International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Potential analysis *Beauveria bassiana* entomopathogen endophyte fungal as phosphate solvent

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Abstract

This research aimed to determine the potency of *Beauveria bassiana* entomopathogen endophyte fungal as phosphate solvent. This was qualitative research with the Pikovskaya medium and observation used to collect data from six isolates of *B. bassiana* from *Triticum* plant (TD312), *Leptocorisa acuta* insect (BbWS), chili leaf (PD114), chili root (PA221), chili stem (PB211), and *Coffea* plant (KT211), from Biological Control Laboratory of Universitas Andalas for 7, 10 and 14 days. The observation variable was carried out to determine the potency of all isolates as phosphate solvents. The result showed that the tested *B. bassiana* entomopathogen and endophyte fungal isolates were capable of being used as phosphate solvents. The highest phosphate solubility index on 14 dai was BbWS isolate (5,42), followed by the TD312 isolate (5,28).

Keywords: *Beauveria bassiana*; entomopathogen; endophyte; phosphate solvent

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1. Introduction

Phosphate solubilizing fungi are capable of easily dissolving phosphate in soil for it to be utilized by plants [1]. Phosphorus (P) is the second vital nutrient needed by plants after Nitrogen. Approximately 95-99 % of this solvent found in the soil is insoluble and absorbed by plants [2]. However, the application of chemical fertilizers to soil is not the best way to dissolve this solvent, because the given phosphate fertilizer acts very quickly with iron, aluminum, manganese, and calcium in acidic and alkaline soils. This makes it difficult for plants to absorb phosphate in the soil during heavy rain (Yasser et al., 2014) [3, 4]. Some bacteria and fungi are capable of solubilizing phosphates that do not dissolve in the soil [5]. The fungus is reported to have a better ability to dissolve insoluble phosphate than bacteria [2]. Elfiati et al. [6] stated that Aspergillus niger is the highest phosphate solubilizing the inoculant biofertilizer from peat soils. Penicillium and Aspergillus fungi have an excellent ability to dissolve phosphate [7]. Biofertilizer technology is developed to facilitate the provision of Nitrogen and Phosphorus nutrients, production of growth hormone (biostimulant), plant pest control (biopesticide), and reformer of organic materials, with their effectiveness determined by the function and technique application in the field.

Several research have been conducted to increase the plant growth and yield of chili plants through the use of biofertilizers. However, this research only focuses on the use of plant growth to promote rhizobacteria (PGPR), such as Azotobacter and Pseudomonas. The use of micromycetes fungi as a biofertilizer in chili production is still very limited. There are several advantages associated with the use of micromycetes as biofertilizers such as the ability to adapt to the soil environment, and the capability of many species to dissolve inorganic P sources, yielding indole-3acetic acid (IAA) and siderophores [8]. One of the potential fungal genera used as soil fertilizer for the production of growth hormone is *Beauveria* spp., also known as entomopathogenic fungus. The research by Felipe et al., [9] showed that Beauveria brongniartii is capable of dissolving P from Ca3(PO4)2, and producing siderophores and Indole Acetic Acid (IAA). Application of B. brongniartii on Capsicumchinense as fertilizer biochemicals increases plant growth and fruit quality. The potential of other fungal species, such as *Beauveria bassiana* as a biofertilizer and biostimulant needs to be investigated. *B. bassiana* is an entomopathogenic fungus usually used as a biological agent to control cabbage pests, such as Crocidolomia pavonana [10]. *Spodopteraexigua* [11], *S. litura* [12], *Nezara viridula* [13], *Eurydema pulchrum* [14], and *B. bassiana* also have the ability to increase plant growth and act as a stimulant. Affandi et al. [15] stated that the fungus of *B. bassiana* entomopathogen is capable of stimulating the plant growth, such as *Phaseolus vulgaris*. This is an exploratory result of endophytic fungi obtained from roots, stems, and seeds chili to stimulate its growth [16]. *B. bassiana* fungi can increase the roots' height, wet weight, and shoots of Vicia faba by foliar spray inoculation [8]. According to Qayyum et al. [17], *B. bassiana* has the ability to stimulate the growth of tomatoes through the spray of foliar. Russo et al. [18] reported that the fungus of *B. bassiana* inoculated on corn plants can increase their height, number of leaves, seed weight, and germination. Several Beauveria sp can also produce oxalic and citric acids,

while *B. caledonia* and *B. bassiana* lead to the formation of formic, lactic, oxalic, and nitrate acids.

