AGRIVITA
Journal of Agricultural Science
www.agrivita.ub.ac.id

Application of Mycorrhizae and Beauveria in Organic Farming System Effectively Control Leafminers and Enhance Shallot Production

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ARTICLE INFO

Keywords:
Biofertilizers
Bioinsecticides
Liriomyza management
Shallot yields
Sustainable farming

Article History:
Received: October 7, 2020
Accepted: December 15, 2020

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ABSTRACT

An increasing studies related with the detrimental effects of agrochemicals suggested the advantage of biofertilizers and biopesticides uses to support the sustainable farming system. This study aimed to evaluate the response of shallot 'Lembah Palu' with the application of arbuscular mycorrhizae (AM) and *Beauveria bassiana* (Bb) under organic farming system. A split-plot experiment was designed to combine Bb at two intervals (every 5 and 10 days) and AM with three rates (0; 5; and 10 g per plant). Leafminer population and infestation, root colonization, root biomass, and shallot yield were observed. Application of the AM and Bb affected shallot production and leafminer infestation, independently. Mycorrhizae application increased the root biomass and yield of shallot but gave negligible effects on population and leafminer attack. The shallot production with no mycorrhizae was significantly lower compared to both mycorrhizae application rates of 5 g and 10 g. The application of *B. bassiana* every 5 days was more effective in suppressing the population and infestation of leafminers compared to that every 10 day-treatment. The study points out the positive contribution of the mycorrhizae and *B. bassiana* in the shallot cultivation and supports the implementation of the organic farming system.

INTRODUCTION

The sustainable farming system proved to be the best way to feed the fast-growing human population in the world because of the increasing concern for healthy food and environmental safety (Gupta, 2012). The development and implementation organic farming that maximizes the use of biofertilizer and biopesticides instead of agrochemicals in plant cultivation (Kawalekar, 2013; Mahdi et al., 2010) has been increasing continuously. The use of biofertilizers and biopesticides is crucial to reduce the high economic and ecological costs of chemical fertilizers and pesticides (Pimentel & Burgess, 2014).

Biofertilizers and biopesticides which are usually isolated and developed from native microbes have some advantages and have proved as the best tools for supporting sustainable agriculture (Bhardwaj, Ansari, Sahoo, & Tuteja, 2014). Mycorrhizae and *B. bassiana* are potentially to harness as biofertilizers and biopesticides. The AM is abundant in natural environments and serves an important component of tropical soil systems (Cardoso & Kuyper, 2006). The capability of plants to obtain water and nutrients, particularly immobile elements such as phosphorus (P) may increase by the existence of mycorrhizae and subsequently promote the growth of plants even in water stress conditions (Marschner & Dell, 1994). The great contribution of the AM on plant growth and crop production in the tropics has been reviewed by Naher, Othman, & Panhwar (2013). However, among horticulture crops, allium species are more sensitive to AM application due to their less growth root system, thus causing low absorption capability of soil nutrients (Golubkina et al., 2020).

ISSN: 0126-0537 Accredited First Grade by Ministry of Research, Technology and Higher Education of The Republic of Indonesia, Decree No: 30/E/KPT/2018

Cite this as: Saleh, S., Anshary, A., Made, U., Mahfudz, & Basir-Cyio, M. (2021). Application of Mycorrhizae and Beauveria in organic farming system effectively control leafminers and enhance shallot production. *AGRIVITA Journal of Agricultural Science, 43*(1), 79–88. https://doi.org/10.17503/agrivita.v1i1.2831

Meanwhile, *B. bassiana* is a soil-borne hyphomycetous fungus with entomopathogenic properties. This fungus has a wide distribution and an extremely large host list of 700 insect species. Since they are a generalist with no strict host preference, *B. bassiana* can be used as a broad-spectrum myco-insecticide against more than 700 insect species (Devi, Padmavathi, Rao, Khan, & Mohan, 2008). For example, their efficacy has been successfully tested against beet armyworms (Shahabuddin & Mahfudz, 2010), cocoa pod borer (Anshary et al., 2017), and mung bean pests (Bayu & Prayogo, 2018). This entomopathogenic fungus has a good perspective to be utilized as a component of leafminer management (Migiro, Maniania, Chabi-Olaye, & Vandenberg, 2010). However, the efficacy of *Beauveria* spp. was varied and depended on several factors including application technique, persistence in the field, and their virulency (Jaronski, 2010; Mascarin & Jaronski, 2016).

