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Effect of Inorganic Fertilizer and VP3 Biofertilizer Applications in Legume on the Population of Indigenous Bacteria

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ABSTRACT

This current study is to examine the effects of inorganic fertilizers which are allegedly able to reduce the population of indigenous bacteria and the application of bacteria from VP3 biofertilizer on three test plants (legumes). In all treatments, the addition of inorganic fertilizer at a dose of 50% and 75% could significantly reduce population of soil bacteria on the 10th and 49th day observations. This also shows that the higher dose of NPK fertilizer also affects the bacteria from VP3 biofertilizer. However, treatment with 25–100% NPK fertilizers caused the decreasing of soil bacteria since the day of planting. In bean and long bean plants, the highest yields were shown at the combination of compost, VP3 biofertilizer and the addition of 75% NPK. Meanwhile, for mung bean, the highest yields were produced from the combination treatment of compost, VP3 and 50% and 75% NPK biofertilizers. However, the treatment of VP3 biological fertilizer with compost without the addition of NPK fertilizer on 3 legumes was able to give higher yields than the treatment of single NPK fertilizer.

INTRODUCTION

In recent years, Indonesia's agricultural lands are experiencing nutritional deficiencies due to the pervasive use of inorganic chemical fertilizers by farmers. Such a behavior is anchored by an assumption that inorganic chemical fertilizers can cater to nutrients in a relatively short time, produce available nutrients that can be absorbed by plants, contain more nutrients than organic fertilizers, and are easy to apply. However, prolonged and excessive use of inorganic chemical fertilizers is known to cause many negative effects. Continuous use of such chemical fertilizers can make the soil harden and lose its porosity (Munthali et al., 2014). Synthetic chemical fertilizers change the pH of the soil into more acidic. The increase of acidity can reduce the presence of soil microorganisms needed by plants, and the availability of nutrients (Roba, 2018). Currently, cultivation and fertilization practices are being pursued to restore soil fertility, among others by applying biological fertilizers together with organic fertilizers such as compost. Such an endeavor is done to minimize the use of inorganic chemical fertilizers in order to maintain the balance of the soil microecosystem so that the availability of nutrients in the soil is not disturbed and agricultural cultivation can be sustainable. The presence of microorganisms in biological fertilizers is needed by organic fertilizers to break down ions from complex to simple forms so that they can be absorbed by plants since organic fertilizers cannot directly provide nutrients for plants (Gupta & Husain, 2014). Biofertilizers applied in the field also take a long time to provide nutrients for plants through nitrogen fixation and phosphate solubilization by

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the microbes contained in them (Peix et al., 2001). This condition is considered a weakness for farmers, that is, enacting fertilizing combined with chemical fertilizers with compost and biological fertilizers.

Biofertilizers are used as a collective name for all functional groups of soil microbes that can serve as providers of nutrients in the soil, making them available to plants (Vessey, 2003). Meanwhile, compost is made from organic material that has undergone a weathering process due to activity of microorganisms (spoilage bacteria and cellulolytic fungi) that degrade organic material (Wang et al.,2015;. Aguilar-Paredes et al. 2023). The organic matter referred to compost is dry grass, dry straw, remnants of twigs and branches, fallen flowers, animal dung, livestock urine, and other organic materials mixed together (Aguilar-Paredes et al. 2023) All these organic materials will experience weathering caused by microorganisms (bacteria, fungi and actinomycetes) that thrive in moist and wet environments , The quality of organic matter affected soil microbial community structure because microbes exhibit metabolite (Wang et al., 2015) However, the use of inorganic fertilizers such as NPK in the long term can result in a decrease in the population of soil microorganisms (Wang et al., 2017; Farmer et al., 2017) and those effect when combined with organic fertilizer is needed to examine

Several studies employing the application of biofertilizers such as beans, long beans, and mung beans showed an increase in plant growth and yield. It was reported that the use of biological fertilizers VAM (Vesicular Arbuscular Mycorrizha) and 75% PSB (Phosphate Solubilizing Bacteria) on beans can increase plant length, number of leaves and weight of chickpea pods (Ramana et al., 2010). Another study also revealed that biofertilizers produced from *Bradyrhizobium* and *Streptomyces griseoflavus* provided a significant increase in the growth and yield of mung bean plants (Htwe et al., 2019). In the same vein, Siregar et al. (2018)'s work reported that the use of *Trichoderma* sp. as a biofertilizer produced better plant height, number of flowers, pods and weight of pods than those with the absent of *Tricoderma* sp. However, the use of other consortium microbe for producing biofertilizer is still limited.

The interaction between plants and soil microorganisms occurs around the rhizosphere. The interaction between soil and microorganisms involves the colonization of different microorganisms. The presence of microorganisms around the roots of plant growth results in sociative, symbiotic, *neutralistic* or parasitic interactions depending on the nutrient status in the soil, soil conditions, plant protection mechanisms and the types of microorganisms that multiply in the rhizosphere zone. It is known that microorganisms close to the epidermis of plant roots will secrete compounds for protection against the invasion of various microbes in the rhizosphere zone (Hayat et al., 2010). Rhizobium bacteria are able to symbiotically interact with legume plants to form positive interactions where rhizobium bacteria are in root nodules. In legume root nodules, nitrogen-fixing bacteria can reduce nitrogen in the form of ammonia so that nitrogen can be available to plants (Sessitsch et al., 2002). Other studies also reported the interaction of legume roots with soil microorganisms such as rhizobia that interact and compete for the formation of root nodules with inoculant lines form antagonistic interactions or synergistic interactions. Diazotrophic bacteria such as *Azotobacter* and *Azospirillum* as well as rhizosphere fungi and bacteria, especially *Pseudomonas* and *Bacillus* species also interact with *Rhizobium* and affect root nodule formation and nitrogen fixation in the soil (Ahmad et al., 2006; Rodriguez & Frioni, 2003).Unfortunatelly, the study of the effect of corsortium bacteria on legume growth and yield are rarely reported.

The present study was designed to examine whether the addition of inorganic chemical fertilizers reduced the population of soil bacteria even when VP3 biofertilizer was applied which derived from vermicomposting activities. Vermicomposting has been reported as a practicable, economical and swift technique for proficient management of the solid wastes (Sharma & Garg, 2018). The biofertilizer was VP3 biofertilizer which then combined with inorganic fertilizer (NPK fertilizer) during application. In particular, this study was conducted to examine the effect of inorganic chemical fertilizer application on long beans, beans and mung beans, as well as on the population of indigenous bacteria in the soil. It is known that these three plants are not allelopathic to the bacteria which was contained in the VP3 biofertilizer (Syafarotin et al., 2018). VP3 Biofertilizer was formulated using vermiwash as a carrier material (Arfarita et al., 2017), where vermiwash was a derived product of cultivating earthworms. Previously, Arfarita et al., (2016) had isolated and identified microorganisms as well as pathogenicity tests of those bioferfilizer. Empirically, it was found that indigenous bacteria which were consisted of P-solubilizing bacteria *Pantoea ananatis*, N-free *Bacillus cereus*, and exopolysaccharide-producing bacteria *Pseudomonas plecoglossicida* had the best activities during VP3 biofertilizer production.

