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Research Article

Application of mealworm frass, mycorrhiza, and vermicompost against Rhizoctonia root rot disease and their effects on the growth parameters of bean plants

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Abstract: Rhizoctonia root rot caused by *Rhizoctonia solani* is one of the critical factors influencing bean plants' yield. This study investigates the effects of some biofertilizers for controlling *R. solani* and their impact on the growth parameters of bean plants in the greenhouse. Biofertilizers, *Funneliformis mosseae* (F. mos), vermicompost (Verm), and mealworm frass (Meal), were used in a completely randomized design with five replications. Compared with diseased control, biofertilizers applied separately or in combination, reduced disease severity (except Meal) and disease incidence (except Verm). The combination of Meal + Verm had the best effect on both indices. All biofertilizer treatments increased the dry root weight (except Verm and F. mos + Meal + Verm), fresh root, and foliage weight. Also, root length, and dry foliage weight was increased only in combination treatments, and stem length in Meal + Verm and F. mos + Verm. The highest growth of foliage parameters, root length, and fresh and dry root weight was observed in Meal + Verm, F. mos + Verm, and F.mos + Meal, respectively. The highest mycorrhizal colonization was in F. mos and F. mos + Verm. Therefore, combinations of biofertilizers had better effects on the plant growth and inhibition of Rhizoctonia root rot. The tested biofertilizers and their combinations could be considered as promising tools for reducing the use of chemicals and enhancing sustainable agriculture and disease management. The appropriate timing and application rates for these biofertilizers must be determined accurately during field experiments.

Keywords: Biofertilizers, Biocontrol, Colonization, *Funneliformis mosseae*, *Rhizoctonia solani*

Introduction

Common bean (*Phaseolus vulgaris* L.) is an important legume crop cultivated worldwide. The seeds contain high protein, carbohydrates, lipids, minerals, and vitamins (El-Benawy *et al.*, 2020). *Rhizoctonia solani* Kühn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is an important plant pathogen that causes seed rot, damping-off, hypocotyl, and root rot on many plant species, especially common beans (Abawi *et al.*, 1985; Matloob and Juber, 2013) and reduces the yield and quality of this crop around the world (Donmez *et al.*, 2015). This pathogen

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produces resistant sclerotia that survive in the soil for long periods and has a wide host range. Therefore, agronomic practices such as rotation do not effectively control the disease (Aljawasim *et al.*, 2020). The development of resistant cultivars is laborious and time-consuming, and the new pathogenic strains may also overcome the resistance in such cultivars (Bilqees *et al.*, 2021). Control of soil-borne plant pathogens by chemicals is also very costly and impractical (Kumar *et al.*, 2017; Kala *et al.*, 2015), and in addition to causing severe environmental and health problems (Bilqees *et al.*, 2021), may harm the beneficial soil microorganisms and increase the resistant populations of the pathogen (Daroodi *et al.*, 2021). Proper nutrition is one of the most efficient agronomic approaches for improving the vigor of plants and their resistance to pathogens and reducing losses. However, in some cases, despite the use of inorganic fertilizers to provide the required nutrients, low crop efficiency, and yield reduction may occur (Kihara *et al.*, 2016; Beesigamukama *et al.*, 2021) due to low amounts of soil organic matter, micronutrients deficiency, and high pH (Wortmann *et al.*, 2019; Vanlauwe *et al.*, 2015). Therefore, developing non-chemical and environmentally friendly alternative strategies seems necessary for inhibiting the disease and enhancing the growth of plants.

Biofertilizers contain many beneficial microorganisms that increase soil productivity and fertility through various processes (Roychowdhury *et al.*, 2017). These microorganisms enhance the accessibility of nutrients for plants in different ways, such as by stabilizing atmospheric nitrogen, dissolving insoluble soil phosphates, and synthesizing growth stimulants (Sadhana, 2014; Raimi *et al.*, 2017). In addition, using biofertilizers in the soil is environmentally friendly and not harmful to crops or other plants. These fertilizers also help the plants' health and protect them against pathogens (Van Wyk, 2018).

Arbuscular mycorrhizal fungi (AMF) are important biofertilizers with symbiotic relationships with many plants' roots. In exchange for photosynthetic products from their host plants, AMF increase plants' water and minerals absorption and enhance their resistance to abiotic and biotic stresses (pests and pathogens) (Van Wyk, 2018; Hage-Ahmed *et al.*, 2019). Therefore AMF improve the health and yield of plants at the presence various pathogens such as Rhizoctonia in beans (Hafez *et al.*, 2013; Moarrefzadeh *et al.*, 2023; Nasir Hussein *et al.*, 2018), and increase soil fertility and reduce the damages caused by pathogens (Rouphael *et al.*, 2015; Van Wyk, 2018).