The leaf miners have become a serious threat to the shallots and other vegetables production in Indonesia (Hidrayani, Purnomo, Rauf, Ridland, & Hoffmann, 2005; Saleh, Anshary, & Made, 2018) and have been developing their resistance to a dozen chemical insecticides (Ferguson, 2004; Grant et al., 2019; Hidrayani, Purnomo, Rauf, Ridland, & Hoffmann, 2005; Roditakis et al., 2018). Since in this experimental sites, there had been no application of chemical fertilizers and pesticides in the last seven years, this study also assesses the compatibility of organic farming system (OFS) on the leaf miner infestation and yields on the local variety of shallots. This is important because farm management practices influence the occurrence and beneficial effect of mycorrhizae on crops (Manoharan, Rosenstock, Williams, & Hedlund, 2017; Plenchette, Clermont-Dauphin, Meynard, & Fortin, 2005). Therefore, this study aims to evaluate the effectiveness of mycorrhizae and *B. bassiana* against leaf miners and their promotion to the growth and yields of shallot and the suitability of OFS in supporting the performance of both useful microbes.

MATERIALS AND METHODS

Location and Experimental Design

The experiment was carried out from March to September 2019 in the farmland implementing the organic farming system since seven years ago located at Bulupountu Jaya village Sigi Biromaru Sub District, Central Sulawesi, Indonesia. A split-plot design involving *B. bassiana* (Bb) at two intervals (every 5 and 10 days) as the main plots and arbuscular mycorrhizael (AM) with three rates (0; 5; and 10 g per plant) as the subplots was set up. The *B. bassiana* was collected from the collection of the Plant Pest and Diseases Laboratory, Agriculture Faculty of Tadulako University, and applied at 10 g/l (w/v). While the mycorrhizae (MycoVir) product by PT. Myco Agro Lestari (contained ca. 50 spores per 5 gram) were applied in each shallot seed before planting. It consists of four genera of mycorrhizae: Acaulospora, Gigaspora, Glomus, and Scutella. Each treatment combination has four replicates. The application doses of AM and *B. bassiana* were based on Saleh, Anshary, & Made (2018).

The shallot was planted using standard cultivation methods at a 1.2 × 3 m^2 plot. Plastic mulch was installed on the plot surface and the shallot seeds were sowed at holes with 15 × 20 cm^2 in spacing for weed control and maintaining microclimatic condition. The main plots and subplots distances were 4 and 1 m, respectively. Organic fertilizers of cow manures (organic-C: 15.23%, C/N ratio: 16.38%, pH:7) enriched with local microorganisms was added at a rate of 4 t/ha at a week before shallot was planted. Additionally, organic fertilizer in liquid form (0.1/l, v/v) was applied three weeks after planting (WAP).

Variable Observation

Variables observed were 1) population and infestation level of leaf miners, 2) shallots yields, 3) root biomass, and 4) root colonization rate by mycorrhizae (RCR). Population and infestation of the leaf miners were observed every week from two WAP until one week before harvest. Yellow sticky traps were used to monitor the leaf miner population while ten shallot plants per plot were selected as samples to measure the leaf miner infestation as described by Saleh, Anshary, & Made (2018). After the fresh organs were weighted, the shoots, bulbs, and root were separated dried at 105ºC for 30 minutes and 70ºC for 48 hours. The dried biomasses were then weighted. For calculating the RCR, the harvested fine roots from each plant were washed and cut into 1 cm long segments, cleared by soaking in 10% (w/v) KOH, and stained in 0.05% (w/v) trypan blue solution (Phillips & Hayman, 1970). AM colonization rate was estimated using the intercept method (Biermann & Linderman, 1981) and calculated using the following formula:

Root colonization rate (%) = (Number of infected root segments/Total number of segments) x 100%............(1)

Data Analysis

An analysis of variance (ANOVA) followed by a mean comparison based on Honestly Significant Difference (HSD) was performed to test the effect of the treatment on all measured variables by using the statistical program, Statistix 10.