MATERIALS AND METHODS

Experimental Design

The study was conducted from January to March 2022. The study was carried out under Glasshouse condition at the Faculty of Agriculture and Microbiology Laboratory, University of Islam Malang. This study used a factorial randomized block design (RBD) with 8 treatments and three replications, thus 24 treatment plots were obtained for each legume plant (bean, long bean and mung bean). The detail treatments in this study are presented in Table 1. The application of VP3 biofertilizer on three test plants were carried out at different time and frequency for each plant according to the best treatment in previous studies (Syafarotin et al., 2018; Nuzula, 2018; Hidayanti, 2018). The dose of VP3 biological fertilizer was 10 ml/liter of water and 10 ml of molasses/10 liter of water. For beans and long beans, the application of VP3 biofertilizer was twice, yet for mung bean, the application was 3 times. On beans, the application of VP3 was at 7 days before planting and 4 days after planting (DAP). At long bean, the application was at planting date and 32 DAP. Meanwhile, on mung bean the VP3 was applied at planting date, 7 and 30 DAP.

Materials

The materials used in this study were fertilizer-free soil (organic or inorganic), compost, VP3 biofertilizer (vermiwash carrier), NPK (inorganic fertilizer), bean variety “Lebat 3”, long bean variety “Kanton Tavi”, mungbean variety “Vima-1”, fungicide, formalin. Meanwhile, the population of soil bacteria were observed using NA (Nutrient Agar) media, 0.05% peptone water and 70% alcohol.

Crop Variables

The growth parameters included plant length (beans and long beans), plant height and number of leaves (mung bean). The yield variables observed were the total number of flowers, and pods and the total weight of the pods (bean and long bean) and seeds (mungbean). Plant height (cm) was observed by measuring from the soil surface to the maximum height at the last branch in each sample of mung bean. Plant length (cm) was observed by measuring from the soil surface to the maximum length at the last branch in each sample of bean and long bean plants. The number of leaves was observed by counting the number of fully developed (opened) leaves in each sample, excluding leaf buds.

Table 1. Description of treatments

No	Plants	Code	Treatments
1.	Beans	TKHA1V	Soil + Compost + 2 times VP3 Biofertilizer
2.		TKHA1V1	Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%)
3.		TKHA1V2	Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%)
4.		TKHA1V3	Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%)
5.		T1	Soil + NPK fertilizer (25%)
6.		T2	Soil + NPK fertilizer (50%)
7.		T3	Soil + NPK fertilizer (75%)
8.		T4	Soil + NPK fertilizer (100%)
9.	Long bean	TKHB1G	Soil + Compost + 2 times VP3 Biofertilizer
10.		TKHB1G1	Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%)
11.		TKHB1G2	Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%)
12.		TKHB1G3	Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%)
13.		T1	Soil + NPK fertilizer (25%)
14.		T2	Soil + NPK fertilizer (50%)
15.		T3	Soil + NPK fertilizer (75%)
16.		T4	Soil + NPK fertilizer (100%)
17.	Mung bean	TKHA2VG	Soil + Compost + 3 times VP3 Biofertilizer
18.		TKHA2VG1	Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (25%)
19.		TKHA2VG2	Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (50%)
20.		TKHA2VG3	Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (75%)
21.		T1	Soil + NPK fertilizer (25%)
22.		T2	Soil + NPK fertilizer (50%)
23.		T3	Soil + NPK fertilizer (75%)
24.		T4	Soil + NPK fertilizer (100%)

Soil Bacterial Population

Soil bacterial population was carried out by taking soil samples in each treatment in a composite manner. Soil bacterial population was observed using spread plate method and calculated using Total Plate Count method (CFU/ml). The samples that were observed for population of soil bacteria were 14 treatments with 3 replications and a series of dilutions up to 10^3. Observations were made on all treatments.

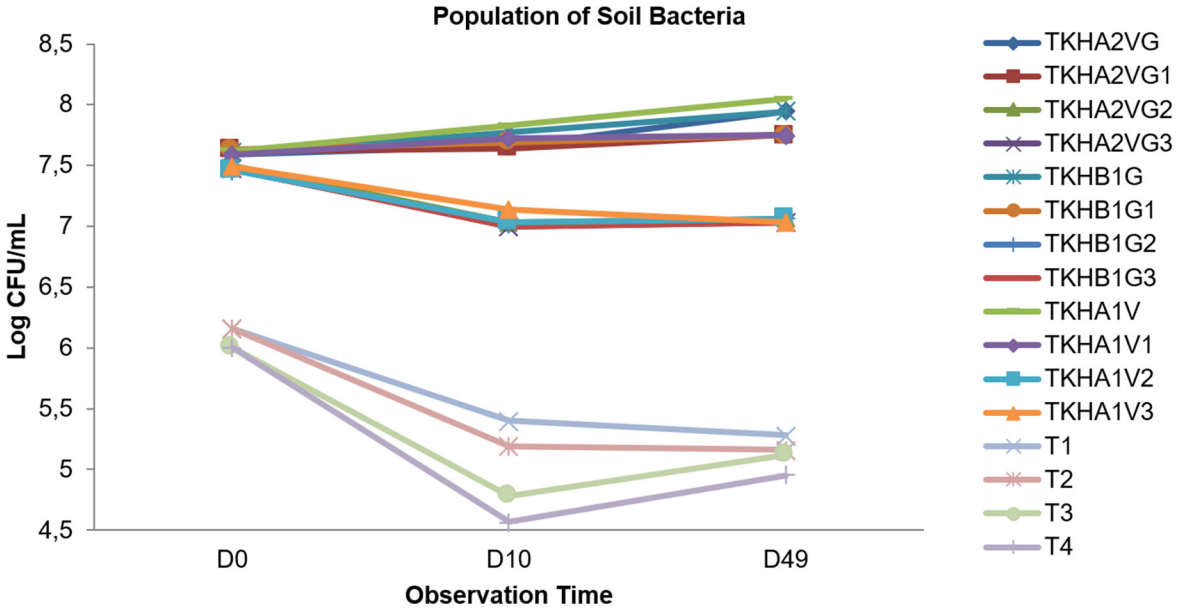
Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) to observe the significant effect of the treatment. Further, the result of ANOVA was continuously tested using the Least Significant Difference (LSD) at a 5% level of significance. Multivariate analysis CVA and Biplot were used to cluster and group the treatment and determine the relationship amongst the parameter using Genstat software version 12.

