



Response of Black Beans (*Phaseolus vulgaris* L.) to Organic Fertilizers and Microbial Inoculant in an Intercropping System with ‘Magallanes’ Pummelo (*Citrus maxima* [Burm] Merr.)

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Abstract: To maximize land use efficiency in the interspaces of ‘Magallanes’ pummelo (*Citrus maxima* [Burm] Merr.) while producing black beans, this study was conducted to evaluate the performance of black beans (*Phaseolus vulgaris*) intercropped with ‘Magallanes’ pummelo applied with microbial inoculant and vermicompost. The different treatments used are T1- Bureau of Soils Recommendation (BSR) (Control); T2- no Fertilizer Application; T3- Microbial inoculant only; T4- Vermicompost (10t ha⁻¹); T5- Microbial inoculant + 75% Vermicompost; T6- Microbial inoculant + 50% Vermicompost; T7-Microbial inoculant + 25% Vermicompost, following the RCBD. Results indicated that black beans applied with microbial inoculant and vermicompost, intercropped with ‘Magallanes’ pummelo performed comparably on growth and yield. A significant increase in the chlorophyll index using an SPAD meter at the vegetative stage was observed. Application of microbial inoculant and vermicompost enhances the growth of ‘Magallanes’ pummelo which indicates a beneficial interaction in the intercropping system. Maximizing the space between ‘Magallanes’ pummelo while at the young stage can be a smart way to effectively use land for black beans.

Keywords: Microbial inoculant; Vermicompost; Chlorophyll content; Intercropping

INTRODUCTION

The world’s largest pulse legume in terms of cultivation and consumption is the black beans (*Phaseolus vulgaris* L.). The alkalase-hydrolyzed black bean protein exhibited the highest ABTS + antioxidant capacity, measuring 63.56%. The maximum antioxidant activity, however, was found in the pepsin-hydrolyzed black bean protein (45.15%). The findings suggest that black bean protein hydrolysates could be utilized as valuable protein (Evangelho *et al.*, 2016).

The Philippines’ dry bean industry is expected to generate USD 324 million in 2020 and USD 384 million in 2021, with a compound annual growth rate (CAGR) of 3.7%. Consumer demand for dry beans is rising in this region due to factors like population expansion, fast urbanization, and industrialization. Additionally, during the past years dry bean producers have had more development. Dry beans are becoming

more and more popular as a source of protein for both vegetarian and non-vegetarian consumers as a result of increased awareness of plant-based diets and protein substitutes. Their acceptance is further supported by their function in advancing sustainable agricultural methods and soil health (6Wresearch, 2022).

The primary problems encountered by most farmers are the increasing inputs, such as inorganic fertilizers, that are important on plants. Biofertilizers were found to provide nutrients as an alternative to synthetic fertilizers. It is mostly found in the soil to enhance soil quality; it reduces the release of nitrogen and helps in the depletion of soil (Devi & Sumathy, 2017).

On the other hand, intercropping, the integration of non-crop plants or alternative crops with cash crops can stabilize and strengthen agroecosystem under climate change, by increasing resource use efficiency, improving soil water holding capacity, and expanding the diversity and quality of habitat for beneficial insects that provide pollination

services and natural pest control. (Huss et al., 2022). Cultivating of leguminous crops such as mung bean and kidney bean as intercrop has been noted to enhance soil nutrient status of a senile orchard (Singh et al., 2016).

Black beans grown organically had higher phenolic compound concentrations (28%) and a better nutritional profile (20% more proteins, 10% less phytate) (Makhaye et al., 2021).

Additionally, Singh (2018) states that on organic manures and mineral fertilizers, as well as the balanced application of various nutrients, helped to maintain and enhance soil health. The primary component of Bio-N is the nitrogen-fixing bacteria *Azospirillum*, which is transported by soil and charcoal. When used as a root dip or a seed inoculant, it can provide 50% of the nitrogen needed by high value crops ("Use Bio-N today,"n.d.).

Longer N availability, as evidenced by higher plant and soil N content at harvest and various crop growth stages, is responsible for the higher grain yield seed for the complete N dose put on biochar + biofertilizer applied as a soil mixture (Khan et al., 2021). According to Devi & Sumathy, (2017), a soil organic material may boost nutrients and improve soil fertility by slowing the release of nitrogen. This would help to prevent soil depletion. Rice grain protein content, plant nutrient uptake, and leaf chlorophyll all increased when biofertilizer containing advantageous bacterial was applied. According to Al-Sayed et al. (2023), adding 5 t ha⁻¹ of biofertilizer can increase rice yield and reduce the consumption of chemical N and P fertilizer by 50%.