RESULTS AND DISCUSSION

Effect of Beauveria Application on Leaf Miners and Shallot

Imago of Liriomyza was initially recorded at 3 weeks after planting (WAP) and reached the highest level at 4 and 5 WAP. The Liriomyza population at 4 and 5 WAP were significantly affected by the interval application of *B. bassiana* but not affected by either the AM or the interaction between both treatments (Table 1). The leaf miner population significantly higher in the shallot plants applied *B. bassiana* every five days (Bb1) compared to those applied every 10 days (Bb2) (Fig. 1). This showed that *B. bassiana* was able to suppress the population of Liriomyza.

Table 1. Summary of the anova testing the effect of mycorrhizae and *B. bassiana* on the population and infestation of leafminers

Variable	Significance		
	Bb	AM	Bb*AM
Leafminers population at 4 WAP	$F_{1,23}$=33.02, P=0.002	$F_{2,23}$=0.65, P=0.534	$F_{2,23}$=1.70, P=0.225
Leafminers population at 5 WAP	$F_{1,23}$=115.17, P=0.011	$F_{2,23}$=1.70, P=0.225	$F_{2,23}$=0.09, P=0.912
Leafminers infestation at 6 WAP	$F_{1,23}$=44.53, P=0.007	$F_{2,23}$=0.07, P=0.932	$F_{2,23}$=0.46, P=0.641
Leafminers infestation at 7 WAP	$F_{1,23}$=110.52, P=0.002	$F_{2,23}$=1.60, P=0.242	$F_{2,23}$=0.17, P=0.846

Remarks: Bb: *B. bassiana*, AM: arbuscular mycorrhizae

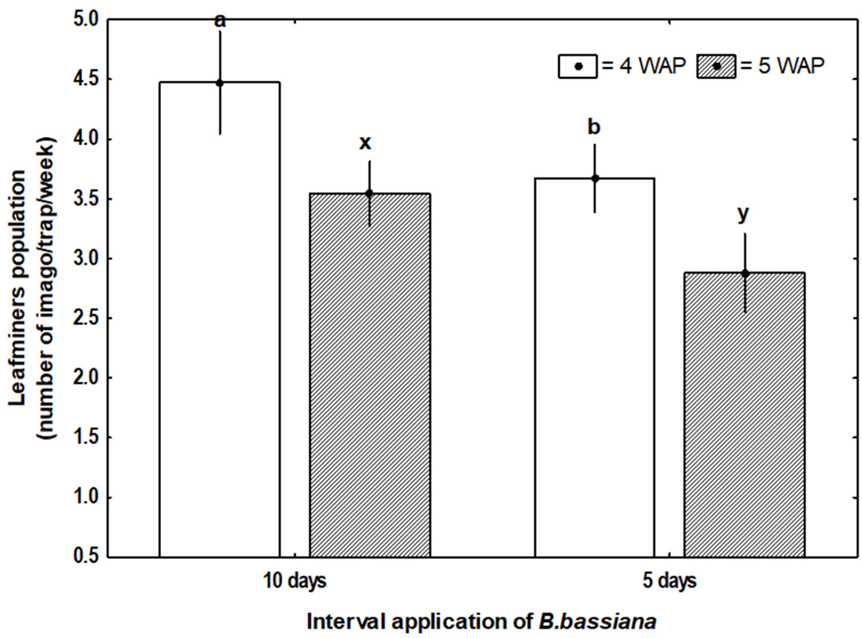

Fig. 1. Leafminer population at 4 and 5 WAP as the response on different interval application of *B. bassiana*. Data are means ± SE. Different letters above the similar bar color is significantly different based on HSD test