It is necessary to determine new technology for the cultivation of chili in a more friendly and sustainable environment, using biological fertilizers in the form of fungi entomopathogen *B. bassiana*. Research need to be conducted on the ability of entomopathogen endophyte *B. bassiana* fungi to dissolve phosphate to obtain the best isolates to be developed as biofertilizer. Therefore, this research aimed to examine and analyze the isolates of *B. bassiana* entomopathogens endophyte to determine its ability to dissolve phosphate and increase the plant growth of chilies.

2. Materials and methods

This research was conducted at the Biological Control Laboratory of Universitas Andalas, from August to December 2021. It aimed to determine the ability of the fungus *B. bassiana* to dissolve phosphate using a qualitative test method. The media used for the solvent test is Politkovskaya consisting of 10 g glucose, 5 g Ca_3 PO_4, 0.5 g $(NH_4)_2$ SO_4, 0.2 g KCl, 0.1 g $MgSO_4H_2O$, 0.01 g $MnSO_4H_2O$, 0.5 g yeast extract, and 0.01 g $FeCl_3$, H_2O in one liter of solution at pH 7.0. The medium was placed in a Petri dish and allowed to harden. *B. bassiana* isolates were used from the Biological Control Laboratory collection. These include entomopathogenic (BbWS), endophytic TD312, PA221, PB211, PD114, and APKo fungal isolates from the wheat stem, chili root, stem, leaf, and coffee leaf rejuvenated on the medium of Saboroud Dextrose Agar Yeast (SDAY). Each isolate was grown on a medium tested by the spot inoculation method and incubated for 7, 10, and 14 days. After the inoculation process, the colony growth forms a holo zone, with isolates capable of dissolving phosphates. The phosphate dissolution qualitative test aims to determine the ability of bacterium and fungus to insolubilize phosphate [19]. The qualitative test was carried out by calculating the average holo zone produced by each isolate on the Pikovskaya medium. The phosphate reduction index is calculated using the following formula.

$$\text{PSI} = \frac{\text{Colony diameter} + \text{holozone diameter}}{\text{colony diameter}}$$

$$\text{PSI} = \text{Phosphate Solubility Index}$$

3. Results and Discussion

3.1. The Ability of Entomopathogenic and Endophyte B. bassiana Fungi as Phosphate Solvent

The ability of *B. bassiana* to dissolve phosphate using a Pikovskaya medium was incubated for 14 days and observed for 7, 10, and 14 days afterward. Pikovskaya medium is used to test microbes with the potential to dissolve phosphate characterized by the formation of the holo zone around the fungus colony. The holo zone produced indicated that *B. bassiana* entomopathogenic and endophyte isolate released Ca, which binds the phosphate on the politkovskaya medium to produce Ca(PO4). The differences between the colonies and holo zone through the treatment of several fungal isolates on Politkovskaya media are seen in Figure 1. Figure 1 shows that all tested *B. bassiana* isolates had the ability to solubilize phosphate at 7, 10, and 14 days after incubation. Holozone diameter of *B. bassiana* isolates increased on the surface of pikovskaya media. Hafsari and Pertiwi [20] stated that the clear zone produced by the fungus phosphate solvent released P bound to the medium of organic acids produced by microbes, which react with $Ca_3(PO_4)_2$, to form an organic chelate with P liberated and dissolved to form a clear colored area. The ability of the fungus to dissolve phosphate is measured by comparing the diameter of the halo zone produced by the fungus with the colony. The measured phosphate solubility index showed the capability of the potential for phosphate solubilizing fungi. According to Alam et al. [21], the formation of the halo zone is due to the dissolution of the tricalcium suspension or iron phosphate. Furthermore, Saragih et al., [22] described the ability of the fungus to qualitatively dissolve phosphate depending on the genetic nature of each fungus in producing organic acids. The production of the most suitable organic acid releases phosphate. The average data of phosphate solubility index from tested *B. bassiana* isolate is shown in Table 1.