Uptake of nutrients of biofertilizer and inorganic fertilizer on maize plant (*Zea mays* L.) revealed that the chemical properties of biofertilizer would be influenced by the microbe consortium (*Enterobacter cloacae*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Aspergillus niger*, and *Trichoderma asperillum*) present in biofertilizers at varying concentrations according to Sondang, et al., 2019.

'Magallanes' pummelo on the other hand, is the world's largest citrus fruit is the pummelo. It is grown in many eastern nations, including China, Japan, India, Fiji, Malaysia, Philippines and Thailand, and it originated in Asia. The 'Magallanes' cultivar was used in Davao, Philippines, to popularize pummelo, Olinares et al. (2018). Pummelo has around double the daily required amount of Vitamin C and is a great source of antioxidant of flavonoids. Vitamins A, B1, B2, B6 and B12 are also present, along with protein, calcium, fiber, folate, K and Iron. Magbalot-Fernandez & Deguzman (2019).

To maximize the area in between the 'Magallanes' pummelo while waiting for fruit production and to produce blackbeans for human consumption and address food production, hence, this study was conceptualized to assess the performance of black beans (*Phaseolus vulgaris*) intercropped with 'Magallanes' pummelo treated with microbial inoculants and vermicompost.

MATERIALS AND METHODS

Experimental Location

The experimental area of the high value citrus project (16°43'42"N 120°23'19"E) of DMMMSU-NLUC, Bacnotan, La Union, Philippines served as experimental site (Figure 1).

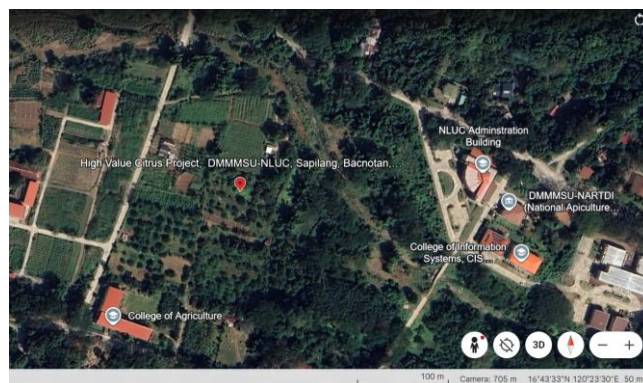


Fig. 1. Study site

The mean temperature, rainfall and %RH under DMMMSU conditions noted the highest temperature of 30.28° C recorded in January and with also the lowest was recorded (19.85° C), Figure 2. The relative humidity reached the highest in November (94.68° C) and the lowest in January (84.20 %) with 90.57 % as average relative humidity. The monthly rainfall ranged from 0.32 mm in February to 1.39 mm in January with a mean rainfall of 1.08mm, means it is generally throughout the monitoring period indicating dry-season conditions. These minimal rainfall levels are characteristics of dry season of the region. Such data is considered favorable for growing of blackbeans and pummelo under DMMMSU conditions.

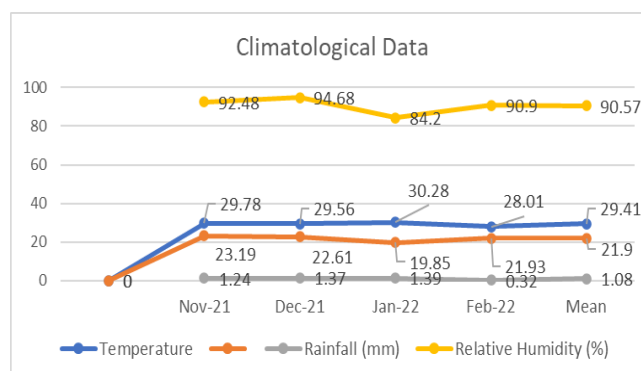


Fig. 2. Minimum, Maximum Temperature, Rainfall and Relative Humidity from November 2021 to February 2022
Source: Agro-meteorological Station, DMMMSU La Union, Philippines, 6°43'32"N 120°23'15"E.

Seeds of blackbeans were procured from the Provincial Agriculture Office, La Union, Philippines (16°36'15.8"N 120°19'14.5"E) and the microbial inoculant, was procured at the University of the Philippines, Biotechnology (UPLB_BIOTECH) and vermicompost was procured from

the Balaoan, Organic and Demonstration Farm, La Union (16°46'38.5"N 120°24'53.7"E).