The leafminer infestation was first detected at 4 WAP as characterized by a small number of the mines at the shallots leaf and the highest attack rates were observed at 6 and 7 WAP. The Liriomyza attack at 6 and 7 WAP was significantly affected by *B. bassiana* main effect (Table 1). The infestation of leafminers was significantly lower on the five-day interval application of *B. bassiana* compared with the ten-days interval (Fig. 2). Meanwhile, the interval application of *B. bassiana* did not significantly affect the level of infection (p = 0.151) and root biomass (p = 0.062) but significantly affected the shallots yield (p = 0.002). The average yield of shallots was significantly higher in the 5 days interval application of *B. bassiana* (Table 2).

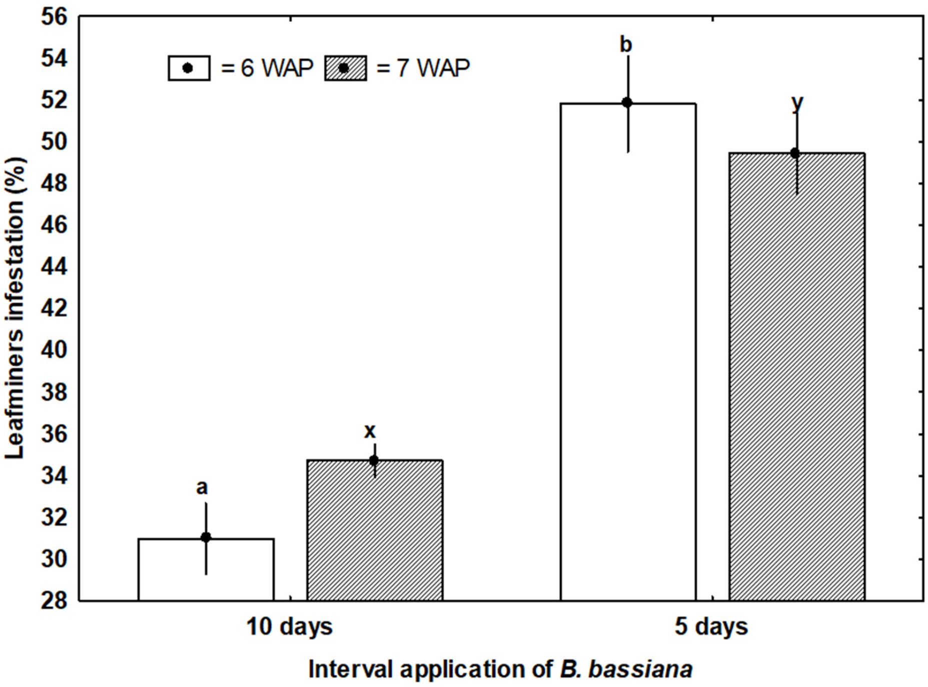

Fig. 2. Leafminer infestation at 6 and 7 WAP as the response on different interval application of *B. bassiana*. Data are means ± SE. Different letters above the similar bar color is significantly different based on HSD test

Table 2. Different effect of mycorrhizae and *B. bassiana* on the root infection, root biomass and yields of shallot

Treatments	Mycorrhizae infection (%)	Roots Biomass (g per plant)	Yields (t/ha)
AM_0	10.10a	1.50a	5.00a
AM_1	16.37b	2.06ab	7.58b
AM_2	28.26b	2.12b	8.01b
Bb_1	16.01a	1.69a	5.77a
Bb_2	20.48a	2.02a	7.97b

Remarks: Mean values ± SE within a similar column and treatments followed by the same letters are not significantly different at $p < 0.01$ based on HSD test. AM_0: without mycorrhizae, AM_1: mycorrhizae 5 g per plant, AM_2: mycorrhizae 10 g per plant, $Bb_{1:}$ *B. bassiana* application every 10 days, $Bb_{2:}$ *B. bassiana* application each 5 days