RESULTS AND DISCUSSION

Population of Soil Bacterial

Analysis of variance presented in Table 2. Fig. 1 shows that the application of inorganic chemical fertilizers (NPK) on legume plant media (beans, long beans and mung beans) significantly affected indigenous bacterial populations as shown in treatments of T1, T2, T3 and T4; and also to the bacteria from the application of VP3 Biofertilizer which was shown in the treatments of TKHA1V1, TKHA1V2, TKHA1V3, TKHB1G1, TKHB1G2, TKHB1G3, TKHA2VG1, TKHA2VG2 and TKHA2VG3.



Remarks: TKHA1V (Soil + Compost + 2 times VP3 Biofertilizer), TKHA1V1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA1V2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA1V3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHB1G (Soil + Compost + 2 times VP3 Biofertilizer), TKHB1G1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHB1G2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHB1G3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHA2VG (Soil + Compost + 3 times VP3 Biofertilizer), TKHA2VG1 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA2VG2 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA2VG3 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (75%), T1 (Soil + NPK fertilizer (25%), T2 (Soil + NPK fertilizer (50%), T3 (Soil + NPK fertilizer (75%), T4 (Soil + NPK fertilizer (100%). Numbers accompanied by the same letter in the same column show that they are not significantly different in the 5% LSD test

Fig. 1. Effect of inorganic chemical fertilizer application on the bacterial population in three test plants (beans, mung beans and long beans)

All treatments with VP3 biofertilizer application and compost had the same initial bacterial population values. However, the bacterial population in these treatments were higher than the single NPK fertilizer treatment (T1, T2, T3 and T4). This happened because the introduction of VP3 biofertilizer and the addition of compost increased the population of soil bacteria. After 10 days after planting, the population of soil bacteria was treated by T3 (75% NPK fertilizer application) and T4 (100% NPK fertilizer application). In treatments of T3 and T4, there were a high decrease on bacterial population compared to treatment of single NPK, namely T1 (NPK 25%) and T2 (NPK 50%). The bacterial population in TKHA1V2, TKHA1V3, TKHB1G2, TKHB1G3, TKHA2VG2 and TKHA2VG3 (application of VP3 biofertilizer and compost with the addition of 50% and 75% NPK) also decreased, but the total number of soil bacteria was still higher than T1, T2, T3 and T4. In contrast to the treatments of TKHA1V, TKHA1V1, TKHB1G, TKHB1G1, TKHA2VG and TKHA2VG1, there was an increase in the soil bacteria population after 10 DAP. This happened because of NPK application with doses of 50%, 75% and 100% greatly affected the life of soil bacteria. The higher the dose of NPK fertilizer applied to the soil would induce greater decrease in the bacterial population. However, the application of NPK fertilizer with a dose of 25% can still increase the population of soil bacteria because there is still additional application of VP3 biological fertilizer with compost (TKHA1V1, TKHB1G1 and TKHA2VG1). It is suspected that this low dose of NPK can still be tolerated by soil bacteria.

Table 2. Bacterial population in the soil under different treatments at 0, 10 and 49 DAP

Treatments	Population of soil bacterial (log)		
	D-0	D-10	D-49
TKHA1V	7.59c	7.66d	7.94f
TKHA1V1	7.62c	7.64d	7.75f
TKHA1V2	7.50c	7.03c	7.06d
TKHA1V3	7.48c	7.00c	7.03d
TKHB1G	7.60c	7.77d	7.94f
TKHB1G1	7.63c	7.69d	7.75e
TKHB1G2	7.46c	7.02c	7.06d
TKHB1G3	7.47c	7.00c	7.03d
TKHA2VG	7.62c	7.83d	8.05g
TKHA2VG1	7.59c	7.72d	7.75e
TKHA2VG2	7.46c	7.03c	7.06d
TKHA2VG3	7.49c	7.14c	7.03d
T1	6.16b	5.40b	5.28c
T2	6.16b	5.19b	5.16b
T3	6.00a	4.78a	5.12b
T4	6.00a	4.57a	4.95a
LSD 5%	**0.16**	**0.62**	**0.11**

Remarks: TKHA1V (Soil + Compost + 2 times VP3 Biofertilizer), TKHA1V1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA1V2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA1V3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHB1G (Soil + Compost + 2 times VP3 Biofertilizer), TKHB1G1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHB1G2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHB1G3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHA2VG (Soil + Compost + 3 times VP3 Biofertilizer), TKHA2VG1 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA2VG2 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA2VG3 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (75%), T1 (Soil + NPK fertilizer (25%), T2 (Soil + NPK fertilizer (50%), T3 (Soil + NPK fertilizer (75%), T4 (Soil + NPK fertilizer (100%). Numbers accompanied by the same letter in the same column show that they are not significantly different in the 5% LSD test

On the observation at 49 DAP, the bacterial population in treatments of T1 (NPK 25%) and T2 (NPK 50%) still decreased, yet increased at treatments of T3 (NPK 75%) and T4 (NPK 100%). This happened since the population was recovery at the treatments of T3 and T4. However, the number of soil bacteria in the T4 treatment was the lowest compared to the T2, T3, and T1 treatments. The T1 treatment still had the highest total soil bacteria value compared to T2, T3 and T4. While the T3 treatment, the total bacteria were not significantly different from the T2 treatment, which showed that the recovery in the T3 treatment was better than the T4 treatment. Other treatments that also experienced an increase in the population of soil bacteria on 49 DAP were TKHA1V, TKHA1V1, TKHA1V2, TKHA1V3, TKHB1G, TKHB1G1, TKHB1G2, TKHB1G3, TKHA2VG, TKHA2VG1, TKHA2VG23 and TKHA2VG3. This shows that the use of NPK fertilizer has an effect on the population of indigenous microbes in the soil. The use of 100% NPK fertilizer in the long term can significantly reduce the population of soil bacteria. The decrease in the soil bacteria population can inhibit the degradation of organic material in the soil from a complex to a simple form that can be absorbed by plants (Gupta & Husain, 2014). The increase and restoration of the number of soil bacteria occurred probably because on the 49th day of observation, the plant canopy had covered the soil and created more suitable environmental conditions for soil bacterial life, such as less sun exposure, the increase of humidity, and more suitable soil temperatures.

The application of inorganic chemical fertilizers has also affected the bacteria from the application of VP3 biofertilizer. This was shown from the observation on day 10, the addition of VP3 biofertilizer, compost and 50% and 75% NPK (TKHA1V2, TKHA1V3, TKHB1G2, TKHB1G3, TKHA2VG2 and TKHA2VG3), resulted in a decrease in total soil bacteria. While in the addition of 25% NPK, the population still increased until the 49 DAP and the increase was higher than the 10th day of observation. This was possible because the plant canopy that has covered the soil created environmental conditions that were more suitable for soil bacterial life, with reduced sun exposure conditions, increased humidity and more suitable temperatures. Inorganic chemical fertilizers provide nutrients that are directly available to plants so that the application of inorganic chemical fertilizers such as NPK fertilizers used in this study greatly affects plant growth and development (Cole et al., 2016: Dubey et al., 2017). However, the continuous use of inorganic chemical fertilizers can lead to a decrease in soil quality, a decrease in the population of microorganisms in the soil and the capacity to absorb groundwater. In addition, the use of inorganic chemical fertilizers with acid containment would make the soil to have a high acidity level and can damage the free N-fixing bacteria (Wang et al., 2017; Farmer et al., 2017). Agricultural cultivation practices that use inorganic chemical fertilizers are considered as unsustainable agricultural cultivation (Smale & Jayne, 2003).

