The effect of VP3 Biofertilizer and its carrying materials on the germination of six plants

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Abstract. This study investigated the growth after application of VP3 biofertilizer and its essential ingredients (vermiwash and vermicompost) and the effect of their pathogenicity on the germination of soybeans, green beans, long beans, kale, mustard greens, and green spinach. Research design was observed on plant height, root length, fresh weight, and signs of pathogenicity. The VP3 biofertilizer inoculation gave better results than other treatments on the high germination and fresh weight. However, in water spinach plants, the application of VP3 biofertilizer had a significant effect only on the height parameters of the seeds. Provision of VP3 biofertilizer and each of its carriers did not show any signs of pathogenicity such as necrosis, wilting, root rot, or stem rot. This finding indicates that VP3 biofertilizer is potential as a seed treatment and is also known to contain soil-borne disease controlling agent bacteria.

1. Introduction

Biological fertilizers (biofertilizer) are formulas that contain active or latent (microbial) organisms, usually in liquid or solid form, can mobilize, facilitate, and increase the availability of nutrients in the soil that are not available so that they become available forms through biological processes [1]. A good biofertilizer formulation can be obtained using a carrier material from vermiwash [2]. The ingredients of this vermiwash have resulted from fermentation from vermicompost, which is a by-product of earthworm cultivation. Arfarita et al. [3] have carried out isolation and identification of functional bacteria, and then formulated VP3 biofertilizer (vermiwash + PEG 1% + three bacterial isolates) containing indigenous bacteria: N-fixation bacteria, namely Bacillus cereus; phosphate-solubilizing bacteria (Pantoea annanatis); and exopolysaccharide-producing bacteria (EPS-producing bacteria).

The VP3 biofertilizer made from vermiwash has not been tested on the germination of soybean, long yard beans, green beans, kale, mustard greens, and green spinach. The application of VP3 biofertilizer to six sprouts aims to prevent disease and stimulate the rapid growth of sprouts to become healthy young plants. The germination phase was chosen for this study because germination involves a series of important processes that occur from seed dormancy until seedlings grow. Seedlings are young plants produced by generative or vegetative reproduction. According to Henny [4], germination is embryo growth and seed components that can grow normally into new plants (seeds). However, the seeds are very vulnerable to various diseases. Seed growth is also influenced by growth regulators (ZPT). This substance stimulates plant growth, such as root growth, shoots, germination, and so on [5]. In the soil, seed germination is also influenced by plant growth-promoting bacteria (PGPR). PGPR is a soil microbe...
found in the roots around plant roots that increase plant growth and protect against specific pathogens [6]. PGPR promotes growth and synthesizes and regulates the concentration of various growth regulators (phytohormones) such as IAA, gibberellins, cytokinins, and ethylene in the root environment [7].

Vermiwash is rich in dissolved nutrients and amino acids available to plants. Vermiwash acts as a tonic for plants and thus helps reduce plant pathogenic fungi. In addition, it can also increase the rate of photosynthesis in plants and increase the number of microorganisms in the soil, where these microorganisms help in the breakdown of soil organic matter [8]. However, vermiwash does not contain inoculants. The purpose of this study was to determine the effect of vermiwash and VP3 biofertilizers on the growth of six plant seeds and their potential as seed diseases.

2. Materials and methods
This research was conducted in the Microbiology Lab, Integrated Laboratory, University of Islam Malang, starting from preparing seeds for germination, Yoshida media, and biofertilizer. Inoculation of the sprouts into the treatment tube when they were 3 days old for soybean, long beans, and beans. At the same time, the sprouts of kale, mustard greens, and green spinach were inoculated when they were 2 days old. Observations were made every day on plant height, root length, fresh weight, and signs of pathogenicity. Observations were stopped at three days after inoculation of green beans, five days after inoculation of long beans, soybeans, and kale, eight days after inoculation on mustard greens, and twelve days after inoculation on green spinach.

This study used a completely randomized design (CRD) consisting of 3 treatments, namely K0 (sterilized aquadest), V0 (sterilized vermiwash), VP3 (vermiwash + PEG 1% + three bacterial isolates). The treatment was repeated 6 times to obtain 18 samples. The variables observed included growth variables and the presence of disease symptoms. Observation of growth variable was root length and fresh weight. Observation of pathogenicity was disease symptoms such as wilting, necrosis, root rot, and stem rot. Also carried out observation of bacterial populations on biological fertilizer VP3 and vermiwash using the Total Plate Count TPC (CFU / ml) method on PCA media. The bacterial population observations were carried out when the storage age was 90 days after bacterial inoculation for VP3 and 7 days after extraction for vermiwash. The data obtained were analyzed using statistical analysis with the F test (ANOVA) level of 5%. If it gave a significant effect, then continued with the LSD test with a level of 5%.

