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The Effect of Entomopathogenic Fungi *Beauveria bassiana* Seed Treatment Duration on Seed Germination and Seedling Growth of Chili

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Abstract

Beauveria bassiana (Balsamo) Vuill. is an entomopathogenic fungus that can live endophytic on different kinds of plants. *B. bassiana* also play a role as growth promoters that improves seed germination and growth of the plant. *B. bassiana* can be introduced onto plants by soaking the seed with fungal suspension. Role as plant growth promoters of *B. bassiana* was affected by fungal strain and duration of the seed treatment. This study aimed to find out about the effect of the duration of *B. bassiana* seed treatment on seed germination and seedling growth in chili. Chili seeds were soaked in the conidial suspension of *B. bassiana* in a concentration of 1 x 10^8 conidial/ml or sterile distilled water for 3, 6, 9, and 12 hours The total germination percentage of chili seeds recorded seven days post-inoculation of fungal suspension. For seedling growth, ten seeds were sown in a mixture of sterile soil and manure (1:1). This research was conducted in a completely randomized design. Radical length, shoot height, and vigor index was measured at 12 days post-inoculation. Seed germination percentages and plant growth were significantly affected by the duration of *B. bassiana* seed treatment. Overall, seed germination, the vigor index, and plant growth of chili were significantly enhanced as the duration of *B. bassiana* seed treatment increased. *B. bassiana* can also accelerate seed germination of chili and an increase of seedling growth. In this study, it concluded that soaking the seeds with a conidial suspension of *B. bassiana* for 9-12 hours is the best duration to increase seed germination and seedling growth of chili plants.

Keywords: endophyte, entomopathogenic fungi, phytohormone, vigor index

1. Introduction

Beauveria bassiana (Bals.) Vuill. (Hypocreales: Cordycipitaceae) is one of the entomopathogenic fungi that has received great attention and has been used for pest control in various plant commodities (Tanada and Kaya, 1993; Zafar *et al.,* 2016). This fungus has been used to control several insect pests such as *Crocidolomia pavonana* (Trizelia and Nurdin 2010). *Spodoptera exigua* (Razak *et al.,* 2016), *S. litura* (Trizelia *et al.,* 2016), *Nezara viridula* (Prayogo, 2013), *Bemisia tabaci* (Zafar *et al.,* 2016), and *Liriomyza* sp. (Trizelia and Nelly, 2017). *B.bassiana* also can exist as an endophyte in many plants and causes no apparent damage to the host. Colonization of fungi in plant tissue can influence pest attacks. Endophytic *B. bassiana* can act as a guard that protects the plant from different pests by producing an array consists of different bioactive metabolites.

Inoculation of fava beans with fungal endophytes reduced *A. pisum* population growth by 33 times compared to the untreated plants. Feeding of *Aphis gossypii* on cotton leaves colonized by *Beauveria bassiana* has also been shown to slow aphid reproduction (Gurulingappa *et al,* 2010). Akello and Sikora (2012) reported that the development and performance of *Acyrthosiphon pisum* and *Aphis fabae* were adversely affected when fed on fava beans plants whose seeds were treated with endophytic fungus *Beauveria bassiana* at planting. *B. bassiana* also has been reported as an endophyte in *Vicia faba* and *Phaseolus vulgaris,* also effective at reducing the population of *Liriomyza huidobrensis* (Diptera: Agromyzidae) (Akutse *et al.,* 2013). Trizelia *et al.* (2020) reported that *B. bassiana* successfully colonized various chili plant parts (leaves, stems, and roots) following seed immersion with conidial suspensions of these fungi and able to suppress development *Myzus persicae*.