Agronomic Practices

Composite soil sample was taken from the area and was brought to the Integrated Laboratory Division, Tebag, Sta. Barbara, Pangasinan, Philippines (15°58'55.8"N 120°27'53.9"E) for soil analysis before planting black beans.

The area was initially prepared through strip clearing and was cultivated two weeks before planting using hand tractor and then was laid-out.

Furrows were set two meters away from the base of 'Magallanes' pummelo following the distance between furrows of 50 cm x 30 cm between hills. The black beans were planted intercropped with five-year old 'Magallanes' pummelo spaced at 5m by 5m.

Watering was done just after planting and every after three days just to irrigate the rootzone of the experimental plants specially at the onset of the flowering stage. Infested plants were eradicated; diseased plants were uprooted, and or burned. Weeding was done through hand pulling of weeds and grass cutting as the need arises. Harvesting was done when the black bean pods turned dried brown and was picked into five priming early in the morning and late afternoon. Sun-drying of pods were done for easy threshing and cleaning.

Treatment Application

The experiment involved seven treatments (Table 1), Vermicompost was applied in furrows two weeks before planting and black bean seeds were treated with solution of one pack of microbial inoculant (200g) diluted to little amount water just to moisten the seeds and were coated for 30 minutes.

Table 1. Treatment used in the study

Treatment	Description
T1	BSR 30-10-30 NPK (Control)
T2	No Fertilizer application
T3	Microbial inoculant only
T4	Vermicompost only (10t ha ⁻¹)
T5	Microbial inoculant + 75% Vermicompost
T6	Microbial inoculant + 50% Vermicompost
T7	Microbial inoculant + 25% Vermicompost

Dibbling method of planting was followed at a distance of 50cm between rows x 30cm between hills at two (2) seeds per hill and seeds were covered with soil about 5cm while application of Inorganic Fertilizer (BSR) was applied following the Bureau of Soils Recommendation as prescribed in the treatment (Control 1) (30-10-30 NPK) while no fertilizer application for Treatment 2 (control 2).

Experimental Design and Statistical Analysis

The study followed the Randomized Complete Block Design (RCBD) with three blocks. Data were arranged following ANOVA in RCBD and analyzed using the computer-based program Statistical Tool for Agricultural Research (IRRI. 2013. STAR 2.0.1). Comparison among means were done using the Tukey's Honest Significant Difference (HSD) Test at $p=0.05$ as pos-hoc analysis.

RESULTS AND DISCUSSION

Growth Parameters of Blackbeans

The mean growth parameters of blackbeans applied with microbial inoculant and different organic fertilizers intercropped with 'Magallanes' pummelo is shown in Table 2. As to days to emergence, days to flower and plant height with means ranging from 3.00 to 4.00 days to emergence, 37.33 to 38.00 days to flower and 95.37 to 123.10 cm plant height, analysis showed comparable result.

Table 2. Growth Parameters of Blackbeans Applied with Microbial Inoculant and Different Rates of Vermicompost Intercropped With 'Magallanes' Pummelo

Treatment	Days to Emergence	Days to Flower	Plant Height (cm)
T1- BSR (Control)	3.33	37.67	100.23
T2- No Fertilizer application	3.00	37.33	104.07
T3- Microbial inoculant only	3.33	37.67	101.23
T4- Vermicompost (10t ha ⁻¹)	3.33	38.00	95.37
T5- Microbial inoculant + 75% Vermicompost	4.00	37.00	108.27
T6- Microbial inoculant + 50% Vermicompost	3.67	37.67	123.10
T7- Microbial inoculant + 25% Vermicompost	3.67	38.00	101.47

This implies that black beans applied with microbial inoculant and vermicompost comparably performed with the control treatments. This might be due to the favorable environmental conditions during the conduct of the study, good soil condition and land preparation.

This is due to the good quality of the soil and the presence of native microorganisms that helps fix Nitrogen in the atmosphere. The germination process is also well established; according to Makhaye et al. (2021), it includes a number of processes, such as imbibition, protein synthesis, and the creation of phytohormones. Extrinsic variables, such as the use of biostimulants, can influence all of these processes. Certain biostimulants can be used to improve germination in many plants by increasing metabolic efficiency. Furthermore, Biofertilizers were found to provide nutrients as an alternative to synthetic

chemical fertilizers. It is mostly found in the soil to enhance soil quality; it reduces the release of nitrogen and helps in the depletion of soil (Devi & Sumathy, 2017). According to Singh et al. (2016), intercropping with appropriate and complementary crops enhances fertility, preserves the orchard top soil and generate extra revenue.