3. Results and discussions

3.1. Total bacterial population in VP3 biofertilizer and vermiwash
Based on the results of total bacterial population observations (Table 1). VP3 biofertilizer and vermiwash contain more bacteria than vermicompost at 7 days after manufacture or 7 days after incubation. Meanwhile, after the storage period of 90 days, the total population of bacteria in VP3 biofertilizer decreased. However, it still contains more bacteria than vermicompost and vermiwash. This reduction is typical in biofertilizer formulations during the storage period. According to Pelczar and Chan [9], microbes will grow well in their environment only if conditions are favorable for growth and survival. If there are physical or chemical changes, such as depletion of nutrients or changes in temperature and pH, it will affect the decline in microbial viability. VP3 biofertilizer contains vermiwash and molasses carriers and added three functional bacteria.

| Table 1. Total bacterial population in VP3 and vermiwash biofertilizer. |
|--------------------------|-------------------------|--------------------------|
| Samples                 | Total of Bacterial Population |
|                         | 7 days (CFU/ml)           | 90 days (CFU/ml)          |
| Vermicompost (Solid)    | 1.2 x 10⁴ CFU/ml          | 8 x 10⁴ CFU/ml            |
| Vermiwash (Liquid)      | 4.3 x 10⁶ CFU/ml          | 7 x 10⁵ CFU/ml            |
| VP3 Biofertilizer (Liquid) | 3.4 x 10¹² CFU/ml      | 1.7 x 10⁶ CFU/ml          |
According to Makkar et al. [10], vermicompost is an environmentally friendly substitute fertilizer usually used in organic agriculture and has shown beneficial effects with 40-60% optimal proportions in the varieties Linum LC-54 and LC-2063. Vermicompost contains microbiota such as fungi, bacteria, and actinomycetes suitable for plant growth [11]. Meanwhile, Vermiwash (V0) is a liquid organic fertilizer extracted from vermicompost fermentation. Vermiwash is a sustainable, non-toxic, and environmentally friendly material. Vermiwash is rich in amino acids, vitamins, and nutrients such as nitrogen, potassium, magnesium, zinc, calcium, copper, and iron and contains growth hormones such as auxins, cytokines, and N-binding bacteria and phosphate solvents (Nitrosomonas, Nitrobacter, and Actinomycetes) [12]. The physicochemical composition contained in vermiwash and vermicompost can be seen in Table 2.

Table 2. Physicochemical properties of vermiwash and vermicompost [13-15].

| Parameter               | Vermiwash       | Parameter               | Vermicompost   |
|-------------------------|-----------------|-------------------------|----------------|
| pH                      | 7.11 ± 0.02     | pH                      | 6.12 ± 0.03    |
| Total salts (ppm)       | 9841.67 ± 123.32| Total salts (ppm)       | 3148.67 ± 48.58|
| Total nitrogen (%)      | 0.02 ± 0.002    | Total nitrogen (%)      | 1.11 ± 0.05    |
| Organic carbon (%)      | 0.18 ± 0.02     | Organic carbon (%)      | 9.77 ± 5.05    |
| Available phosphate (ppm)| 48.86 ± 0.13   | C/N ratio               | 8.80           |
| Calcium (ppm)           | 192.4 ± 30.22   | Available phosphate (ppm)| 597.67 ± 0.58 |
| Magnesium (ppm)         | 142.53 ± 38.90  | Calcium (ppm)           | 322.33 ± 24.91 |
| Potassium (ppm)         | 245.67 ± 9.50   | Magnesium (ppm)         | 137.33 ± 19.50 |
| Manganese (ppm)         | 0.04 ± 0.02     | Potassium (ppm)         | 3428.33 ± 326.28|
| Iron (ppm)              | 2.21 ± 0.04     | Manganese (ppm)         | 0.69 ± 0.01    |
| Copper (ppm)            | 0.35 ± 0.01     | Iron (ppm)              | 0.11 ± 0.01    |
| Zinc (ppm)              | 0.03 ± 0.01     | Copper (ppm)            | 0.01 ± 00      |
| Total Heterotrophs (CFU/ml)| 1.79 x 10³     | Zinc (ppm)              | 2.13 ± 0.05    |
| Nitrosomonas (CFU/ml)   | 1.01 x 10³      |                         |                |
| Nitrobacter (CFU/ml)    | 1.12 x 10³      |                         |                |
| Total Fungi (CFU/ml)    | 1.46 x 10³      |                         |                |