The data in Table 1 and Figure 1 show the difference in phosphate solubility index at 7, 10, and 14 days post inoculation (dpi). At each observation, the results showed that the differences increase in the phosphate solubility index at 7 days. Based on the magnitude, it was discovered that there were insignificant differences between BbWS, TD312, PB211, PD114, and KT211 isolates. Meanwhile, TD312 was significantly different from PA221 isolate. The highest PSI was found in the TD312 isolate, which was 4.65 mm. All the tested fungal isolates significantly varied from the control at 7, 10, and 14 days. At 10 dpi observations, there was a significant difference between TD312, PA221and BbKo isolates. Similarly, PA221, PB211, PD114, BbWS, and KT211 isolates were different but insignificant. The PSI between KT211 and PA221 isolates was insignificantly different. The highest PSI of 5.07 mm was found in TD312 isolate. PSI observation on 14 day, between BbWS and TD312 isolates, were insignificantly different, while those from APKo and PA211 isolates were significant. PSI differences between BbWS, TD312, PD114, and PB211 isolates were insignificant. Meanwhile, the PSI between isolates of BbWS, TD312, PD114, and PB211 was insignificantly different. The highest PSI of 5.42 mm was found in the BbWS isolate on the 14th day. In comparison with the research by Evan [23], the highest phosphate solubility index from Aspergillus sp isolate from corn root endophytic fungi found on the 10th day of observation was 7.58 mm. Handayani et al. [7], stated that one endophyte fungal from rice plant roots has the ability to dissolve phosphate by 20.45%. The results showed that the endophytic fungal obtained from the roots of different isolates comprises a phosphate solvent. The research by Syamsia et al. [24] on local aromatic rice plants found 12 endophytic fungi from rice roots, stems, and leaves, dissolvable in phosphate between 8.92 to 10.86 mg-^{1}. Endophytic fungi isolate, which had the highest phosphate solubilizing ability, was an endophytic fungal isolate, from Pare Lambau stem. Data of PSI in Table 1 shows the difference in phosphate solubility index (PSI) by fungi with P $Ca_3(PO_4)_2$.

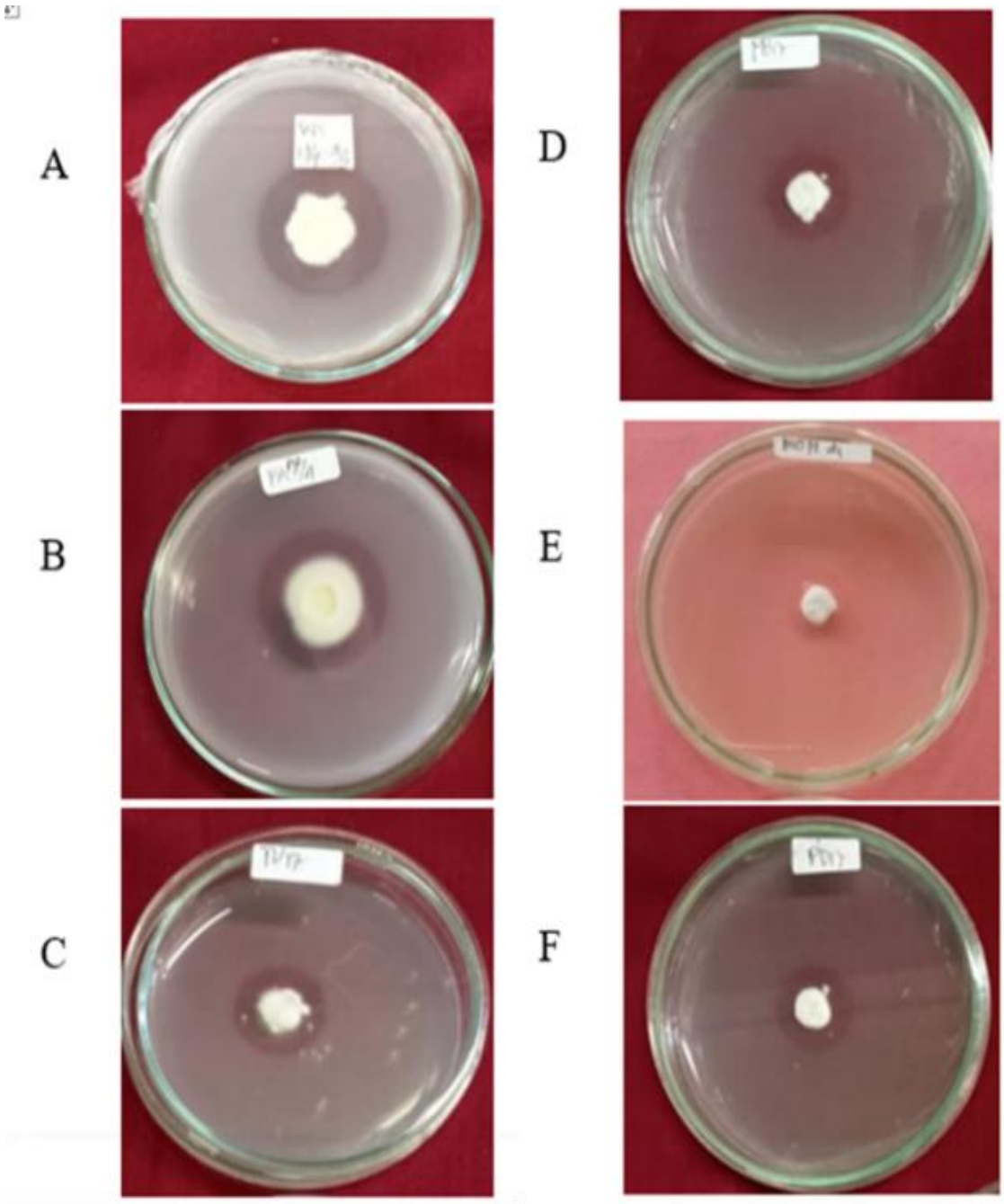
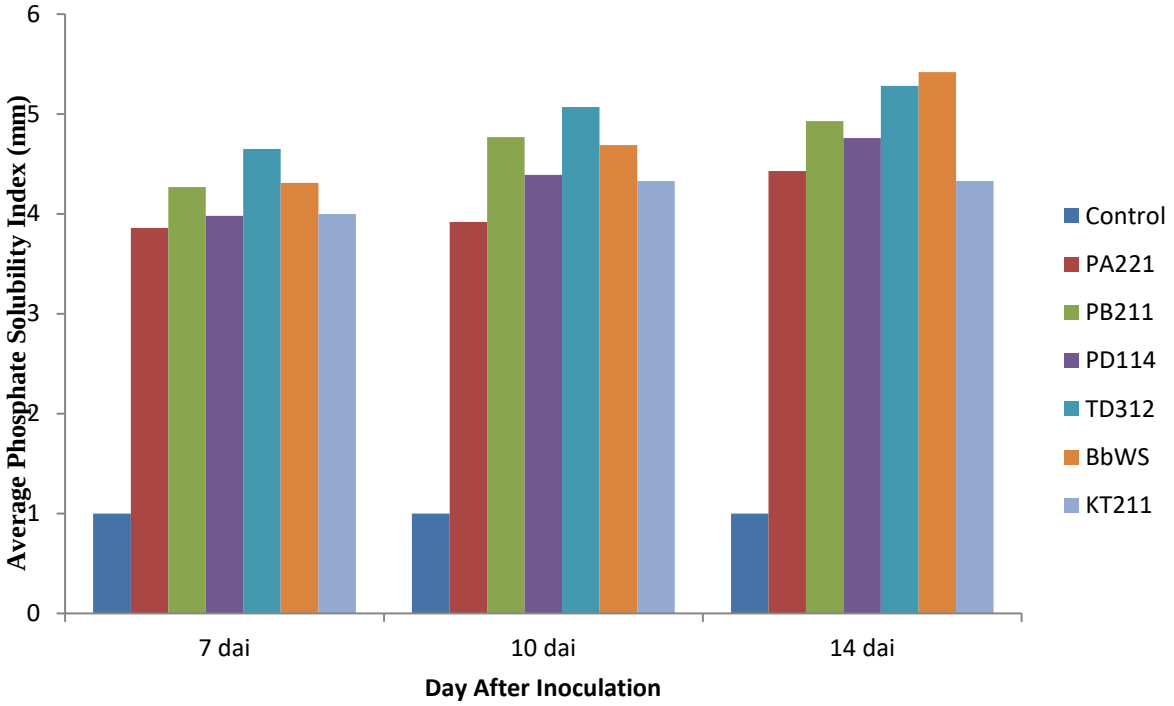