Sondang et al. (2019) found that inorganic fertilizer affects the nutrient uptake of N and P maize plants, whereas biofertilizers influences the nutritional uptake of P and K in maize plants. Additionally, INM is a tool that can provide alternative and cost-effective choices to give plants the nutrients they need. Additionally, it can reduce overall expenses, improve soil physiochemical conditions and the environment, eliminate barriers, preserve the soil nutrient balance, and find safe methods of getting rid of agricultural waste (Selim, 2020). This conforms to the statement of Khan (2011) that compared to garden soil and pit compost, vermicompost has a significant impact on *Pisum sativum* development, improves crop output, and plays a critical role in enhancing soil qualities.

In a study on N source and algae extracts application on broccoli, the results show that the application of compost + ammonium nitrate + biofertilizer resulted in the largest increases in NPK absorption. Plant height, leaf area, number of leaves per plant, shoot fresh weight, chlorophyll a and b curd weight and diameter, vitamin C, TSS total phenol, antioxidant content, and head yield all increased concurrently as a result of these enhancements Shams & Morsy (2019). Abd El-Rahman, et.al. (2016) found that, in comparison to 100% ONF (t 8) or 100% MN only, sunflower (*Helianthus annuus* L.) seed production and quality considerably increased yield, yield characteristics, and protein and oil yields.

Chlorophyll content of Blackbeans

Table 3 shows the chlorophyll content of black beans at vegetative, flowering and maturity stages. Results revealed a significant variation among the treatment means, blackbeans applied with microbial inoculant only recorded highest chlorophyll content but not differ to blackbeans applied with microbial inoculant + 75% vermicompost while all other treatments showed comparable chlorophyll content of blackbeans with the control treatments in its vegetative stage with means ranged from 53.84 to 62.98. In flowering and maturity stages, blackbeans recorded comparable with the control treatment. This implies that legume plants responded with microbial inoculant applied bio-fertilizer in terms of its chlorophyll contents specifically on its vegetative stage. In a related study, vermicompost at 50% + peat moss at 50% significantly increased total chlorophyll content in banana plants, Hassan et al.,(2020).

Table 3. Chlorophyll Content of Blackbeans Applied with Microbial Inoculant and Different Organic Fertilizers Intercropped With ‘Magallanes’ Pummelo

Treatment	Chlorophyll content (Vegetative stage) *	Chlorophyll content (Flowering Stage)	Chlorophyll content (Maturity Stage)
T1- BSR (Control)	56.40 ^b	47.81	43.67
T2- No Fertilizer application	57.12 ^b	49.72	47.33
T3- Microbial inoculant only	65.93 ^a	51.77	40.00
T4- Vermicompost (10t ha ⁻¹)	57.08 ^b	50.75	43.40
T5- Microbial inoculant + 75% Vermicompost	62.98 ^a	51.64	41.37
T6- Microbial inoculant + 50% Vermicompost	55.64 ^b	50.74	46.40
T7- Microbial inoculant + 25% Vermicompost	53.84 ^b	53.40	48.37

*Means with the same letter are not significantly different at 5% level (Honest Significant Difference (HSD) Test)

Number Of Root Nodules, Length of Primary and Secondary Roots

Table 4 shows the number of root nodules, length of primary and secondary roots of black beans at vegetative and flowering stage of growth. Results revealed no significant differences of blackbeans applied with microbial inoculant and vermicompost with the control treatments. This implies that legume plants responded comparably among the treatment means which soils might have already contain effective native bacteria which is capable of forming symbiotic relationship with the blackbeans.

By forming a symbiotic relationship, some bacteria fix atmospheric nitrogen in tree root nodules. While Frankia is an actinomycete that is recognized for its actinorhizal symbiosis with nonlegumes, Rhizobium often infects legume trees for nodulation. *Azospirillum*, on the other hand, is a nitrogen-fixing associative symbiosis. Fungi known as vesicular arbuscular mycorrhizal (VAM) aid in the uptake of phosphorus by plants (Shah et al., 2007).

