeters in B_1 (Control).

The interaction effect of different biofertilizers and biostimulants showed beneficial effect in terms of plant heights. In soil amended with *Rhizobium* added to humic acid favorably enhanced plant growth with 55.78 cm (A_2B_2). This was due to the effect of rhizobium in secreting phytohormones and siderophores and solubilizing insoluble phosphate. Malik and Sindhu (2011) reported that the promotion of plant growth after inoculation was attributed to the biosynthesis and secretion of IAA by Rhizobacteria.

Table 1. Effect of biofertilizers and biostimulants on the height (cm^2) of the plants

Treatments	Plant height (cm^2)	
	30 DAS	60 DAS
	Biofertilizers	
A_1- Control	49.39 c	57.53 b
A_2-*Rhizobium*	54.42 a	61.78 a
A_3-*Azospirillum*	51.39 b	57.80 b
	Biostimulants	
B_1-Without biostimulants	49.19 c	55.03 c
B_2-Humic acid	54.30 a	62.70 a
B_3-Carrageenan	51.03 bc	57.55 bc
B_4-AMO organic supplement	52.40 b	60.85 b
Results		
C.V. (a)	0.51	0.51
C.V. (b)	0.70	0.63

**high significant at 5% level, Means with the same letter are not significantly different with each other using Tukey's HSD

Table 2. Number of nodules as affected by biofertilizers and biostimulants on the plants

Treatments	Number of nodules	
	30 DAS	60 DAS
	Biofertilizers	
A_1-Control	44.45 c	56.03 b
A_2-*Rhizobium*	55.25 a	62.17 a
A_3-*Azospirillum*	50.94 b	57.67 b
	Biostimulants	
B_1-Without biostimulants	47.48 c	53.56 c
B_2-Humic acid	52.77 a	61.81 a
B_3-Carrageenan	50.45 b	57.96 b
B_4-AMO organic supplement	50.15 b	61.15 a
Results		
C.V. (a)	2.09	1.45
C.V. (b)	1.44	1.06

**high significant at 5% level, Means with the same letter are not significantly different with each other using Tukey's HSD

Similar trend was recorded on the height of the plants at 60 days after sowing. Results indicated that the addition of *Rhizobium* to the recommended rate of inorganic fertilizer positively produced taller plants with mean of 61.78 centimeters (A_2) which consistently followed by the plants inoculated with *Azospirillum* (A_3) with 57.80 cm while 57.53 cm in A_1 (Control). There was a difference among treatments wherein among the three biostimulants materials, humic acid showed the best compared with carrageenan, AMO organic supplement and the control group. The treatments with humic acid as biostimulants produced taller plants with mean of 62.70 cm over 60.85 cm (B_4), 57.55 cm (B_3) and 55.03 cm (B_1).

The interactions with biofertilizers to biostimulants resulted to significant increase in heights of the plants. Considered environmentally friendly and cost-effective alternatives to synthetic products such as fertilizers, crop protection products and plant growth regulators (Ronga *et al.*, 2019).

Number of nodules

The result on the analysis of variance indicated that biofertilizer affected the number of nodules. The consistent performance of biofertilizers was exhibited significant difference at 30 and 60 days after sowing. Plants inoculated with rhizobium registered the greatest number of nodules between *Azospirillum* and the control treatment. In like manner, humic acid (B_2) was the most appropriate biostimulants that forms more numbers of nodules.

Researches determined that both nodulating and non-nodulating *Rhizobium leguminosarum* strains produce indole-3- acetic acid (Wang *et al.*, 1982) which promotes the growth of the plants and plays the crucial role in the production and expansion of root nodules. Also, rhizobium has the ability to protect plants from ethylene stress. According to Sri Purwansih *et al.* (2021), the application of rhizobium inoculum can increase root nodules, which function to fix nitrogen for plants.