B. bassiana is one of the most effective entomopathogens against some group of pests including the leafminers (Devi, Padmavathi, Rao, Khan, & Mohan, 2008; McGuire & Northfield, 2020; Migiro, Maniania, Chabi-Olaye, & Vandenberg, 2010; Saleh, Anshary, & Made, 2018) but some factors such as the application technique and persistence, determined their efficacy in the fields (Jaronski, 2010). Our study revealed that 5 days interval application of *B. bassiana* is more effective in suppressing population and infestation of the shallots leafminer compared to 10 days interval. These results could be related to the fungal persistence in the fields.

It was well-known that propagules (conidia) of the *B. bassiana* are were strongly influenced by solar radiation. The UV-A (320–400 nm) and UV-B (280-320 nm) of the sunlight are the main factors causing the conidial death and germination delay (Jaronski, 2010). It is about 9%, the daily loss of *B. bassiana* conidia due to solar radiation. Therefore, more frequent application of *B. bassiana* would increase number of potentially viable conidia and prevented the viability loss of the conidia over time. The conidial density in a 10-day schedule is considered low and may not be sufficient to control the pest. Interval application of *B. bassiana* was reported influencing the daily conidia lost on melon (Jaronski, 2010). Air temperature and humidity were also reported determining the efficacy of *B. bassiana* against lesser grain borer, *Rhyzopertha dominica* (Zaman et al., 2020). Therefore the environmental effect should be considered when planning an integrated pest management strategy based on the application of entomopathogenic fungi such as *B. bassiana* and *M. anisopliae* (McGuire & Northfield, 2020). Both entomopathogenic fungi have high pathogenicity against leafminers of pea bean (Migiro, Maniania, Chabi-Olaye, & Vandenberg, 2010), common bean, *Phaseolus vulgaris* (Gathage et al., 2016), and major pests of vegetable legumes and brassicas (Srinivasan, Sevgan, Ekesi, & Tamò, 2019).

The higher efficacy of *B. bassiana* to manage the crop pests compared to chemical insecticides is due to its the ability to infect the target insects by direct contact of conidia and therefore it is suitable for leaf spraying methods. Additionally this fungus is also able to colonize in the plant tissues and potentially to spread throughout the tissues (Allegrucci, Velazquez, Russo, Perez, & Scorsetti, 2017). The presence of *B. bassiana* spora on colonized crops and persists in leaf tissue for a longer time has made the fungus has a high possibility to infect offspring from any succeeding infestations by an insect (Wagner & Lewis, 2000). This study detected the significant contribution of *B. bassiana* to the shallot yields. These entomopathogen may reduce the yield loss due to pest infestation such as leafminers. Another advantage of *B. bassiana* is it can be combined with botanical pesticides or other biopesticides in pest management programs (Afandhi, Pratiwi, Hadi, Setiawan, & Puspitarini, 2020; Zaman et al., 2020).

Effect of Mycorrhizae on Shallot Roots and Yields

The application of AM successfully colonized the shallot roots. It was indicated by the development of hypha and vesicle on colonized roots (Fig. 3). The developments of roots and bulbs of shallot applied with AM was better than those without AM application (Fig. 4). Root biomass and onion production were also significantly higher in shallots that were applied by AM (Table 1). These fungi significantly affected the level of mycorrhizael colonization ($p = 0.001$), root biomass ($p = 0.001$), and the yields ($p = 0.001$) of shallot but there was no interaction between AM and *B. bassiana* on these three parameters. However, the average values of those three parameters on AM treatments were significantly higher than those without AM.

Despite the variability response of crops to the mycorrhizae application (Estaún, Calvet, & Camprubí, 2010; Kokkoris, Hamel, & Hart, 2019; Saia et al., 2020), our result is in line with number of studies related with significant contribution of mycorrhizae in increasing the crops yield (Bhardwaj, Ansari, Sahoo, & Tuteja, 2014; Saleh, Anshary, & Made, 2018; Samanhudi, Yunus, Pujiasmanto, Cahyani, & Lestariana, 2017; Wang et al., 2019). This study also confirmed the importance of AM application on the allium species that have limited expansion of the root system as one obstacle to absorb sufficient nutrients for plant growth (Golubkina et al., 2020).