In addition to its role in improving plant resistance against insect pests, the application of *B. bassiana* also affects the growth of plants. The growth-enhancing effect can be attributed to the ability of fungi to mobilize valuable nutrients for plant growth (Moloinyane and Nchu, 2019). Russo *et al.* (2019) reported that corn plants that inoculated with *B. bassiana* have seed germination percentage about 89% while non-inoculated (control) corn only 77%. Afandhi *et al.* (2019) demonstrated that *B. bassiana* can be introduced onto plants by seed-soaking, leaf-spraying, and soil-wetting. The method of inoculation of *B. bassiana* can impact the growth of common bean plants. Inoculation of *B. bassiana* had a positive effect on plant growth parameters, which included plant height, number of leaves, and root length. Lopez and Sword (2015) reported that *B. bassiana* enhanced cotton growth when plants were inoculated with the fungus as seed treatment and grown under greenhouse conditions. Raya-Dıaz *et al.* (2017) demonstrated that sorghum plants inoculated with *B. bassiana* by seed dressing were significantly taller than the ones that were inoculated by leaf spraying and soil treatment. Jaber and Enkerli (2016) reported that fungal strain of fungi and seed treatment duration also affects plant growth enhancement.

The effect of *B. bassiana* on plant growth of chili is important, especially in the nursery. The improvement of plant vigor to overcome biotic and/or abiotic stresses results in the production of stronger plants and increasing plant productivity and yields. Therefore, this study was designed to find out the effect of the duration of seed treatment with *B. bassiana* on the germination percentage rate and seedling growth in chili plants.

2. Materials and Methods

A. Plant and Fungal Material

Chili seeds used in this study were local chili varieties that were obtained from farmers in Korong Gadang village, Kuranji District, Padang City. Chili seeds were sterilized by soaking in a solution of 1% NaOCl for three minutes and then the seeds were washed three times with sterilized distilled water, then were dried in a laminar airflow cabinet for 60 minutes.

Entomopathogenic fungi *B. bassiana* (BbWS) were isolated from *Leptocorisa oratorius* in Duku, Padang Pariaman District provided by the Laboratory of Biological Control, Plant Pest and Diseases Department, Agriculture Faculty, Andalas University. The fungi were propagated in Sabouraud dextrose agar plus yeast extract (SDAY) selective medium contains 0.1% antibiotic chloramphenicol. Fungi cultures were incubated for 3 weeks and conidia germination is counted before the applications. The fungal suspension was obtained by adding 5 ml of distilled water and a 0.01% Tween 80 to a petri dish containing the cultured fungus, then the cultured fungus conidia were removed using a soft brush. The conidial suspension was filtered and then homogenized with a magnetic stirrer for 5 minutes. The concentration of conidia in fungal suspension was determined under the microscope using a Neubauer hemocytometer and adjusted to a concentration of $1x10^8$ conidia/ml.

B. Plant inoculation with *B. bassiana*

To determine the increase of seed germination influenced by *B. bassiana* seed treatment, 25 surface-sterilized seeds were soaked in the *B. bassiana* conidial suspension and or in sterile distilled water containing

Table 1. Effect of *B. bassiana* seed treatment duration on seed germination of chili.

Seed Treatment Duration	Seed Germination (%) ± SD			
	4 dpi	5 dpi	6 dpi	7 dpi
Control	37.5 ± 8.26 c	78.0 ± 6.41 c	91.5 ± 2.34 d	91.5 ± 3.33 c
3 hours	41.5 ± 8.54 bc	78.5 ± 4.75 c	94.5 ± 2.98 cd	95.5 ± 2.56 b
6 hours	49.5 ± 7.39 ab	82.5 ± 4.75 bc	96.5 ± 4.50 bc	97.0 ± 3.55 b
9 hours	54.0 ± 6.41 a	92.0 ± 7.09 a	100.0 ± 0.00 a	100.0 ± 0.00 a
12 hours	38.0 ± 8.82c	87.0 ± 8.21 ab	98.0 ± 2.14 ab	98.0 ± 2.14 ab

Means followed by the same letter within the same column are not significantly different at P=0.05 (Duncan's Multiple Range Test).

0.1% Tween 80 (control treatment) for 3, 6, 9 and 12 hours. Then the seeds were air-dried inside the laminar airflow cabinet. The air-dried seeds were placed on 9 cm Petri dishes lined with moist sterile filter paper. Sterile water was added as needed to keep the filter paper moist. Eight replicate dishes were prepared for each treatment and the Petri dishes were kept in the dark condition at 20 °C for a week. A total of 200 seeds were used per treatment. The total germination percentage was recorded seven days post-inoculation.

