Colonization of *Beauveria bassiana* (Bals.) Vuill on chili (*Capsicum annum*) and its effect on populations of *Myzus persicae*

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ABSTRACT

Beauveria bassiana (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) is one species of entomopathogenic fungi that can live endophytically by colonizing plant tissues. Colonization of fungi in plant tissue can heavily influence pest attacks. This study aimed to study the endophytic ability of *B. bassiana* isolates in chili and its effect on *Myzus persicae* population. Three isolates of *B. bassiana* fungus (TD312, APKo and PD114) were applied through seed immersion for 6 hrs with a concentration of 10^8 conidia / mL. Observed parameters included seed germination, seedling growth, colonization ability of this fungi in the roots, stems and leaves of chili and *Myzus persicae* population. The results showed that all *B. bassiana* isolates were found effective to enhance the germination percentage of chili seeds. This fungi colonized all parts of the plant with the rate of fungal colonization higher in the leaves compared to the stem and roots. *B. bassiana* TD312 isolate proved to be the best isolate in suppressing the development of *Myzus persicae* population.

Keywords: *Beauveria bassiana*, endophytes, chili, *Myzus persicae. Macrophomina phaseolina,* cowpea, neem, *in-vivo*.

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INTRODUCTION

Red chili (*Capsicum annuum L.*) is a commodity vegetable with a high economic value and as such, is a source of income for most vegetable farmers in Indonesia. In 2018, Indonesian red chili production amounted to 8.77 tons / ha, which is low compared to its potential yield of 12 tons / ha (Badan Pusat Statistik, 2019). Low productivity in chili plants is often due to aphids (*Myzus persicae*). These pests primarily attack the leaves of chili plants. Direct damage to the chili plants due to this pest attack can cause chlorosis, necrosis, wilting, stunting, flower and fruit abortion, leaf distortion and defoliation. This aphid also produce honeydew that can stimulate the growth of sooty mold, contaminating fruit and reducing the photosynthetic capability of plants. (Frantz *et al*., 2004). In addition to direct injury on the plants, aphids are able to transmit more than 200 plant viruses (Blackman and Eastop, 2000) such as cucumber mosaic virus (CMV), tobacco etch virus (TEV), pepper mottle virus (PepMoV) and potato virus Y (PVY) (Frantz *et al*., 2004). Losses directly caused by aphids range from 6-25%, and as vectors, can reach more than 80% (Blackman and Eastop, 2000).

One of the various methods to reduce losses caused by aphids is the biological control of using entomopathogenic fungus such as *Beauveria bassiana* (Bals) Vuill. *B. bassiana* is an entomopathogenic fungus with the broadest range of hosts and can live on host insects as well as in the soil (Tanada and Kaya, 1993). Some studies also report *B. bassiana* living endophytically on various types of plants such as corn (Bing and Lewis, 1991), coffee (Vega *et al*., 2008), cocoa (Trizelia and Winarto, 2016) and wheat (Trizelia *et al*., 2017). In addition to infecting and killing insects, *B. bassiana* fungus also increases plant resistance to pest attacks.

Research conducted by Akello *et al*. (2007) showed that *B. bassiana* was able to colonize banana plants 4 months after inoculation. The highest colonization was found in roots (91.5%) followed by rhizomes (75.6%) and pseudo stem (58.9%). According to Posada and Vega (2007), its colonization is influenced by application techniques and increasing plant age. Further study by Akutse *et al*. (2013) showed that colonization of *B. bassiana* fungi was influenced by fungal strains. Bing and Lewis (1992) reported corn plants by colonized by stem injection were able to provide resistance and control populations of corn stem borers (*Osrinia nubilalis*). Gao *et al*. (2012) showed that a *B. bassiana* RSB strain was able to colonize broccoli leaves and was effective in suppressing the growth of thrips, (*Frankliniella occidentalis*) thereby increasing this plant's resistance to pests. Application of this fungus has also been shown to influence plant growth. The purpose of this study was to investigated the ability of *B. bassiana* to endophytically colonize chili and the impact on populations of aphids (*Myzus persicae)*