The significant contribution of mycorrhizae in increasing the shallots yields maybe related to the high colonization level of AM at the roots of inoculated shallots. The root colonized by the mycorrhizae rises the absorptive surface area and promotes the nutrient uptake by the hyphae from the soil. Soil penetration by mycorrhiza hyphae may increase the absorbing ability of surface host roots as much as ten times (Naher, Othman, & Panhwar, 2013). However, farming practices that highly use chemical fertilizer tend to decrease the diversity and ecosystem services of the mycorrhizae (Manoharan, Rosenstock, Williams, & Hedlund, 2017; Plenchette, Clermont-Dauphin, Meynard, & Fortin, 2005). On the other hand, organic farming systems potentially promote the beneficial contribution of AM to soil health and crop productivity (Gosling, Hodge, Goodlass, & Bending, 2006). This also confirmed the study of Samanhudi, Yunus, Pujiasmanto, Cahyani, & Lestariana (2017) that the utilization of mycorrhizae and organic manure significantly increases the growth and nutrient uptake of soybean in Indonesia.

This is the first study in Central Sulawesi implementing the organic farming system on shallots cultivation. Interestingly, the implementation of this eco-friendly system obtained quite higher yields compared with high external chemical input practices using the same shallot variety of shallots on previous studies. Using chemical fertilizer and pesticide, Azwar, Pasigai, & Lasmini (2018) and Entaunayah, Barus, & Adrianton (2015) only obtained 6.4 and 3.8 t/ha of shallot yields, respectively. Lasmini, Wahyudi, Rosmini, Nasir, & Edy (2019) obtained 10 t/ha but this study applying synthetic insecticides combined with a high dose of chemical and organic fertilizer.

Fig. 3. Colony structure of AM colonize the shallot root ; (a) cortex cell, (b) hyphae, (c) vesicle

Fig. 4. The development of shallot roots and bulbs at the age of 8 WAP as a result of different treatments. AM_0: without mycorrhizae, AM_1: mycorrhizae 5 g per plant, AM_2: mycorrhizae 10 g per plant, Bb_1: *B. bassiana* application every 10 days, Bb_2: *B. bassiana* application each 5 days

In general, organic farming produces lower yields compared to conventional agriculture (Reganold & Wachter, 2016). Surprisingly this study showed that shallots cultivation in the organic farming system has high competitiveness against the conventional system that uses agrochemicals. Even if the yields of conventional systems higher than the organic system, the later still has economic advantages because of higher prices awarded to organic foods. But the most important contribution of this farming system is it's high potency to decrease the extensive usage of the agrochemicals and their residue on the production of allium species (Jamaluddin, Ariefa, Ibrahim, & Yuyun, 2015; Muliana, Anwar, Hartono, Susila, & Sabiham, 2018) and replaced it with the eco-friendly farming system (Etana, Aga, & Fufa, 2019; Mahfudz et al., 2019).

CONCLUSION

Application of mycorrhizae and *B. bassiana* affects leafminer infestation on and the production of shallot 'Lembah Palu', independently. Shallot yields increased for about 50% (from 5 to 7.5 t/ha) by application 5 g per plant of the AM. Population and infestation of leafminers significantly decreased by application of 10 g/l of *B. bassiana* every five days. Accordingly, AM and *B. bassiana* applications support the implementation of organic farming systems on shallot cultivation. Further research needs to explore the indigenous AM and other microbes potentially as biofertilizers or biopesticides for developing sustainable farming systems on shallot and other crops.

ACKNOWLEDGEMENT

This research was financially supported by the Applied Research Grant of The Directorate General of Higher Education, Ministry of Research, Technology and Higher Education, Indonesia (Contract No. 100/SP2H/LT/DRPM/2019).

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