Fig. 1 presents that the role of VP3 biofertilizer in conjunction with compost and NPK on the population of soil bacteria. The treatment with VP3 biofertilizer and compost like TKHA2VG could increase the population of soil bacteria, but the treatment single NPK could reduce the population of soil bacteria. This happened because the VP3 biofertilizer with vermiwash carrier (VP3) functions to maximize the ability of bacteria to survive during storage (Arfarita et al., 2016; Hartatik, 2017). Vermiwash has the potential as a medium for propagation of biological agents because it contains a complex nutritional composition and is good for bacterial growth such as water, carbohydrates, fats, proteins, amino acids, mineral, mineral salts, micro-elements and other nutrients. In addition to the VP3 biofertilizer with a vermiwash carrier, there was compost that plays an important role for microbeslife as a porosity creator to regulate the circulation of oxygen in the soil and as a source of energy and food for microbes in the soil with the availability of sufficient compost (organic matter), the activity of soil organisms can take place properly (Anwar, et al., 2005; Chen et al., 2021; Birkhofer et al., 2008; Liu et al., 2007). The population of soil bacteria decreased when the soil was applied with high doses of inorganic N fertilizer (Wang et al., 2017). Farmer et al., (2017) also reported that the use of inorganic fertilizers in the long term can reduce the bacterial population and bacterial diversity in the soil. However, the population of soil bacteria can increase when inoculated with biological fertilizers (*Azotobacter, Azospirillum and Rhizobium*) in the soil (Kuzyakov, 2010; Kaur et al., 2017).

Plant Growth

Plant Length and Plant Height

The results in Table 3 show that the best treatment on the plant length of beans (*Phaseolus*

vulgaris L.) was the TKHA1V and TKHA1V3, but not significantly different from TKHA1V1, TKHA1V2 and T4 treatments (100% application of NPK fertilizer). In long bean plants, treatment of VP3 biofertilizer application and compost had an effect on plant length, but it was not significantly different from T4 treatment (100% NPK). At 56 DAP, the height of mung bean plant of TKHA2VG, TKHA2VG1, TKHA2VG2, and TKHA2VG3 treatments were not significantly different. This shows that the substitution or addition of NPK at doses of 25%, 50% and 75% applied with VP3 biofertilizer and compost was not significantly different from the treatment with VP3 biofertilizer and compost without the addition of NPK fertilizer. However, when compared with plant height in T1, T2, T3 and T4 treatments, the plant height in treatment of VP3 biofertilizer and compost supplemented with NPK had a higher value.

Treatment of VP3 biofertilizer and compost affected the plant length of beans and long bean as well as the height of mung bean plant. The application of VP3 biofertilizer (with a *vermiwash* as carrier material) can increase plant growth because the microbes added to the biofertilizer are not only capable of increasing nutrient availability, but also increasing the efficiency of nutrient uptake (uptake) by plants thus, increase fertilization efficiency. In the formation phase, plant length and plant height require N and P elements, whose availability were supported by bacteria from biofertilizer VP3 which was applied with compost. The N-free fixing bacteria and phosphate solubilizing bacteria contained in the VP3 biofertilizer can degrade compost during the first week incubation period before planting and make the N and P nutrients needed by plants can be directly available at planting for the growth of legumes (bean, long bean and mung bean). Compost which is applied together with biological fertilizers serves as a source of nutrients for the survival of microbes in the soil (Aguilar-Paredes et al., 2023; Anwar et al., 2005). Compost can also improve soil structure and can improve micro and macro pores thus, increase groundwater holding capacity increases and proper air movement (aeration) in the soil (Sharma, & Garg, 2018; Chen et al., 2021; Birkhofer et al., 2008). Plant height as an indicator of growth showed that plants without organic fertilizer grew slower. Organic fertilizers improve soil conditions so that they can provide nutrients for plant growth (Cole et al., 2016). The addition of NPK fertilizer serves as an addition of nutrients in the soil to meet nutrient needs that can be utilized directly by legumes (Krestini, et al., 2020; Anatalia et al., 2021; Cole et al., 2016)

Number of Leaves

Table 4 shows that application of biofertilizer with compost compared to the application of NPK has an effect on the number of leaves of all legumes (test plants). The results showed that TKHA1V3 treatment gave the highest number of leaves on bean. However, it was not significantly different from TKHA1V, TKHA1V1 and TKHA1V2 treatments. In long bean plants, TKHB1G3 treatment was the best treatment in terms of number of leaves. However, it was not significantly different from the TKHB1G, TKHB1G1 and TKHB1G2 treatments. Similar to bean and long beans, the treatment of VP3 biofertilizer and compost applied gave higher number of plant leaves than single NPK fertilizer on mungbean. The number of leaves on day 56 DAP, treatments TKHA2VG, TKHA2VG1, TKHA2VG2 and TKHA2VG3 were not significantly different. This happens because the application of VP3 biofertilizer containing N-fixing bacteria or N-fixing bacteria can provide N nutrients that can increase the number of leaves (Souza et al., 2014; Htwe et al., 2019).

In the treatment of TKHA1V3 and TKHB1G3, and compost was applied twice combined with 75% NPK fertilizer, and affected the number of leaves in bean plant. This happened because VP3 biofertilizer contains N-fixing bacteria, thereby increasing the availability of N nutrients, then increasing leaf formation (Souza et al., 2014; Htwe et al., 2019). In addition, addition of 75% NPK fertilizer can also affect the number of leaves because NPK fertilizer is able to meet the optimum state of plant nutrient needs and the nutrients was available directly to be absorbed by plants. Krestini et al., (2020) stated that the role of nitrogen (N) for plants is to stimulate overall growth, especially stems, branches and leaves (Jakubus & Bakinowska, 2020). When NPK was combined with VP3 biological fertilizer and compost they can have a positive effect in increasing the number of leaves.

Crop Yield

The results showed that application of inorganic chemical fertilizers and VP3 biofertilizers on legumes affected the total number of flowers, the total number of pods, the total weight of the pods and the total weight of seeds of the legumes. Table 5 shows that TKHA1V3 treatment was the best treatment for the total number of flowers, total the number of pods and the total pod weight of beans.

TKHA1V3 was not significantly different from the TKHA1V1 and TKHA1V2 treatments. In long bean plants, TKHB1G2 and TKHB1G3 treatments gave the best total number of flowers but were not significantly different from TKHB1G and TKHBiG1 treatments. Meanwhile, in mung bean, TKHA2VG, TKHA2VG1, TKHA2VG2 and TKHA2VG3 gave the best total number of flowers compared to treatments T1, T2, T3 and T4.