MATERIALS AND METHODS

Fungal inoculums

Three isolates of *B. bassiana* (TD312, PD114 and APKo) obtained from the collection of entomopathogenic fungal culture maintained by Biological Control Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Andalas University. The cultures were grown *Sabauraud dextrose agar plus Yeast extract* (SDAY) medium and the culture was incubated for 3 weeks. The fungal suspension was obtained by adding 10 ml of distilled water and 0.1% Tween 80 to petri dishes containing the fungus culture and conidia were harvested by scraping the surface of the plate with a steril spatula. The conidial concentration was determined using improved Neubauer hemacytometer and adjusted to 10^8 conidia / mL.

Chili Seeds Chili seeds were obtained from farmers in the village of Korong Gadang, Kuranji District, in Padang City. Before being treated, the chili seeds were immersed in a 1% NaOCl solution for three minutes then washed three times with aquades that had been sterilized in an autoclave (121°C, pressure of 1.02 atm for 15 minutes). Washed seeds were dried in a laminar air flow cabinet for 60 minutes.

Effect of *B. bassiana* on seed germination

Chili seeds were then soaked in *B. bassiana* suspension for 6 hrs. The treated seed were then dried in a laminar air flow cabinet for 60 minutes. A control group of seeds was soak with a sterile distilled. After that, seeds put in sterilized Petri dishes containing moistened filter paper. A total of 25 treated chili seeds were arranged in petri dishes. The seeds were then incubated for seven days. Total number of seeds used for each treatment was 200 and germination of seeds was calculated after seven days. The percentage of seed germination was calculated as the percentage (%) of seeds that germinated and produced normal seedlings at the end of the test period

Colonization of *B. bassiana* on chili

Application of *B. bassiana* on the chilli was conducted by a seed immersion method. Chili seeds were first soaked in the suspension of each *B. bassiana* isolate for 6 hours (Hernawati *et al*., 2011). Soaked seeds were then dried in a laminar air flow cabinet for 60 minutes prior to being planted in a pot-tray containing a mixture of soil and sterile manure (1:1). For control, another group of chili seeds were soaked only in sterile aquades. The establishment of three *B. bassiana* isolates as endophytes in chilli was examines at 28, 42 and 56 DAI (days after inoculation). At each evaluation time, 3 plants were selected from each treatment. Plant were washed under running tap water . The leaf, stem and root of plant were surface sterilized with 1% sodium hypochlorite for 5 minutes, with 70% alcohol for 2 minutes and then washed three times with sterile distilled water. Samples were dried in a laminar air flow. Then the samples were cut into 1 cm and transferred into petri dishes containing selective medium Oatmeal agar (OA), consisting of oatmeal and 0.6 g / L cetyl trimethyl ammonium bromide (CTAB)

(Posada *et al*., 2012). Plates were incubated at 25ºC for 10 days to observe and record fungal outgrowth. Fungal outgrowth from the plated plant samples were identified as *B. bassiana* based on colony morphology and microscopic conidia. Each petri dish contained five pieces of leaves, stems or roots. After 10 days, the presence of *B. bassiana* is confirmed by the presence of mycelium or conidia at the tips of the leaf, stem or root tissue. Percent colonization of plant tissue by fungus was calculated as number of sampled plant tissue (leaves, roots or stems) showing fungal outgrowth divided by the total number of plant tissue samples x 100 (Petrini and Fisher, 1986)

Effect of *B. bassiana* on *M. persicae* populations

Chili seeds that had been soaked in endophytic fungal suspension for 6 hrs were then sown in pot-trays containing a planting medium of soil and sterile manure (1: 1) and watered daily. After four weeks, they were replanted in 5 kg capacity poly bags, each containing one chili plant. Plants were with fertilized N (3g/poly bag), P (4.5g/poly bag), and K2O (3g/poly bag). Fertilizer was spread around the plant at a distance of 10 cm and a depth of 5 cm twice following planting, once at week one and again at week four. Mechanical weeding was performed every two weeks. Plants were watered in the morning and evening according to plant needs. 10 days after planting, the chili plants were infested with up to five individu of *M. persicae* for each plant. The plant was then covered with 50 x 100 cm of mica plastic and gauze. Observation of *M. persicae* populations (nymphs and adults) began one week after infestation at a rate of twice weekly.