While the composition of VP3 biofertilizer consists of a carrier comprised of vermiwash + PEG 1% + 3 functional bacterial isolates and molasses. The VP3 biofertilizer has a higher number of microbes because, in addition to the microbes in the vermiwash, three functional bacterial isolates are also added (Table 1). Additional ingredients such as PEG and molasses can also support the growth and viability of bacteria contained in the liquid formulation. PEG 1% is a carrier material often used as an additive to a formulation to increase solubility, which is inert, not easily hydrolyzed, and prevents fungal growth [16]. Meanwhile, molasses contain nitrogen, which is beneficial for bacterial growth and useful for plants. Molasses contain vitamins and pigments [17]. In addition, molasses act as an excellent chelating agent because it can change chemical forms into readily available forms [18]. Molasses acts as a source of energy that supports the survival of microbes. Giyanto et al. [19] also stated that biological fertilizers from organic liquid waste have potential as a medium for propagating biological agents. Biological fertilizers contain good nutritional composition for bacterial growth, such as carbohydrates, protein, water, amino acids, fats, mineral salts, and other nutrients.

Martin et al [16] formulated VP3 biofertilizer with the addition of 3 functional bacteria, namely N-free fixing bacteria (Bacillus cereus), phosphate solubilizing bacteria (Pantoea ananatis), and EPS-producing bacteria, namely Pseudomonas plecoglossicida. These bacteria can help to fix N in the air and dissolve P to meet plant nutrient needs. The N is a constituent of proteins, nucleic acids, and protoplasm to increase plant height [20]. VP3 biofertilizer (vermiwash + PEG 1% + three bacterial
isolates) can act as a biocontrol agent that is not harmful to ecological processes and the environment. It is potential as a Seed Treatment. In addition, VP3 biofertilizer most likely produces secondary metabolites beneficial to plants because they contain more bacteria.

### 3.2. Pathogenicity test

Based on the results of observations (Table 3 and Figure 1), it shows that the VP3 biofertilizer treatment did not show any pathogenicity effects (such as wilting, necrosis, root rot, or stem rot) on the six test seeds: soybeans, long beans, green beans, kale, mustard greens, and green spinach. VP3 biofertilizer is a liquid formulation containing functional bacteria such as *Bacillus cereus*, *Pantoea ananatis*, and *Pseudomonas plecoglossicida*. Many studies have reported that these bacteria can act as biological control agents for plant diseases.

Several studies have reported that *Bacillus cereus*, not only as an N-fixing bacteria, is also an agent with great potential for use as biological control. Christina [21] reported that *B. cereus* strain W35 could control plant diseases. Emmert and Handelsman (2006) [22] also reported that *B. cereus* can produce two kinds of antibiotic compounds that can inhibit the growth of the *Phytophthora* fungus, which causes sprouts and root rot in soybean plants. Meanwhile, Meyer et al. [23] reported that *Pseudomonas plecoglossicida* produces siderophores under Fe deficient growth conditions. Microbes capable of producing siderophores have implications for controlling microbes that cause plant pathogens. Limura and Hosono [24] stated that *P. ananatis* isolate from wheat germ has been shown to have vigorous antifungal activity against *Rhizopus* spp. in vitro. Another study also showed that *Pantoea ananatis* has antagonistic activity against the pathogen *Rhizoctonia solani* [25]. *P. ananatis* bacteria can be applied as an effective biocontrol agent. After all, it can protect the host plant from infection with various pathogens such as bacteria and fungi because it has antifungal and antibacterial properties. The application of biofertilizer is also reported to stimulate plant growth, protect plant root systems, and increase plant resistance to disease attacks [26].

| Treatments | Soy Bean | Long Yardbeans | Bean |
|------------|----------|----------------|------|
|            | NK       | L              | BA   | BB   |
| K0         | -        | -              | BA   | BB   |
| V0         | -        | -              | BA   | BB   |
| VP3        | -        | -              | BA   | BB   |

**Table 3.** The presence of disease symptoms in each treatment on the test seeds.

| Treatments | Kale | Mustard Greens | Green Spinach |
|------------|------|----------------|---------------|
|            | NK   | L              | BA            | BB            |
| K0         | -    | -              | BB            |
| V0         | -    | -              | BB            |
| VP3        | -    | -              | BB            |

Note: NK: Necrosis, L: Wither, BA: Root rot, BB: stem rot; (-) no symptoms; (+) symptomatic.