Table 3. Effect of VP3 biofertilizer and NPK (inorganic fertilizer) applications on plant length of beans and long beans; and plant height of mung beans

Plants	Treatments	Plant Length/Plant Height (m) at Days After Planting (DAP)							
		7	14	21	28	35	42	49	56
Beans	TKHA1V	0.12	0.42	1.03	1.85	2.49 b	3.63 cd	3.89 c	4.07 d
	TKHA1V1	0.12	0.47	1.05	1.86	2.52 b	3.46 bcd	3.61 bc	3.74 bcd
	TKHA1V2	0.12	0.42	0.89	1.92	2.95 c	3.71 d	3.88 c	4.03 cd
	TKHA1V3	0.12	0.40	1.24	2.20	3.04 c	3.81 d	4.09 c	4.22 d
	T1	0.14	0.52	0.98	1.58	2.30 ab	3.01 abc	3.12 ab	3.27 abc
	T2	0.12	0.38	0.92	1.47	1.95 a	2.64 a	2.71 a	2.78 a
	T3	0.10	0.33	0.83	1.59	2.43 b	2.86 ab	2.99 ab	3.06 ab
	T4	0.11	0.46	0.93	1.72	2.40 b	3.23 bcd	3.38 abc	3.46 bcd
LSD 5%		NS	NS	NS	NS	42.02	67.45	75.94	77.10
Long Beans	TKHB1G	0.12	0.23	0.48	1.19 b	2.03 c	2.66 cd	2.90 c	3.01 d
	TKHB1G1	0.12	0.22	0.48	1.27 b	2.32 d	2.79 cd	3.00 c	3.10 d
	TKHB1G2	0.12	0.23	0.37	1.13 b	2.12 cd	2.70 cd	2.90 c	3.04 d
	TKHB1G3	0.10	0.20	0.31	1.12 b	1.90 c	2.84 d	2.99 c	3.11 d
	T1	0.10	0.22	0.26	0.56 a	1.30 a	1.79 a	2.00 a	2.08 a
	T2	0.10	0.21	0.23	0.56 a	1.43 ab	1.90 a	2.19 a	2.35 b
	T3	0.11	0.20	0.23	0.63 a	1.49 ab	2.33 b	2.53 b	2.63 c
	T4	0.10	0.22	0.28	0.67 a	1.59 b	2.48 bc	2.78 bc	2.88 cd
LSD 5%		NS	NS	NS	20.76	26.66	32.95	30.04	27.29
Mung Beans	TKHA2VG	0.10	0.20	0.24 cd	0.31 c	0.37 b	0.44 b	0.50 b	0.52 b
	TKHA2VG1	0.11	0.19	0.24 d	0.28 c	0.37 b	0.43 b	0.49 b	0.51 b
	TKHA2VG2	0.10	0.19	0.24 d	0.30 c	0.41 b	0.48 b	0.54 b	0.54 b
	TKHA2VG3	0.10	0.20	0.24 d	0.31 c	0.40 b	0.47 b	0.56 b	0.59 b
	T1	0.11	0.18	0.20 ab	0.22 ab	0.27 a	0.30 a	0.34 a	0.38 a
	T2	0.10	0.19	0.21 bc	0.23 b	0.28 a	0.32 a	0.37 a	0.39 a
	T3	0.091	0.17	0.18 ab	0.20 ab	0.26 a	0.29 a	0.32 a	0.34 a
	T4	0.096	0.16	0.17 a	0.19 ab	0.26 a	0.29 a	0.34 a	0.34 a
LSD 5%		NS	NS	3.05	3.37	5.22	8.02	10.86	11.72

Remarks: TKHA1V (Soil + Compost + 2 times VP3 Biofertiliser), TKHA1V1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA1V2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA1V3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHB1G (Soil + Compost + 2 times VP3 Biofertiliser), TKHB1G1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHB1G2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHB1G3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHA2VG (Soil + Compost + 3 times VP3 Biofertiliser), TKHA2VG1 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA2VG2 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA2VG3 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (75%), T1 (Soil + NPK fertilizer (25%), T2 (Soil + NPK fertilizer (50%), T3 (Soil + NPK fertilizer (75%), T4 (Soil + NPK fertilizer (100%). Numbers accompanied by the same letter in the same column show that they are not significantly different in the 5% LSD test

The application of VP3 biofertilizer and compost affects the number of flowers and the number of pods of all test plants, because the beginning of generative phase is marked by the beginning of flower formation. In these phase, P and K nutrients are needed more by plants compared to nitrogen (Jakubus & Bakinowska, 2020). As an evidence, phosphorus and potassium in compost are decomposed by microbes and make them available for bean plants thus can stimulate flower growth P functions as a raw material for the formation of certain proteins, helps assimilation and respiration and accelerates flowering and ripening of seeds and fruit. The element K acts as a building block for proteins and carbohydrates. Potassium also serves to strengthen the plant body so that leaves, flowers and fruit would not fall off easily (Jakubus & Bakinowska, 2020). The number of flowers determines the number of pods produced by plant. The more flowers that appear on plant would result more pods formation. Carbohydrates from photosynthesis will be used for the growth and development of other organs. With a sufficient number of leaves, plants can carry out photosynthesis optimally to improve the quality of flowers and pod development. The use of biofertilizers has also been applied to legumes. For example, the use of VAM and 75% PSB in bean plants was reported to increase plant length, number of leaves and weight of bean pods (Ramana et al., 2010).

Table 4. Effect of Application of VP3 Biofertilizer and NPK (An-organic Fertilizer) on Number of Leaves on Beans, Long Beans and Mung Beans