To invests the effect of seed treatment duration on the vigor index of chili, 10seeds were sown in pots that had contained a mixture of sterile soil and manure (1: 1). In this experiment, there were 60 seeds were used for each treatment. This experiment using a completely randomized design. The percentage of seedling emergence and vigor index was measured and observed 12 days post-inoculation. Vigor Index is calculated using the formula Abdul-Baki and Anderson (1973) which is Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] × percentages of seed germination.

To examine the effects of increased seed treatment duration on seedling height, inoculated or control seeds were planted in a mixture of sterile soil and manure (1:1) in pots. Two seeds of chili were sowed per pot. Plants were not fertilized through the experiment. After emergence, seedlings were thinned to one per pot and allowed to grow for 28 days. Plant height was measured in 28 days post-inoculation. Plant height of chili was measured from the base of the stem to the growing point

3. Results and Discussion

A. Effect of *B. bassiana* seed treatment duration on seed germination of chili.

Inoculation of *B. bassiana* onto chili seeds can increase the germination percentage and speed of the germination process. Generally, the seed germination process of treated seed was significantly enhanced compared to control. Seed germination percentage of chili caused by the duration of *B. bassiana* seed treatment is shown in Table 1.

Table 1 shows the effect of seed treatment duration on the percentage of seed germination at 4, 5, 6, and 7 days post-inoculation (dpi). Generally increasing seed treatment duration increased the percent value of seed germination. An increase in the germination of seeds after the application of *B. bassiana* can be caused by the fungus ability. in producing phytohormones that stimulate seed germination. Soaking the seeds of chili with *B. bassiana* conidia suspension for 9 -12 hours produces the best seed germination compared to the others. Espinoza *et al.* (2019) reported that germination of chili seeds inoculated with *B. bassiana* BB09 and *Metarhizium anisopliae* MA28 strains was almost 10% greater than non inoculated control. Russo *et al.* (2019) also reported that corn plants that inoculated with *B. bassiana* showed an increase in the percentage of seed germination compared to control plants. Percentage of seed germination of corn in the non-inoculated plant (control) only 77% while the inoculated plant with *B. bassiana* has the percentage of seed germination about 89%. In this study, the treated seed germinated consistently faster and more uniformly than untreated seeds. Seed germination of chili from seeds that soaked in the suspension of *B. bassiana* for 9-12 hours was higher compared to seeds that soaked for 3 and 6 hours.

B. Effect of *B. bassiana* seed treatment duration on the seedling growth of chili

In seed germination, the entomopathogenic fungus *B. bassiana* also increase the seedling growth of chili. All growth parameters assessed after inoculation were significantly higher than control. Seedling growth of chili (radical length, shoot height, and vigor index) was affected by seed treatment duration of *B. bassiana* (Table 2.)

Table 2. Effect of *B. bassiana* seed treatment duration on the seedling growth of chili in 12 days post-inoculation

Seed Treatment Duration	Radical length (cm) ± SD	Shoot height (cm) ± SD	Vigor index ± SD
Control	21.88 ± 6.29 c	28.53 ± 1.81 c	48.85 ± 8.37 c
3 hours	33.07 ± 4.63 b	32.98 ± 3.49 bc	62.63 ± 5.30 b
6 hours	30.68 ± 6.37 b	37.77 ± 5.04 ab	67.20 ± 5.20 b
9 hours	41.42 ± 7.35 a	41.07 ± 5.45 a	82.48 ±10.76 a
12 hours	31.20 ± 6.32 b	31.58 ± 4.93 c	61.85 ± 8.03 b

Means followed by the same letter within the same column are not significantly different at P=0.05 (Duncan's Multiple Range Test).

It is noted that increasing seed treatment duration resulted in a marginally significant increase in the growth of inoculated plants. The growth-enhancing effect of *B. bassiana* on chili can be attributed to the ability of fungi to mobilize valuable nutrients for plant growth and also probably depends on the availability of nutrients in the growth medium. It is suspected that *B. bassiana* produces plant growth regulators such as auxins and cytokinins that promote plant growth There are several studies regarding the effect of *B. bassiana* on plant growth.