The carrier material for biological fertilizers is one of the determining factors for the successful use of biological fertilizers. Biological fertilizers play an important role in maintaining the viability and effectiveness of microbes in their formulations. Microbes inoculated into the rhizosphere have a positive impact (mutualism or commensalism), adverse impacts (parasitism, competition, or amensalism) or have no impact (neutralism) [27]. In this study, Vermiwash played a role in maximizing the ability of bacterial inoculants to survive, agreed with Giyanto et al. [19], which states that vermiwash contains a good nutritional composition for bacterial growth such as carbohydrates, protein, water, amino acids, fats, mineral salts, and other nutrients.
3.3. Growth of six seedling

The formulation of biofertilizer VP3 consists of carrier material of vermiwash + PEG (1%) + 3 bacterial isolates [28]. The liquid formulation of biological fertilizers with the carrier (vermiwash) and 1% PEG is easily absorbed by plants. In the vegetative phase, plants require nutrients for metabolic processes such as cell division to form plant organs that support photosynthesis [29].

![Pathogenicity test on seedling of six plants.](image)

Figure 1. Pathogenicity test on seedling of six plants.

Table 4 and Figure 1 show that the application of VP3 biofertilizer and Vermiwash gave significantly different results than controls on seed height and fresh weight parameters for almost all of the tested plants. However, kale gave different results only in the height parameter of the seeds. The root length parameter of each treatment gave the same or not significantly different results for almost all of the tested plants. While the application of VP3 biofertilizer compared to Vermiwash (V0) showed no significant difference in the parameters of seed height and fresh weight of soybeans, beans, kale, and mustard greens. However, it gave significantly different results on the parameters of seed height, root length and fresh weight of string beans, seed height, and fresh weight of green spinach.

Arancon et al. [30] state that soil microorganisms play an important role in the availability and recycling of soil nutrients also the ability to store soil nutrients. The effectiveness of biological agents is influenced by microbial strain factors present in the formulation, environment conditions, and plant genotype. Vermiwash is a clear, transparent, and pale yellow liquid. It consists of various earthworm excretion and secretion products from soil organic molecules and micronutrients that are beneficial to plants [31]. Vermiwash contains a lot of nitrogen, phosphorus, potassium, calcium, magnesium, zinc,
and alkaline. Freshly extracted vermiwash contains many beneficial microbes which promote plant growth and prevent infection. Vermiwash acts as a tonic for plants and thus helps in reducing plant pathogenic fungi. In addition, it can also increase the rate of photosynthesis in plants and increase the number of microorganisms in the soil, where these microorganisms help in the process of decomposition of soil organic matter [8].

Table 4. Effect of treatments on growth of six plant

| Treatments | Soybean (5 dap) | Long Yard Bean (5 dap) | Bean (3 dap) |
|------------|----------------|-----------------------|-------------|
|            | SH  | RL  | FW  | SH  | RL  | FW  | SH  | RL  | FW  |
| K0         | 5.333 a | 2.350 | 0.620 a | 3.367 a | 2.037 a | 0.570 a | 7.100 a | 3.683 | 0.808 a |
| V0         | 9.400 b | 2.767 | 0.863 b | 6.450 b | 1.950 a | 0.677 a | 9.783 b | 4.867 | 1.022 b |
| VP3        | 7.817 b | 3.750 | 0.715 b | 8.817 c | 4.133 b | 0.860 b | 10.500 b | 5.133 | 1.132 b |
| LSD 5%     | 1.744 | NS  | 0.114 | 2.045 | 1.415 | 0.187 | 1.301 | NS  | 0.127 |

| Treatments | Kale (5 dap) | Mustard (8 dap) | Green spinach (12 dap) |
|------------|--------------|-----------------|------------------------|
|            | SH  | RL  | FW  | SH  | RL  | FW  | SH  | RL  | FW  |
| K0         | 6.467 a | 1.767 | 0.243 | 4.467 a | 1.233 | 0.043 a | 2.300 a | 1.100 b | 0.004 a |
| V0         | 7.367 b | 1.883 | 0.273 | 5.067 ab | 1.333 | 0.055 b | 2.383 a | 0.950 a | 0.005 a |
| VP3        | 7.550 b | 1.900 | 0.293 | 5.650 b | 1.383 | 0.060 b | 2.667 b | 1.133 ab | 0.007 b |
| LSD 5%     | 0.750 | NS  | NS  | 0.681 | NS  | 0.013 | 0.270 | 0.152 | 0.001 |

Keterangan: The numbers accompanied by the same letter in the same column show no significant difference in the LSD test of 5%; NS: Not Significant; SH: Seedling height; RL: Root length; FW: Fresh Weight.