Plants	Treatments	Number of Leaves (Strands) at Age (Days After Planting)							
		7	14	21	28	35	42	49	56
Beans	TKHA1V	1.83	6.75	14.92 c	25.92 ab	41.67 bc	62.83 cd	76.33 bc	80.83 bc
	TKHA1V1	2.00	7.00	16.00 bc	27.92 abc	41.67 bc	64.92 cd	73.42 bc	68.25 ab
	TKHA1V2	2.00	7.50	14.92 bc	29.08 bc	47.42 cd	60.58bcd	85.50 c	78.42 bc
	TKHA1V3	2.00	7.42	17.33 c	31.17 c	50.33 d	68.25 d	83.75 c	87.25 c
	T1	2.00	6.00	12.25 a	24.08 a	36.25 ab	49.92 a	54.50 a	59.92 a
	T2	2.00	5.75	13.17 ab	24.75 ab	35.42 a	51.67 ab	55.67 a	56.75 a
	T3	2.00	6.25	12.50 ab	25.00 ab	38.00 ab	49.92 a	55.92 a	55.75 a
	T4	2.00	6.75	12.92 ab	25.00 ab	40.00 ab	56.58abc	67.92 ab	65.33 ab
LSD 5%		TN	TN	2.44	4.51	6.03	10.26	15.58	16.11
Long Bean	TKHB1G	2	7.27c	10.60b	19c	35.33d	47.58c	51.83b	53.17ab
	TKHB1G1	2	6.60bc	9.80b	20.27c	39.25d	58.08d	66.33c	60.50bc
	TKHB1G2	2	6.53bc	9.73b	20.07c	37.25cd	48.50c	66.67c	61.18c
	TKHB1G3	2	5.80ab	10.53b	19.93c	36.33cd	52.17c	67.83c	61.92c
	T1	2	5.00a	6.40a	11.53a	20.50a	29.17a	36.75a	45.25a
	T2	2	5.00a	6.87a	13.73ab	24.00ab	33.00ab	37.42a	45.33a
	T3	2	5.00a	7.13a	13.93b	24.17ab	37.50b	46.75ab	46.08a
	T4	2	5.00a	6.67a	12.47b	25.83b	37.33b	48.08ab	46.08a
LSD 5%		TN	0.30	1.11	1.94	3.87	4.60	12.08	9.35
Mung Bean	TKHA2VG	3.00	5.00	8.00 b	11.60 b	16.20 b	20.33 c	22.53 b	23.40 b
	TKHA2VG1	3.00	5.00	8.00 b	11.00 b	14.80 b	18.00 bc	20.80 b	21.33 b
	TKHA2VG2	3.00	5.00	8.00 b	11.93 b	15.93 b	20.93 c	23.40 b	23.67 b
	TKHA2VG3	3.00	5.00	7.40 b	11.00 b	15.20 b	19.20 c	22.40 b	23.00 b
	T1	3.00	5.00	5.20 a	8.33 a	12.07 a	14.13 a	16.40 a	17.67 a
	T2	3.00	5.00	5.60 a	8.40 a	12.20 a	14.60 ab	16.80 a	17.40 a
	T3	3.00	5.00	5.80 a	8.80 a	11.60 a	14.67 a	16.80 a	17.40 a
	T4	3.00	5.00	5.20 a	8.20 a	11.67 a	14.60 a	17.00 a	17.60 a
LSD 5%		TN	TN	0.85	1.33	2.03	2.72	3.41	3.80

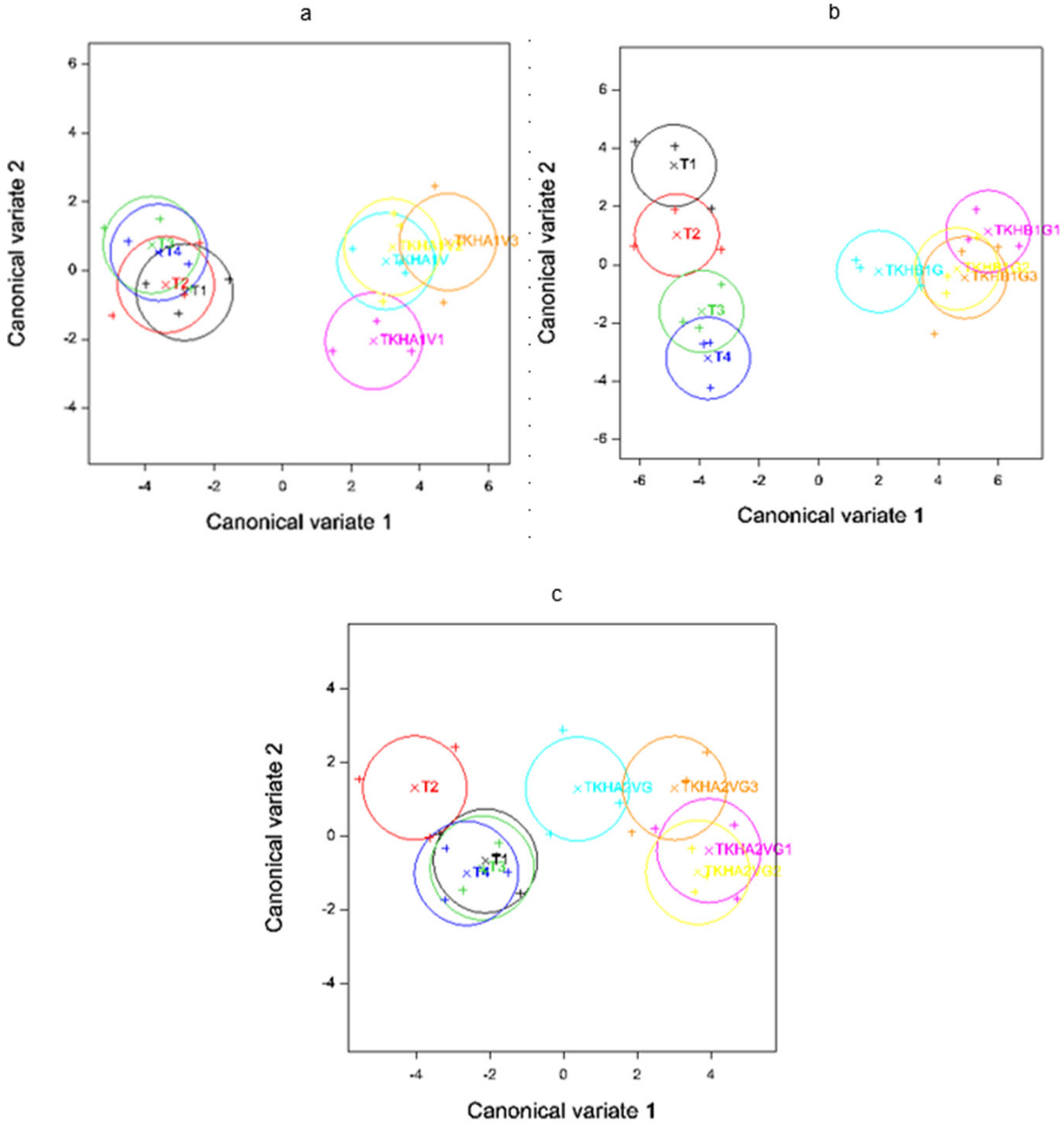
The use of biofertilizers in agricultural cultivation has an economic potential. Therefore, the use of biofertilizers is increasingly popular among farmers, especially for agricultural and food production. Furthermore, the long term biofertilizer use has the prospect of becoming alternative fertilizers and environmentally friendly fertilizers to increase the efficiency of fertilizer use to achieve sustainable agriculture. However, in the application of biological fertilizers and chemical fertilizers, their dosage and time application should be considered. This is related to their effects on the population of bacteria in the soil.