Jaber and Enkerli (2016) reported that the strain of entomopathogenic fungi and seed treatment duration had significant effects on the plant growth of *Vicia faba*. Inoculation with *Metarhizium brunneum* and *B. bassiana* significantly enhanced seedling emergence, plant height, number of leaf pairs, and fresh root weight. Increasing seed treatment duration had a significant effect on plant growth (plant height, number of leaf pairs, fresh shoot weight, fresh root weight) of *Vicia faba*. Increasing seed treatment duration might have caused conidia to swell and begin to germinate, and produce mucilage that could have facilitated conidial attachment to the seed. It causes the enhanced plant colonization rate and growth responses.

Kumar *et al.* (1999) reported that the conidia of *B. bassiana* are germinated 8 hours after inoculation on the cuticular surface of *Bombyx mori* larvae, but the conidial germination period varied according to the texture of the substrate and the presence or absence of nutrients. Pus (2017) reported that the root drench application of *Trichoderma* spp. or *B. bassiana* isolates to *Brassica oleracea* var. *capitata* did not significantly affect the total number of true leaves and shoot lengths, however, there was a significant effect on the root lengths. All *Trichoderma* spp. and *B. bassiana* treatments, apart from *B. bassiana* J18, significantly increased root length compared with the untreated control. Russo *et al.* (2019) reported that corn plants that inoculated with *B. bassiana* showed an increase in height, the number of leaves, grain weight, yield, and percentage of seed germination compared to control plants. In this study, seed treatment duration also affects the vigor index (VI) of the chili plant from treated seed with *B. bassiana* compared to control. The highest VI values were recorded when the chili seed that soaked in a conidial suspension of *B. bassiana* for 9-12 hours. The lowest vigor index was recorded in control.

C. Effect of *B. bassiana* seed treatment duration on the seedling height of chili

Application of entomopathogenic fungi in chili by soaking the seeds also can promote the growth of seedling height. Increasing seed treatment duration also produced a significant increase in plant height (Table 3). Soaking the seeds of chili for 6 hours has been able to increase the height of chili seeds. The enhancement of plant growth of chili may be influenced by phytohormones produced by *B. bassiana*. The ability of the *B. bassiana* in increasing the plant height suspected by the production of growth regulators compound or the availability of nutrients for plant growth. But, the improvement of plant growth is not only resulted by *B. bassiana* isolates and plant species, but also the complex interaction of many factors such as environmental parameters, soil microorganisms, and soil-plant interaction. Raya-Díaz *et al.* (2017) stated that endophytic *B. bassiana* and *M. brunneum* can and can improve the iron availability, the chlorophyll content, the length of roots, and the abundance of fine roots in sorghum. Jaber and Enkerli (2016) also reported that increasing seed treatment duration of *Vicia faba* that were soaked in suspensions of *B. bassiana* had a significant effect on plant height. Russo *et al.* (2019) observed an increase of soybean plant growth after inoculated with *B. bassiana* also increase the yield of soybean plants under fields condition, with no adverse effects in the inoculated plants.

Table 3. Effect of *B. bassiana* seed treatment duration on the seedling height of chili at 28 days post-inoculation

Seed Treatment Duration	Seedling height (cm) ± SD
Control	6.18 ± 0.67 b
3 hours	6.19 ± 0.64 b
6 hours	7.50 ± 1.30 a
9 hours	8.30 ± 0.67 a
12 hours	8.19 ± 0.93 a

Means followed by the same letter within the same column are not significantly different at P=0.05 (Duncan's Multiple Range Test).

4. Conclusions

In this study, inoculating *B. bassiana* onto chili plants by seed-soaking treatment had a positive effect on seed germination and plant growth. Overall. increasing duration for seed treatment with *B. bassiana* was significantly enhanced the seed germination, the vigor index, and the plant growth of chili. *B. bassiana* also can accelerate seed germination of chili and an increase of seedling growth. The best duration of seed-soaking treatment with *B. bassiana* to increased the seed germination process and increase plant growth of chili is 9-12 hours.

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