Vermicompost is the final product of non-thermophilic biodegradation of organic materials through interactions between different species of earthworms and related microorganisms (Arancon *et al.*, 2004). This biofertilizer has attracted much attention due to its numerous effects, such as the increased microbial and antagonistic activity of soil (Pathma and Sakthivel, 2012), richness in nutrients and humic acid, cation exchange capacity, high humidity (García *et al.*, 2014), strong water absorption capacity, and high porosity (Zhang *et al.*, 2020; Esmaielpour *et al.*, 2020). Other advantages include proper drainage, having essential nutrients such as nitrogen, phosphorus, and potassium (Shishehbor *et al.*, 2013; Van Wyk, 2018), improving soil fertility and plant's growth and yield (Esmaielpour *et al.*, 2020) and its inhibitory effects on some soil-borne plant pathogens (Rivera *et al.*, 2004; Zhang *et al.*, 2020).

Insect production as a sustainable protein source is among the fastest-growing industries worldwide and a promising solution for effectively recycling organic wastes. The most abundant byproduct of insect production is the frass or the residues from rearing their larvae (Blakstad, 2021). This substance, which is converted to a microbial-rich substance during digestion (Chavez and Uchanski, 2021), is mixed with uneaten food substrate and pieces of the insect-shed exoskeleton and is rich in chitin (Blakstad, 2021; Diener *et al.*, 2009) and large amounts of nutrients that plants could easily absorb (Poveda *et al.*, 2019). Several studies have shown the effect of insect frass as stimulants for resistance to biotic (Xuan *et al.*, 2022; Quilliam *et al.*, 2020; Ray *et al.*, 2016) and abiotic (Poveda *et al.*, 2019) stresses and their role as biofertilizers

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in improving plant growth (Poveda *et al.*, 2019; Houben *et al.*, 2020; Xuan *et al.*, 2022). Yellow flour beetle (*Tenebrio molitor* L., (Col.: Tenebrionidae)) is an important storage pest with a global distribution that contaminates stored food products (Gao *et al.*, 2018). The larvae of this beetle, known as mealworms (Han *et al.*, 2014), are widely used in converting plant biomass into protein (Osimani *et al.*, 2018). Mealworm frass contains macronutrients such as NPK (Liu *et al.*, 2003) and has beneficial effects on the growth of plants (Houben *et al.*, 2020; Poveda *et al.*, 2019). This substance slowly releases the nutrient elements and improves soil structure and therefore, it could be considered a noticeable fertilizer (Liu *et al.*, 2003). Considering the diversity of the populations of microorganisms in mealworm frass (Osimani *et al.*, 2018) and their beneficial effects on promoting plant growth (He *et al.*, 2020), it is possible that mealworm frass directly or indirectly affects the population of plant pathogens.

One of the limitations of applying the agents for biological control and stimulating plant growth is that when used separately, these agents have relatively little activity range compared to chemicals or may not show their beneficial effects due to inadequate root colonization. In addition, they face many problems in activating their biocontrol mechanisms in different environmental conditions, and ultimately their performance becomes inadequate or unstable. One of the strategies to overcome this instability is to use combinations of biological agents. A consortium of beneficial microorganisms with different patterns of root colonization, different optimal environmental conditions, and growth-enhancing properties, or disease inhibition mechanisms could help improve plant growth and control various pathogens in a wide range of ecological conditions (Szczech, 2008; Akhtar and Siddiqui, 2008; Atwa, 2018; Etesami and Maheshwari, 2018).

The effect of insect frass on plant growth and inhibition of plant diseases has not been studied widely. According to our literature review, the impact of mealworm frass on plant diseases had not been investigated previously. Despite the beneficial effects of different biofertilizers on plant growth, they may show variable effects on soil-borne plant pathogens (Bonanomi *et al.*, 2010; Termorshuizen *et al.*, 2006). Thus, their effects must be evaluated before applying them to prevent plant diseases. The activity and function of biological agents may increase or decrease under the influence of rhizosphere microorganisms (Raaijmakers *et al.*, 1995; Janisiewicz and Bors, 1995). Using biofertilizers with high microbial populations likely affects other biofertilizers' efficiency in disease control. Considering the above issues, three biofertilizers (mycorrhiza, vermicompost, and mealworm frass) were investigated for disease control potential and their impact on bean growth parameters. The effect of vermicompost and mealworm frass on mycorrhizal root colonization was also investigated.

Materials and Methods

Preparing the inoculum of pathogen isolate

An isolate of *Rhizoctonia solani* AG-2, previously characterized by its morphology and pathogenicity on bean plants, was obtained from the collection of fungi in the Plant Protection Department, Faculty of Agriculture, Razi University (Kermanshah, Iran). This isolate was grown in Petri dishes containing potato dextrose agar (PDA) and incubated at 25 °C. For preparing the inoculum of *R. solani*, millet seeds were soaked in water for 24 hours and, after transferring them to glass flasks, were sterilized twice at 24 h intervals by autoclave (121 °C for 20 min). Five discs (1 cm in diameter) separated from the growing margins of *R. solani* colony on PDA medium were transferred into flasks containing sterilized millet seeds. These were stored at 25 °C for two weeks. Millet seeds entirely colonized by mycelia were used as the pathogen inoculum in the greenhouse test (Ardalan *et al.*, 2017).