Multivariate Analysis

Principal component analysis axis was employed to cluster and distinguish the position of treatment among others based on multivariate parameters such as: plant height, number of leaves, number of flowers, number of pod and weight of pod along with the bacteria population. In term of beans, the (CVA-1) responded to 86.68% while the second principal components analysis axis (CVA-2) was compounded by 6.15% of the total variance, providing a cumulative percentage of both CVA-1 and CVA-2 in 100% of the total variance. CVA-1 was accounted mostly for the above parameters, which successfully split the treatments along the X axis and separate into two different groups. The first group located on the left side were overlapping one to another, which means there are no significant differences across those treatments. The first cluster were consisted of 4 treatment which were: T1, T2, T3 and T4, while the other group was located at far-right positions. The second group also overlapped one another (TKHA1V and TKHA1V2) except for the treatment of TKHA1V1 and TKHA1V3 (Fig. 2a).

Table 5. Effect of VP3 Biofertilizer and NPK (an-organic fertilizer) applications on total number of flowers, total number of pods, total weight of pods and total weight of seeds on legumes

Plants	Treatments	Total number of flowers per plant	Total number of pods per plant	Total weight of pods/seeds per plant
Beans	TKHA1V	39.00bc	28.42b	150.18b
	TKHA1V1	43.58bcd	32.17bc	150.22b
	TKHA1V2	52.33cd	33.33bc	173.79bc
	TKHA1V3	57.75d	42.17c	221.55c
	T1	22.50a	16.58a	76.94a
	T2	20.42a	14.25a	66.38a
	T3	27.67ab	16.25a	84.38a
	T4	21.25a	16.42a	82.63a
LSD 5%		18.03	10.92	55.31
Long Bean	TKHB1G	19.08cd	10.25cd	147.96c
	TKHB1G1	17.83cd	11.75d	193.50cd
	TKHB1G2	19.92d	11.70d	190.15cd
	TKHB1G3	20.50d	12.51d	196.75d
	T1	7.17a	3.72a	36.33a
	T2	8.00a	3.02a	34.63a
	T3	11.75ab	4.49ab	64.48ab
	T4	14.58bc	7.89bc	88.30bc
LSD 5%		4.92	3.41	47.81
Mung Bean	TKHA2VG	14.20b	10.40b	7.07b
	TKHA2VG1	17.00b	14.40c	8.95bc
	TKHA2VG2	18.00b	15.93c	10.42c
	TKHA2VG3	18.47b	15.07c	10.26c
	T1	9.07a	7.00ab	4.04a
	T2	8.47a	4.13a	2.46a
	T3	9.60a	7.13ab	4.11ab
	T4	8.67a	7.00ab	4.09ab
LSD 5%			3.69	3.01



Remarks: TKHA1V (Soil + Compost + 2 times VP3 Biofertiliser), TKHA1V1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA1V2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA1V3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHB1G (Soil + Compost + 2 times VP3 Biofertiliser), TKHB1G1 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (25%), TKHB1G2 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (50%), TKHB1G3 (Soil + Compost + 2 times VP3 Biofertilizer + NPK fertilizer (75%), TKHA2VG (Soil + Compost + 3 times VP3 Biofertiliser), TKHA2VG1 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (25%), TKHA2VG2 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (50%), TKHA2VG3 (Soil + Compost + 3 times VP3 Biofertilizer + NPK fertilizer (75%), T1 (Soil + NPK fertilizer (25%), T2 (Soil + NPK fertilizer (50%), T3 (Soil + NPK fertilizer (75%), T4 (Soil + NPK fertilizer (100%)

Fig. 2. CVA analysis on three test plants (a. beans; b. long bean; c. mung bean)

A similar pattern was observed at the long yard bean experiment, which also indicated a clear separation of two different groups. The first group was located at the left side, and not overlapping (T1, T2, T3 and T4) indicating that those treatment were significantly different based on the selected parameter above. The other cluster was consisted of overlapped treatment of TKHB1G2 and TKHB1G2 which were separated by the treatment of TKHB1G and TKHB1G1. The first and second CVA analyses contributed to 81.91 % and 14.17 %, respectively (Fig. 2b). Unlike the beans and long bean experiments the CVA assessment of mung bean experiment showed that the first group (T1, T3 and T4) was overlapped except T2 treatment. Meanwhile on the opposite position, the treatment of TKHA2VG1 and TKHA2VG2 were on the top of each other, while the other treatment was not overlapped (TKHA2VG and TKHA2VG3). The (CVA-1) as the first axes, responded 80.57 %. Meanwhile, the second principal components analysis axis (CVA-2) was compounded by 9.57% of the total variance (Fig. 2c). Multivariate analysis (PCA/CVA) is one of the prevailing statistical tools which could distinguished the reciprocal influence of various treatment involved in the experiment. Those method could accommodate a great number of calculation from various variable involved following an accurate analysis with low risk of loss many information. It could present 2 dimensional graphs which show two axis of principal components in which each of them contains the value of the magnitude of treatment separation variance. Moreover, using a multivariate PCA/CVA biplot, it could determine the relationship among the variables if they were had a similar magnitude and direction. Based on Principal Component Analysis (PCA) Biplot, the relationship between two variables were determined by the angle formed. If the angle formed less than 90°, there was a strong relationship and if not then the relationship is in the opposite manner (Afriliyanti et al., 2021).

The Principal Component Analysis (PCA) or CVA (Canonical Variate Analysis) has been successfull to examine interrelationship between the contents of various nutrients on others. Moreover, as a part of the PCA/CVA analysis for elements, graphs illustrating individual observations of clustering were created based on selected variables following the effect of those treatments. Plotted PCA/CVA of confidence circle allowed for identification of groups of plants or fertilization (Jakubus & Bakinowska, 2020). This method could identify two homogeneous fertillizer groups which were T2, T4 and T6 for the first group, and groups of T0, T1, T3, T5 for the second. The treatment of T0 is soil with no fertilization, T1 is soil under the application of NPK I, T2 is soil under the application of NPK II, T3 is soil which is combined with Organic Fertilizer (OF) I, T4 is soil under the combination OF II, T5 is soil combined with M–OF I, and T6 is soil combined with M–OF II, whereas the fertilizer dosage was as much as to about 70 kg N/ha (I) and to 170 kg N/ha (II). The study was carried out using three different crops which were: Camelina (*Camelina sativa* L.) (C), White Mustard (*Sinapis alba* L.) (WM) and spring barley (*Hordeum vulgare* L.) (B). It was also reported that between N and P, as well as N and K have a significant impact. This phenomenon was to justify the major roles of these macronutrients in affecting the changes of crop chemical composition. This reciprocal influences of the treatments were successfully identified by the Principal Components Analysis (PCA) graph.