Table 4. Number of Root Nodules, Length of Primary and Secondary Roots of Blackbeans Applied with Microbial Inoculant and Different Rates of Vermicompost Intercropped With ‘Magallanes’ Pummelo

Treatment	Number of Root Nodules at Flowering	Length of Primary Roots (cm)	Length of Secondary Roots (cm)
T ₁ - BSR (Control)	17.00	13.97	7.82
T ₂ - No Fertilizer application	13.00	13.55	7.28
T ₃ - Microbial inoculant only	9.33	16.43	10.48
T ₄ - Vermicompost (10t ha ⁻¹)	11.33	15.55	7.48
T ₅ - Microbial inoculant + 75% Vermicompost	8.00	14.40	7.75
T ₆ - Microbial inoculant + 50% Vermicompost	7.67	14.88	8.84
T ₇ - Microbial inoculant + 25% Vermicompost	12.57	14.80	9.13

Yield and Yield Components of Blackbeans

Presented in Table 5 are the number of pods and pod length (cm) of black beans applied with microbial inoculant and vermicompost with the control treatments.

Table 5. Number Of Pods and Pod Length (Cm) Of Blackbeans Applied with Microbial Inoculant and Different Rates of Vermicompost Intercropped With ‘Magallanes’ Pummelo

Treatment	Number of Pods	Pod length (cm)
T ₁ - BSR (Control)	112.33	17.83
T ₂ - No Fertilizer application	102.00	18.44
T ₃ - Microbial inoculant only	134.33	18.40
T ₄ - Vermicompost (10t ha ⁻¹)	114.33	17.10
T ₅ - Microbial inoculant + 75% Vermicompost	106.33	16.70
T ₆ - Microbial inoculant + 50% Vermicompost	137.00	19.29
T ₇ - Microbial inoculant + 25% Vermicompost	131.90	16.05

Results revealed that the number of pods and pod length (cm) of blackbeans applied with microbial inoculant and vermicompost at various rates performed comparably with the control treatments with means ranging from 102.00 to 137.00 pods and 16.05 to 19.29 cm pod length, respectively.

In terms of pod and seed yield (g/plot) and weight of 100 seeds (g) of black beans applied with different organic fertilizers intercropped with “Magallanes” pummelo showed no significant variations (table 5) with means ranging from 5133.33 to 9200.00 g plot pod yield, 4266.67 to 6633.33 g/plot seed yield and 26 to 31.67g weight of 100 seeds. This is due to the good quality of the soil and the presence of native microorganisms that helps fix Nitrogen in the atmosphere. In related study, Hassan et al., (2022) states that adding vermicompost to the culture mix enhanced the in vitro banana plants grown in greenhouses.

Table 6. Pod and Seed Yield (g/plot) And Weight Of 100 Seeds (g) Of Blackbeans Applied with Microbial Inoculant and Different Rates of Vermicompost Intercropped With ‘Magallanes’ Pummelo

Treatment	Pod Yield/ g/plot	Seed Yield/ g/plot	Weight of 100 seeds (g)
T ₁ - BSR (Control)	6766.67	5050.00	28.67
T ₂ - No Fertilizer application	8933.33	6633.33	30.67
T ₃ - Microbial inoculant only	9000.00	6333.33	26.00
T ₄ - Vermicompost (10t ha ⁻¹)	9200.00	6233.33	30.67
T ₅ - Microbial inoculant + 75% Vermicompost	7866.67	5900.00	29.33
T ₆ - Microbial inoculant + 50% Vermicompost	8033.33	5533.33	31.67
T ₇ - Microbial inoculant + 25% Vermicompost	5133.33	4266.67	30.43

According to Al-Sayed et al. (2023), applying compost along with biofertilizer may be an effective way to boost nutrient availability, growth parameter and yield. Sahoo, et.al. (2022) asserted that the application of 50% of the prescribed fertilizer in combination with vermicompost (1.25 t ha⁻¹) and biofertilizer inoculated farmyard manure (5 t ha⁻¹) can boost onion bulb yield.

Furthermore, according to Singh (2018), in place of chemical fertilizers, vermicompost is an excellent organic medium for growing vegetable crops since it is abundant in nutrients, has pesticidal properties, and releases nutrients gradually, while artificial fertilizers deplete nutrients more quickly. Additionally, crops cultivated in vermicompost enhance plant health, which has a major effect on a variety of plant growth and yield.

Leguminous crops have the ability to fix nitrogen from the air which means there is no need to apply fertilizer materials, the nutrients present in the soil is already enough to sustain the growth and development of the crops.