RESULT AND DISCUSSION

Seed Germination

Aplication of *B. bassiana* have significant effect on seed germination of chili (P<0.0009). Results showed that all *B. bassiana* isolates were found effective to enhance the germination percentage of chili seeds compared to control. However among the three *B. bassiana* isolates, the highest mean percent germination was recorded in the chili seeds dipped in suspension of *B. bassiana* TD312 isolates and the least mean percent germination was recorded in the chili seeds dipped in suspension of *B. bassiana* PD114 isolates as compared to control that recorded 91.5 % germination (Table 1).

Table 1. Effect of *B. bassiana* isolates on chili seed germination 7 days after treatment

Treatment	Seed germination
TD312	100.0 a
APKo	99.5 a
PD114	92.5 b
Control	91.5 b

Means with the same letter are not significantly different (P<0.05) by Duncan's Multiple Range Test

Higher percent germination of chili seeds after application of *B. bassiana* indicates the positive impact of these entomopathogenic fungus on seed health of chili. It is suspected that *B. bassiana* secrete plant growth hormones such as cytokinins, auxins and gibberellins which promote seed germination and growth in crop plants. Besides acting as an entomopathogen, *B. bassiana* also have an additional role in plants as growth promoters. Additionally, several studies revealed the potential of endophytic fungi in enhancing the seed germination and growth of plants Russo *et al*. (2019) reported that corn plants inoculated with *B. bassiana* showed an increase in percentage of seed germination compared to control plants. At control percentage of seed germination of corn only 77% and at corn inoculated with *B. bassiana* percentage of seed germination of corn was 89%. The percentage of seed germination could not be influenced directly by *B. bassiana*, and may instead be an indirect effect of the presence of the fungus in the parent plant. On the contrary Jaber and Enkerli (2016) reported that seed germination of *Vicia faba* was not affected by *B. bassiana* but growth parameters (plant height, number of leaf pair, and fresh shoot and root weight) were significantly enhanced in plants grown from seeds soaked in the suspension of *B. bassiana*

Colonization of *B. bassiana* in chili plants

The results showed that the fungus *B. bassiana* successfully colonized various chili plant parts following seed immersion with conidial suspensions of each fungal strain.

Table 2. Percentage colonization of *B. bassiana* isolates in leaves, stem and root of chili at 28, 42 and 56 days after inoculation (dai)

Isolates	28 dai			42 dai			56 dai		
	Root	Stem	Leaves	root	stem	leaves	root	stem	Leaves
TD312	13,3	6,7	20,0	6.7	6.7	33.3	0.0	0.0	13.3
APKo	6,7	20,0	26,7	0.0	6.7	20.0	0.0	0.0	0.0
PD114	0,0	26,7	40,0	6.7	6.7	40.0	0.0	6.7	6.7
Control	0,0	0,0	0,0	0.0	0.0	0.0	0.0	0.0	0.0

Colonization of fungus on chili was influenced by isolates. In the control group, *B. bassiana* fungus was not seen as an endophyte in chili plants (Table 2). Colonization tended to be highest in the leaves compared to the stems and roots, at 28, 42 and 56 days after inoculation respectively. Comparatively, the lowest percentage of colonization was found in the roots.