Figure 1. The Holozone of *B. bassiana* Fungal Isolates on Pikovskaya Medium. A = BbWS isolate; B = PA221 isolate; C = TD312 isolate; D = PB211 isolate; E = KT211isolate; F = PD114 isolate

Table 1. Average of Phosphate Solubility Index

Treatment	Average Phosphate Solubility Index (mm)
Control	
7 dai	1.00 c
10 dai	1.00 d
14 dai	1.00 c
PA221	
7 dai	3.86 b
10 dai	3.92 bc
14 dai	4.43 b
PB211	
7 dai	4.27 ab
10 dai	4.77 ab
14 dai	4.93 ab
PD114	
7 dai	3.98 ab
10 dai	4.39 abc
14 dai	4.76 ab
TD312	
7 dai	4.65 a
10 dai	5.07 a
14 dai	5.28 a
BbWS	
7 dai	4.31 ab
10 dai	4.69 ab
14 dai	5.42 a
KT211	
7 dai	4.00 ab
10 dai	4.33 bc
14 dai	4.33 b

*dai=day after inoculation



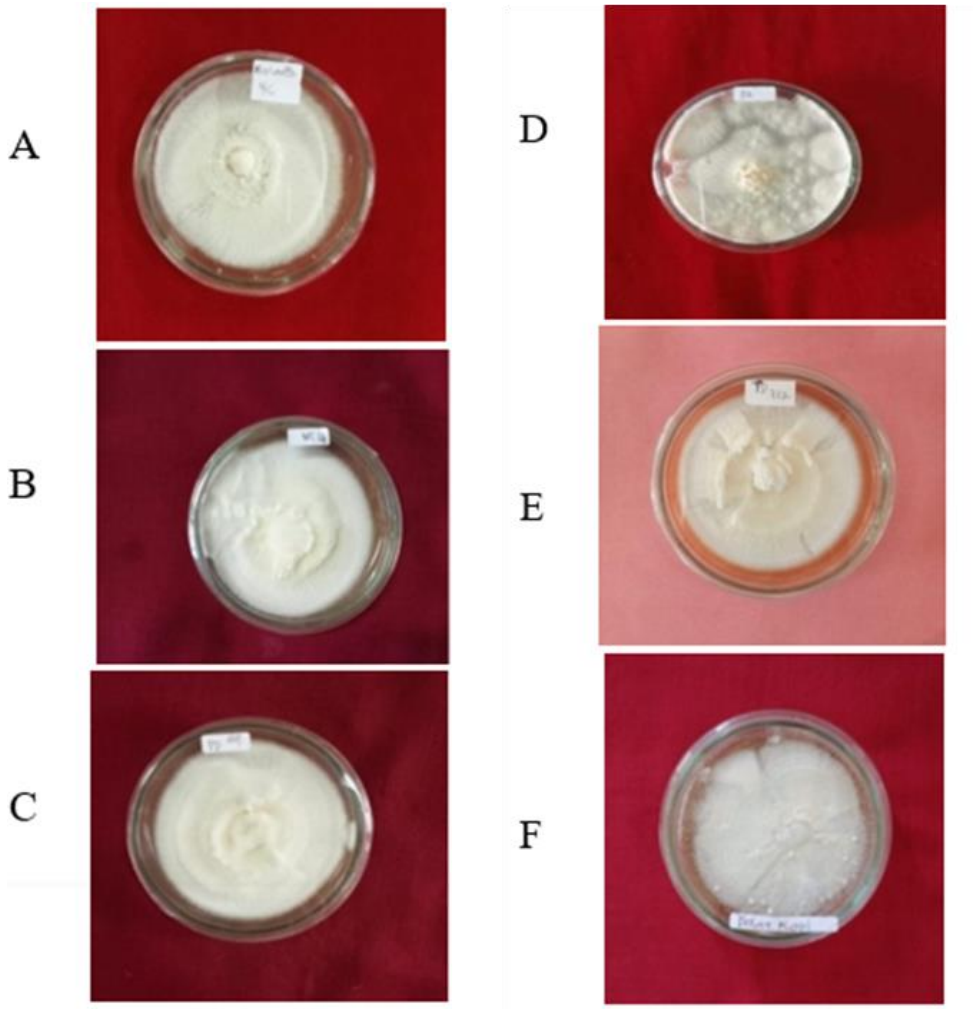


Figure 2. Morphology of *B. bassiana* on SDAY Media
A = PB 211 isolate; B = Bb WS isolate; C = PD 114 isolate; D = PA211 isolate; E= TD 312 isolate; F = KT211 isolate

The greater value of the Fosfat dissolution index described the ability to release Phosphate bonds as greater [25]. Phosphate solubilization ability is due to differences in the ability of fungi to produce organic acid sand phosphatase enzymes. The research results on the ability of *B. bassiana* entomopathogens endophyte fungal isolates to dissolve phosphate indicated its potential as a biofertilizer. Subsequent research need to conduct in vivo or field tests on chili plants to determine the morphological character of each fungal isolate *B. bassiana* as shown in Figure 2. Colony growth of *B. bassiana* fungal isolates spread on the surface of SDAY media with the white colony. Macroscopic observations were made based on the shape and the color of the colony [26].

4. Conclusions

Based on the research result, the conclusions obtained are as follows:

1. All tested *B. bassiana* fungal isolates can act as a phosphate solvent.
2. The highest phosphate solubility index of 5.42 from BbWS isolate was found 14 days after inoculation, followed by 5.28 of TD321 isolate.
3. *B. bassiana* entomopathogen fungal isolates as phosphate solvent has the potency of a biofertilizer. The use of entomopathogen endophytic fungal is an alternative to the biofertilizer and biostimulant demonstrated in chili plant

Acknowledgments

The authors are grateful to Dr. Yusniwaty, Prof. Trizelia, and Prof. Nurbailis for the support and guidance provided, as well as to Dana Hibah Program Doktor Ilmu Pertanian Universitas Andalas for funding this research.

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