Table 3. Effect of biofertilizers and biostimulants on the root length ((cm2) of the plants

Treatments	Root length (cm^2)	
	30 DAS	60 DAS
	Biofertilizers	
A_1-Control	29.83 c	38.44 c
A_2-*Rhizobium*	38.36 a	46.31 a
A_3-*Azospirillum*	35.06 b	42.20 b
	Biostimulants	
B_1-Without biostimulants	32.11 c	38.59 c
B_2-Humic acid	36.44 a	41.22 b
B_3-Carrageenan	34.07 b	43.22 a
B_4-AMO organic supplement	35.04 b	43.22 a
Results		
C.V. (a)	2.21	4.87
C.V. (b)	3.74	3.30

**high significant at 5% level, Means with the same letter are not significantly different with each other using Tukey's HSD

Root length

The data shows the effects of *Rhizobium* as biofertilizers on mungbean. In comparable, the longest roots were those in A_2 with a mean of 38. 36 cm followed by 35.06 cm (A_3) and 29.83 cm (A_1) at 30 DAS. Same manner at 60 DAS with a mean of 46.31 cm (A_2), 42.20 cm (A_3) and 38.44 at A_1 (Control). Sri Purwansih *et al.* (2021) highlighted rhizobium is capable to produce indole acetic acid (IAA) and gibberellic acid (GA), which are growth hormones for germination and plant growth and enhancing the growth of root hair and root branches that extends the root's range.

Number of pods

The number of pods was recorded and exhibited significant difference from first, second and third priming. The plants applied with rhizobium registered the greatest number of pods between *Azospirillum* and the control treatment. This was also noted on the application of humic acid as biostimulants having the highest number of pods produced.

Plants inoculated with rhizobium produced the highest number of pods as well as those applied with humic acid (A_2B_2).

Table 4. Effect of biofertilizers and biostimulants on the number of pods of the plants

Treatments	Number of pods		
	1st Priming	2nd Priming	3rd Priming
	Biofertilizers		
A_1- Control	18.41	60.92	20.58
A_2-*Rhizobium*	22.42	75.58	27.58
A_3-*Azospirillum*	21.83	63.91	25.40
	Biostimulants		
B_1-Without biostimulants	18.67	54.33 b	21.44
B_2-Humic acid	23.66	87.56 a	27.33
B_3-Carrageenan	20.66	66.00 b	24.56
B_4-AMO organic supplement	20.55	59.33 b	24.78
Results			
C.V. (a)	8.41	34.05	5.88
C.V. (b)	6.53	15.69	5.99

**high significant at 5% level, Means with the same letter are not significantly different with each other using Tukey's HSD

Table 5. Effect of biofertilizers and biostimulants on the weight of pods (g) of the plants

Treatments	Weight of pods (g)		
	1st Priming	2nd Priming	3rd Priming
	Biofertilizers		
A_1-Control	14.50 b	54.00 b	16.25 b
A_2-*Rhizobium*	18.09 a	66.09 b	24.00 a
A_3-*Azospirillum*	18.43 a	57.25 a	22.25 a
	Biostimulants		
B_1-Without biostimulants	14.78 b	47.67 b	16.22 b
B_2-Humic acid	19.22 a	77.89 a	24.11 a
B_3-Carrageenan	17.44 b	53.00 b	21.55 b
B_4-AMO organic supplement	16.56 b	57.89 b	21.44 b
Results			
C.V. (a)	8.41	43.13	7.20
C.V. (b)	6.53	15.82	5.37

**high significant at 5% level, Means with the same letter are not significantly different with each other using Tukey's HSD

The result showed that the increase of pods of the plants is associated with the growth hormones production characteristics, given by the bacteria inoculant specifically rhizobium.

Purwansih *et al.* (2021) emphasized rhizobium can increase the absorption of phosphate. Phosphate is a primary nutrient in root development and formation of legume pods such as soybean. Same results were observed using the biostimulants specifically humic acid in which resulted in maximum number of pods. Jung *et al.* (2014) mentioned that humic acid application methods at the rate of 3kg ha^{-1} resulted in higher number of pods plant. Humic acid enhance plant growth by promoting the bioavailability of nutrients via reform of the soil environment at the roots (Chen *et al.,* 2004). Humic acid was the main source of nitrogen (N), phosphorus (P) and potassium (K) (Panuccio *et al.,* 2001) and HA enhanced absorption of micro and macro-elements in plants (Varani and Pinton, 1995).