We observed that all *B. bassiana* isolates can be successfully inoculated as an endophyte in chilli by means seed inoculation. The entomopathogenic fungus, *B. bassiana,* was shown to colonize the entire tissue of chili plants (roots, stems and leaves). Therefore, this fungus has the ability to live systemically and spread throughout plant tissue. The fungal isolates that successfully colonized the plant might have been translocated across the plant together with photosynthates. The colonization of *B. bassiana* in plants through seed immersions has also been reported by Jaber and Enkerli (2016), *B. bassiana* can systemically colonize different plant parts of *Vicia faba* and improve plant growth when applied as a seed treatment. Russo *et al*. (2019) also reported the ability of this fungus to colonize soybean plants following seed application. The ability of *B. bassiana* colonization in plants is strongly influenced by plant physiology. Akusse *et al*. (2013) reported that colonization of the host plant species varied according to fungal isolate, host plant and different parts of the host plant. Colonization ability of *B. bassiana* in chili decreased with time. In 28 dai, TD312 and APKo isolates were able to colonize all parts of the chili plants (roots, leaves and stems) compared to PD114 isolates which only colonized stems and leaves. Colonization of TD312 isolates in leaves increased to 33.3% at 42 dai while colonization of APKo isolates in leaves, roots and stems decreased. Colonization ability of *B. bassiana* in chili plants at 56 dai was reduced significantly. The APKo isolate is not found in plants. A possible explanation for the low recovery we recorded 56 days post-inoculation could be a result of competition from other fungi and bacteria in the system, leading to inhibition of *B. bassiana* growth.

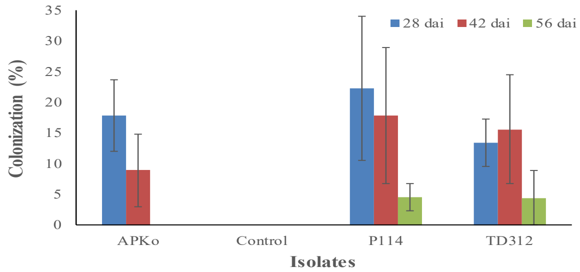

Fig. 1. Colonization of *B. bassiana* isolates in chilli

Overall, average colonization abilities of each isolate in chili plants is seen in Figure 1. PD114 and TD312 isolates were shown to colonize chili plant tissue for up to 56 dys after inoculation, whereas APKo isolates were only able to colonize plant tissue for up to 42 days after inoculation . The colonization ability of PD114 isolate was higher compared to TD312 and APKo. *B. bassiana* colonization was higher in leaves compared to roots and stems. These findings are in line with research conducted by Zhang (2014), which showed that the colonization of *B. bassiana* endophytic fungi was highest in leaves (90%, by strain EABb04) compared to the buds and roots of cabbage plants. In contrast, Jaber and Enkerli (2016) reported higher colonization in stems compared to roots and leaves from observations made 14 days after inoculation and a 16 hurs immersion time.

From this research, the rates of *B. bassiana* colonization have been shown to decrease with increasing plant age. Research by Renuka *et al*. (2016) showed that *B. bassiana* can colonize and persist in stem and leaf tissues of maize. Persistence of inoculated fungal isolates decreased with increase in age of the plant. In general, all isolates showed higher percent colonization in maize stem and leaf at 30–45 days of crop age and reduce at 60-90 days. Similar results were also found by Gomez-Vidal *et al*. (2006), from *B. bassiana* colonized in the stem tissue of date palm plants.