Cobs/plant, cob length, grains/cob, 1000-grains weight, grain yield/ha, and stover yield/ha of maize were all significantly impacted by intercropping the two crops. In both years, maize plus two rows of mungbeans produced

more stover and grain than maize plus one row of mungbeans and sole maize. In both trial years, the Integrated Nutrient Management (INM) had a considerable impact on maize yield and yield characteristics. The maximum number of cobs/plants, number of grains/cob, cob length, 1000-grains weight, and grain and stover yield of maize were generated in both years by applying 120 kg N/ha along with biofertilizers (*Rhizobium* + *Azospirillum* + PSB) (Jat et al., 2014).

In addition to increasing crop yields, mixed fertilization (organic + inorganic) also improved soil health and reduced the need for chemical fertilizers (Anisuzzaman et al., 2021).

Additionally, Basco et al. (2017) found that biofortified vermicompost treated tomato significantly influence the reduction of disease incidence, improvement in growth and yield, and higher stimulation of antioxidants when compared to the control. According to Rahaman & Barmon (2019), vermicompost greatly boosts productivity when combined with other traditional inputs, and its users are comparatively more technically proficient.

Growth of Pummelo

Table 7 presents the growth of 'Magallanes' pummelo before planting blackbeans (cm), and growth increment after five months. Result showed that 'Magallanes' pummelo increase in height after five months of intercropping blackbeans with height increment of 32.34cm, 2.59cm increment on stem diameter, 22.67 crown diameter increment, while chlorophyll index through SPAD index was decreased by -1.09, from initial chlorophyll index of 75.05 to 73.96 SPAD index.

Intercropping of legumes enhance the growth of pummelo, legumes have several uses in intercropping systems, such as enhancing soil quality, biologically fixing nitrogen, creating functional diversity, and generating extra yields like protein (Maitra et al., 2021).

Lower chlorophyll content explains why larger nutrient contents are available during the vegetative growth of legumes, while lower chlorophyll content occurs during the maturation stage of legumes. Plant photosynthetic activity is impacted when chlorophyll content declines. The green pigment of the plants disintegrated as they get older (Maitra et al., 2021). The breakdown of thylakoid membrane-associated chlorophyll-binding proteins, which necessitates the concurrent catabolism of chlorophyll, is poorly understood. According to studies, senescence causes plants to respire more, which results in a considerable amount of carbon being lost from the leaves. This indicates that the disintegration process for chlorophyll and chloroplast proteins is partly connected (Amad Bhat et al., 2019). Numerous investigations have been conducted to comprehend the molecular mechanisms linked to leaf senescence, such as the breakdown of chlorophyll, the control of phytohormones and transcription, the interaction

with environmental cues, and related metabolic changes. The nutrient recycling and metabolic reprogramming that take place during leaf senescence underscore the critical function of this development stage for the overall plant nutrition economy.

Table 7. Height, Stem Diameter and Crown Diameter of Magallanes Pummelo Before Planting, After Three Months and Increment Planted In Between Black Beans

Growth	Initial	After	Increment
Height (cm)	283.13	315.47	32.34
Stem Diameter(cm)	28.09	30.68	2.59
Crown Diameter (cm)	254.13	276.80	22.67
Chlorophyll index (SPAD)	75.05	73.96	-1.09

This shows that intercropping of legumes enhance the growth of pummelo. However, in terms of chlorophyll content, this possibly due to the effect of availability of higher nutrient contents during the vegetative growth of legumes while reduction of chlorophyll content when legumes are in its maturity stage. Decline of chlorophyll content affects the photosynthetic activity of the plants.

Transcripts coding for enzymes involved in the breakdown of chlorophyll and many genes associated with senescence were found to be significantly up-regulated as senescence progressed (Palmer et al., 2015). About 70% of the proteins in green leaves are found in chloroplasts. Accordingly, nitrogen and carbon remobilization depend heavily on the enormous breakdown of chloroplasts during leaf senescence (Kusaba et al. 2009). The breakdown of macromolecules like as proteins, lipids, and nucleic acids occurs concurrently with the onset of chloroplast degeneration in aging leaves

CONCLUSION

Blackbeans applied with microbial inoculant only and microbial inoculant + 75% vermicompost significantly increase the chlorophyll content of blackbeans at vegetative stage. Application of microbial Inoculant and vermicompost enhance the growth of 'Magallanes' pummelo intercropped with blackbeans. Maximizing the area dedicated to 'Magallanes' pummelo, especially during the vegetative stage can be effective strategy for improving land utilization, food production, and overall land use efficiency.

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Conflict of Interest

There is no conflict of interest declared by the author.

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