3.4. Seedlings height

The application of VP3 biofertilizer and Vermiwash (V0) showed different seedling heights from the control (Table 4). As stated by Meirina [32], nutrients produced by microbial activity contained in the biofertilizer in the form of nitrogen (N), phosphorus (P), and potassium (K) are absorbed by plants for metabolic processes in plants. In addition, vermiwash also contains growth hormones such as auxin, gibberellin, and cytokinins, where these hormones can stimulate plant vegetative growth if available in optimal doses for plants. Cytokinin hormones stimulate cell growth in plants, and then the dividing cells will develop into shoots, branches, and leaves [33]. It is hoped that the absorbed elements can be used to encourage the division and formation of new cells to form better plant organs such as leaves, stems, and roots to accelerate the photosynthesis process [29].

Meanwhile, biofertilizer VP3 and Vermiwash (V0) application to soybeans, green beans, kale, and mustard greens showed no significant difference in seedling height parameters. Still, they gave significantly different results for long beans and green spinach. This finding indicates that each plant seed responds differently to plant height parameters to biofertilizer VP3 and Vermiwash applications.

3.5. Root length

Observations on the root length parameters of beans, kale, soybean, and mustard greens showed that all treatments gave not significantly different results (Table 4). Inhibition of root growth can be caused by ethylene formed from auxin production, and most of the ethylene in plants can inhibit root elongation [34]. Auxins are synthesized at the apex of the canopy and roots to be transported through the embryo process. In this study, the seedlings were tested into a transparent test tube covered with cotton so that the seeds were exposed to direct sunlight and were likely to be damaged. This is in accordance with Riyadi [35], which states that auxins are easily damaged when exposed to direct light. These conditions may not be suitable for the root system of beans, kale, soybean, and mustard greens seedlings.

However, the root length parameters of long beans and green spinach gave significantly different results. Masfufah [36] states that if a plant is placed in favorable conditions with appropriate nutrients and mineral elements, the plant will experience upward growth and become taller. Conversely, suppose
the plant is placed in a condition of excessive nutrient availability. In that case, it will result in the plant being reluctant to absorb it because the nutrients are already available in the root area, resulting in a low root length.

3.6. Fresh weight
The observation of fresh weight showed that the application of biofertilizers VP3 and Vermiwash (V0) gave results that were not significantly different for soybeans, beans, and mustard greens but significantly different for long beans and green spinach (Table 4).

Plants need sufficient and balanced nutrients. If nutrients are given in excessive or low amounts, it will cause the plant's fresh weight to decrease. However, the availability of nutrients in a balanced manner allows plant growth and production to occur well. The optimal photosynthesis process will affect the fresh weight of the plant. The available water conditions will be more easily absorbed by plants so that automatically the water content in the plants will increase so that the plant's fresh weight increases [37]. An increase in biomass can be caused by plants absorbing more water and nutrients, nutrients that stimulate the development of organs in plants such as roots so that photosynthetic activity increases and increases the wet weight and dry weight of plants [38].

Vermiwash is the result of the fermentation of vermicompost, which is a by-product of cultivating earthworms. Vermiwash contains enzymes and earthworm secretions that stimulate the growth and yield of plants. Vermiwash contains macro and micronutrients such as nitrogen, phosphorus, potassium, calcium, sodium, magnesium, and micronutrients such as Fe, Cu, Zn, and Mg [39]. However, after undergoing the sterilization process, there may be some damaged ingredients. Therefore, giving VP3 biological fertilizer is better because it contains bacteria and additional vermiwash.

4. Conclusions and suggestions
Inoculation of VP3 biofertilizer gave better results than vermiwash and control on the height and fresh weight of seedlings tested (six plants). However, in water spinach plants, the application of VP3 biofertilizer had a significant effect only on the height parameters of seedlings. Inoculation of biofertilizers VP3 and vermiwash did not show any signs of pathogenicity such as necrosis, wilting, root rot, or stem rot on the 6 types of seedlings tested. Biofertilizer VP3 is potential as a Seed Treatment because it is not pathogenic to the test plant seeds and is also known to contain soil-borne disease-controlling agent bacteria. The application of VP3 biofertilizer is more effective in the seedlings of Long Beans and Green Spinach because the effectiveness of biological agents is influenced by the microbial strain factors present in it, the growing environment, and plant genotypes.

Vermiwash is a carrier material in biofertilizer VP3 with the addition of 3 bacterial isolates, possibly containing the IAA hormone even though it has undergone sterilization. It is necessary to carry out further tests regarding the content of growth regulators (phytohormones) IAA in the liquid formulation of biofertilizers VP3 and vermiwash.

Acknowledgments
The authors wish to thank Miftahur Rohmah and Syafarotin for their contribution to assist in laboratory work.

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