Moreover, a clear grouping between the treatments using PCA indicated that metabolite compositions were affected by the type of fertilizer in Radish, Komatsuna and Mizuna crop. The results indicated that the levels of manure amendments, nitrogen, and fast release organic fertilizers was detected in the leaf metabolites of Mizuna. PC1 represented for 33.4% of the total variance and showed a significant correlation ($P<0.01$) with N absorption, while PC2 only contributed 15.6% of the total variance and discriminated between C and O treatments (Okazaki et al., 2016). In the case of the green bean experiment, there was a strong relationship between the number of leaves and the number of flower and number of pods and weight of pod. Moreover, most of agronomic parameter at green bean experiment did not correspond closely with bacteria population except for the plant height (Fig. 3a). In term of long yard bean experiment, there was a strong relationship between plant height and number of flowers, number of pod and the weight of pod and bacterial population and number of leaves (Fig. 3b). Under the mung bean experiment, most agronomic parameters were closely linked with bacterial population (number of leaves, plant height and number of flower) except the number of pod and weight of pod (Fig. 3c).

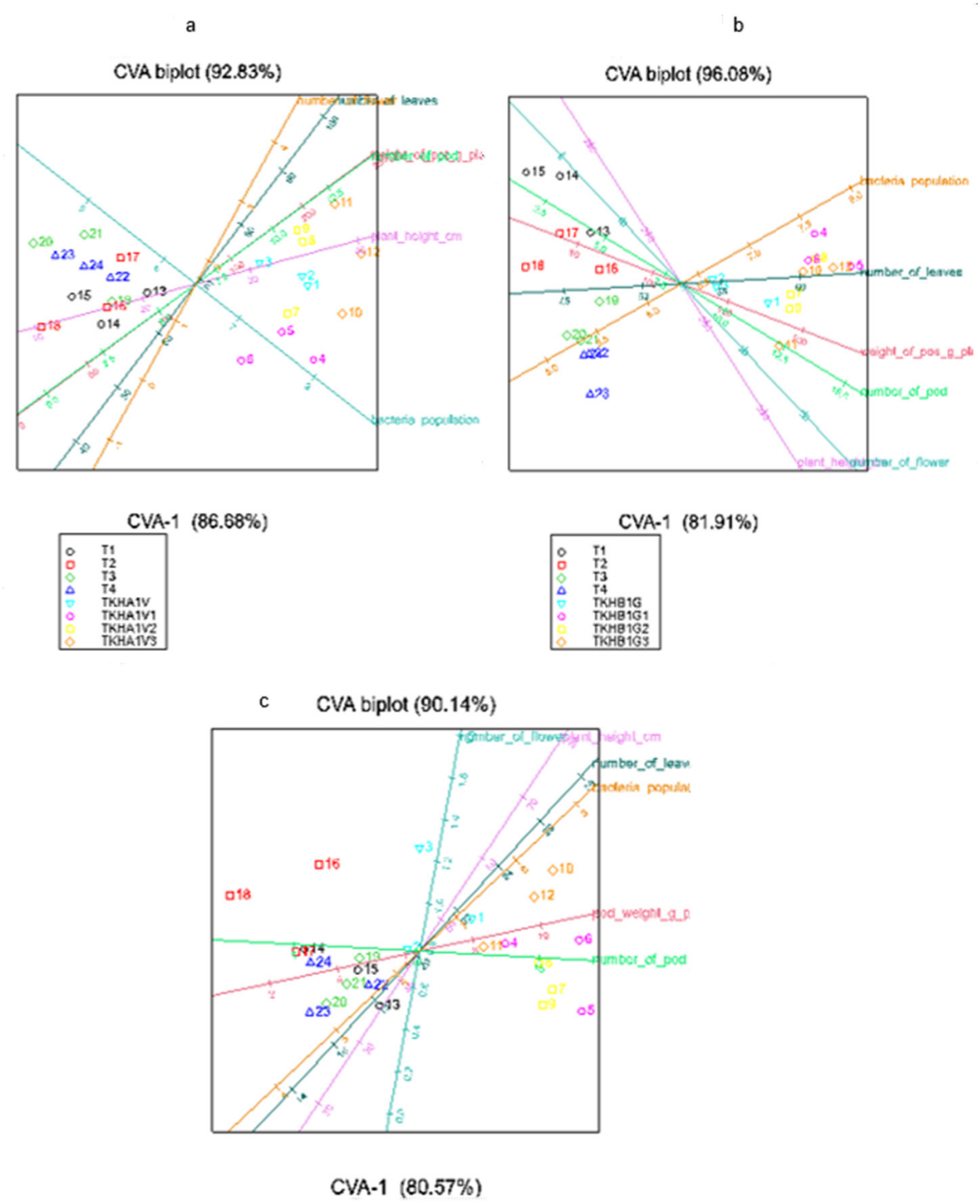


Fig. 3. BiPlot analysis on 3 different legumes (a. beans; b. long bean; c. mung bean)

A recent study concluded that the use of PCA Biplot can determine a relationship between soil parameters under long-term tillage and nitrogen fertilizer under Cowpea cultivation. There was a close relationship between cowpea production, soil respiration, fungal population, and soil organic C content, other four variables which were in similar trend such as the number of leaves, plant height, bacterial population, and total N content of the soil. The higher respiration rate indicates that the high activity and number or population of bacteria were occurred (Afriliyanti et al., 2021).

CONCLUSION

The study concluded that all treatments with the addition of inorganic fertilizers with high doses of 50% and 75% can reduce the population of soil bacteria significantly at observations day-10 (vegetative phase of legumes) and day-49 (phase of legumes or generative legumes). However, treatments without VP3 biofertilizer, a decrease bacterial population had occurred since the observation of day-0 (at planting), indicating that the application of NPK fertilizer greatly affected the indigenous bacteria of the soil which resulted in a decrease in their population. Applying biofertilizer VP3 and compost without additional application of NPK fertilizer can significantly increase the population of indigenous bacteria and biofertilizer bacteria. The role of VP3 biofertilizer in conjunction with compost and the addition of 25% NPK fertilizer was still able to increase the population of indigenous bacteria and VP3 biofertilizer bacteria but the effect was negligible. This shows that the higher the dose of NPK fertilizer greatly affects the bacteria from the applied VP3 biofertilizer formulation and decreases its population. In the bean and long bean plants, the highest yields was obtained from the treatment of compost, VP3 biofertilizer and the addition of 75% NPK. Meanwhile, for mung bean, the highest yields were shown in the combination treatment of compost, VP3 and 50% and 75% NPK biofertilizers. The treatment of VP3 biological fertilizer and compost without NPK fertilizer on 3 legumes was able to give higher yields than the single of NPK fertilizer. Application of VP3 biofertilizer and compost can replace 100% NPK fertilizer in all test plants. Therefore, the use of VP3 biofertilizer with compost is very economically potential and can support a sustainable agricultural system. The use of biological fertilizers and the application of chemical fertilizers must consider of dosage and time of application. The effect of using VP3 Biofertilizer compared to NPK fertilizer on 3 legumes are significantly different. Using multivariate PCA/CVA, it can be seen that there was a clear separation between the treatment group under the application of VP3 Biofertilizer and NPK fertilizer on the 3 different legumes (green bean, long yard bean, and mung bean). This also released the role of macronutrient (N, P, and K), plant physiology and development, which could also influence crop yields.

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