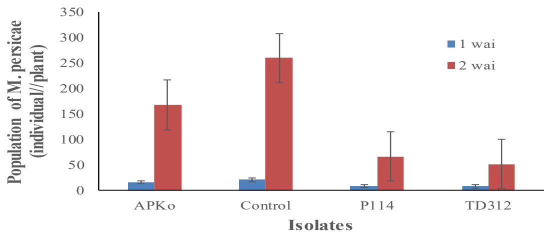

Figure 2. Population growth of *M. persicae* on chilli 1 and 2 weeks after infestation

Effects on *Myzus persicae* populations

The results showed that the overall application of *B. bassiana* on chili seeds significantly lowered populations of *M. persicae* in plants grown from treated seeds compared to controls (Figure 2). In particular, it was shown that *B. bassiana* TD312 isolates more effectively suppressed the development of *M. persicae* populations on chili plants compared to PD114 and APKo isolates. One week after infestation, the *Myzus persicae* population in control was 21.2 individu/plant whereas in plants treated only ranged from 8.4-16.2 individu/plant *Myzus persicae* population increased significantly in the 2nd week after infestation. The highest population of aphids was in the control (260 individu/plant) and the lowest as in the chilli plants treated with TD312 isolate

Presumably, inhibitory effect of *B. bassiana* on population growth of *M. persicae* is mostly due to toxin produced by fungal endophyt and a changes in the chemical profiles of treated plants. Tan and Zou (2001) argued that highly diverse groups of toxin produced by fungal endophyte ia alkaloids, terpenoid, steroid, quinone, and flavonoid, phenylpropanoids and lignans, peptides, phenol, phenolic acids and aliphatic compounds. Bing and Lewis (1991) reported that the entomopathogen *B. bassiana* can colonize the corn plant, move within the plant and persist to provide season long suppression of *Ostrinia nubilalis*. Significant reductions in *O. nubilalis* tunneling at harvest occurred when *B. bassiana* was applied foliarly or by injection. According to the research of Akello and Sikora (2012), endophyte treated fava beans had a significantly lower number of *Acyrthosiphon pisum* when compared to the untreated controls. The highest reduction effects were observed among plants treated with *Trichoderma asperellum*, *Gibberella moniliformis* and *Beauveria bassiana* isolates, while all *Metarhizium anisiopliae* and *Hypocrea lixi* isolates had the least effects on *A. pisum* population growth. endophyte seed treatment had a detrimental effect on offspring fitness, development and fecundity of *Acyrthosiphon pisum*. Hernawati *et al*. (2011) reported that some leaf endophytic fungi of chili play a role in protecting chili against *Aphis gossypii*. This endophytic fungi is able to suppress the growth, development and population growth of *Aphis gossypii*. Main mechanism in increasing host resistance against insects mediated by fungal endophyte is antixenosis. Antixenosis is proven in this research showed by suppression of fecundity, prolonged life cycle and decreased body size.

In the laboratory experiment, *Plutella xylostella* did not show any preference in laying egss on the treated and untreated cauliflower plants by *Beauveria bassiana*. However, no larvae were able to develop on treated leaf. The larval mortality over time developed on treated cauliflower plants could be attributed to the internal growth of the fungus in the plant tissues following germination and penetration and production of secondary metabolites (Gautam *et al*. 2016). Reduction in feeding and reproduction by *Aphis gossypii* (Hemiptera: Aphididae) has also been reported on cotton endophytically colonized by either *B. bassiana* or *Lecanicillium lecanii* (Hypocreales: Clavicipitaceae) (Gurulingappa *et al*., 2010). Akutse *et al*. (2013) reported that *B.*

bassiana isolates that endophytically colonized *Vicia. faba* were also pathogenic to *Liriomyza huidobrensis*, causing 100 % mortality. *B. bassiana* G1LU3 significantly reduced the number of pupae produced by female *L. huidobrensis* and their emergence from pupal skins In addition to pupal mortality, fungal endophytes reduced survival of the *L. huidobrensis* adults, *B. bassiana* G1LU3 had the largest negative effect on emergence of *L. huidobrensis* and on insect population and can be considered as promising biocontrol candidates for the management of *L. huidobrensis* populations. Pus (2017) reported that *Myzus persicae* fed on *Brassica oleracea* var. *capitata* plants inoculated with *T.richoderma hamatum* LU593, and *Beauveria bassiana* isolates FRh2 and BG 11, had reduced longevity compared to aphids fed on uninoculated control plants.

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