Weight of pods

Significant interaction between factors were also observed in relation to weight of pods of the plants at first, second and third priming. It shows that the superiority of A_2B_2 (*Rhizobium* and humic acid) obtained the heaviest weight pods at second priming of harvesting followed by A_2B_4, A_2B_3 and A_2B_1, while the least was recorded from the rest of the treatments at first and third priming.

The results showed that the increase of weight of plant pods was benefited by inoculation of rhizobium and application of humic acid. The application of rhizobium can significantly increase the weight of mungbean pods compared to *Azospirillum* and uninoculated treatment.

Likewise, the application of humic acid application resulted in a higher number of pods plant, thousand grain weights and grain yield and yield components of mungbean (Lee *et al.*, 2014).

Number of seeds per pods

The overall average number of seeds per pod for treatments with or without inoculation as affected by biofertilizers shows that significantly affected the number of seed per pod particularly those inoculated with rhizobium with mean of 10.85 per pod. It was followed with the application of *Azospirillum* (A_3) and the uninoculated treatments (A_1). Satter and Ahmed (1992) reported that rhizobium inoculation significantly increased seed yield of mungbean as compared to the uninoculated control. Rewari *et al.* (1981) found the seed yield between 70 to 350 kg/ha due to inoculation. Solaiman *et al.* (2003) found significantly higher seed yield in lentil due to the inoculation of seeds with rhizobium strain.

Table 6. Effect of biofertilizers and biostimulants on the number of seeds per pod of the plants

Treatments	Number of seeds per pod		
	1st Priming	2nd Priming	3rd Priming
	Biofertilizers		
A_1- Control	9.17 b	9.35 b	9.08 b
A_2-*Rhizobium*	10.79 a	10.85 a	11.08 a
A_3-*Azospirillum*	10.75 a	10.69 a	10.92 a
	Biostimulants		
B_1-Without biostimulants	9.73 b	9.72 b	9.78 c
B_2-Humic acid	10.67 a	10.70 a	10.89 a
B_3-Carrageenan	10.26 a	10.41 a	10.33 b
B_4-AMO organic supplement	10.28 a	10.35 a	10.44 ab
Results			
C.V. (a)	4.35	5.40	4.83
C.V. (b)	2.36	3.20	3.08

**high significant at 5% level, Means with the same letter are not significantly different with each other using Tukey's HSD

Table 7. Effect of biofertilizers and biostimulants on the weight of seeds of the plants

Treatments	Weight of seeds (g)		
	1st Priming	2nd Priming	3rd Priming
	Biofertilizers		
A_1- Control	7.50 b	41.75 b	6.83 b
A_2-*Rhizobium*	11.67 a	50.42 a	11.92 a
A_3-*Azospirillum*	10.58 a	45.67 b	10.00 ab
	Biostimulants		
B_1-Without biostimulants	8.22 b	38.44 b	7.11 b
B_2-Humic acid	12.78 a	57.44 a	13.00 a
B_3-Carrageenan	8.89 b	40.56 b	8.34 b
B_4-AMO organic supplement	9.78 b	47.33 ab	9.89 b
Results			
C.V. (a)	15.03	45.58	19.91
C.V. (b)	10.63	17.74	8.34

**high significant at 5% level, Means with the same letter are not significantly different with each other using Tukey's HSD

As to the effect of biostimulants, the results showed significant difference in which humic acid had obtained the highest number of seeds per pod while comparable mean difference was noted in Carrageenan, AMO organic supplements and without application of biostimulant.

Weight of seeds

There was a significant effect of inoculation of biofertilizers on the weight of seeds of the plants. It showed that rhizobium added to the recommended rate of inorganic fertilizer produced heaviest weight of seeds while no further difference noted in A_3

(*Azospirillum*) and the control treatments. Hoque *et al.* (1982) and Solaiman *et al.* (2003) found increased 1000-seed weight of soybean and lentil, respectively, due to inoculation with rhizobium strain. The same trend of significant difference was recorded as affected by humic acid as biostimulants and the interaction of the both factors.

Conclusion

Based on the results of the study specifically the strain of rhizobium and humic acid is an appropriate combination supplemented to the recommended rate of inorganic fertilizer, thus increasing the growth and yield parameter of mungbean.

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