Fungus, vermicompost, and mealworm frass materials

The frass of *Tenebrio molitor* larvae (mealworm) was harvested from an experimental farm at the Faculty of Agriculture, Razi University, after

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rearing and was stored for six months before use. Mealworm frass included bran particles used for insect-rearing beds, remnants of shells from different larvae stages, and even whole insects. The *Funneliformis mosseae* and vermicompost substrates were purchased from Turan Biotech Company (Shahroud, Iran) and Surin Khak Company (Tehran, Iran), respectively.

Evaluating the biofertilizers

To evaluate the effect of separate and combined applications of biofertilizers against Rhizoctonia rot of beans and their potential for improving the growth traits of bean plants in the presence of the pathogen, a pot experiment was performed in greenhouse conditions based on a completely randomized design with nine treatments and five replications.

Bean seeds (variety Early Khameneh 535) were disinfected with 70% ethanol and 0.5% sodium hypochlorite (each for one minute). After thorough washing with distilled water three times, the seeds were dispersed inside sterile containers with a thin layer of water and incubated for two days.

A sterile mixture of perlite and peat moss (2:1) was used as the basal medium for plant cultivation. Depending on the treatment, the basal medium was mixed with other materials before pouring into plastic pots (8 cm diameter, 13 cm height, and 450 ml volume). From the mycorrhizal substrate, 36 grams (containing 100 spores per gram) were mixed with the basal medium in each pot (Moarrefzadeh *et al.*, 2021b). Vermicompost and mealworm frass were added to the basal medium in the appropriate treatments by 25% (Fekrat *et al.*, 2017) and 2% (Poveda *et al.*, 2019) of the total volume, respectively. A plastic tube (15 cm in length and 1 cm in diameter) was first placed in the center of each pot, and then the growing mixture was poured into the pot to keep a blank space for the further addition of the pathogen inoculum. Four bean seeds were placed in each pot and covered with a layer of sterile basal medium. The basal medium was used with no biofertilizers in diseased and healthy control treatments. After germination, three seedlings were kept in each pot, and the excess seedlings were discarded. Two weeks later, plastic tubes were removed from the center of the pots, and in the space in the middle of each pot, 50 mg of millet seeds colonized by *R. solani* were added. It was added uniformly with some sterile vermiculite. Only sterile vermiculite was added to the healthy control treatment. The inoculated plants were kept in greenhouse conditions at 25 ± 5 °C, and irrigated daily by a gentle flow of water containing 100 ppm of a complete fertilizer (Fermolife NPK 18-18-18 + TE, received from Baharan Co., Isfahan, Iran).

Evaluation of disease and plant growth indices

Three weeks after pathogen inoculation and the appearance of disease symptoms on diseased control treatment, the plants were carefully removed from the pots, and their roots were washed. Disease severity was evaluated by a 0 to 4 scale as described by Yildirim and Erper (2017) as follows: 0) healthy seedlings without disease, 1 very few and superficial spots or lesions on the roots or hypocotyl, 2) large and deep spots or lesions on the roots or hypocotyl, 3) lesions surrounding hypocotyl, severe root rot and relatively restricted root length, 4) complete root rot and seedling death.

The disease severity index (DSI) was calculated according to Silva *et al.* (2020) as follows:

DSI (%) = [Σ (degree of the scale × frequency) / (total number of units evaluated × maximum degree of the scale)] × 100

The disease incidence (DI) rate was determined according to Ali and Nadarajah (2013) as follows:

DI (%) = (Number of infected plants / total number of observed plants) × 100

The growth indices of bean plants were measured, including root wet and dry weight, root length, foliage wet and dry weight, and stem length. The Data were analyzed by SAS 9.4. Duncan's multiple range test was used to compare the means, and the statistical probability level in all calculations was 5%.

Determination of mycorrhizal root colonization

The method described by Phillips and Hayman (1970) was used with slight changes to determine

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the colonization rate of bean roots by *F. mosseae* in treatments including this fungus. Briefly, after removing the roots from the soil and washing them, and before measuring the growth indices of the plant, 0.1 g of the roots were separated and thoroughly washed with distilled water. The roots were cut into 1 cm pieces and transferred to tubes containing 10% potassium hydroxide. The tubes were heated at 90 °C for one-hour (water bath). The samples were washed five times with water and placed in 1% hydrochloric acid solution for five minutes. With no further washing, the root pieces were kept in lactoglycerol solution containing 0.01% acid fuchsin for one hour, placed in 90 °C water bath for one hour, and in lactoglycerol solution for 30 minutes for destaining. One hundred fragments of the stained roots were placed on glass slides and observed by microscope at 100 to 400 X magnification to count the mycorrhizal roots. The percentage of root colonization was determined by dividing the number of infected mycorrhizal roots by the total number of roots multiplied by 100.

Results

The effect of biofertilizers on disease incidence and severity

Except for Meal, all other treatments, including biofertilizers applied against *R. solani* in greenhouse conditions separately or in combination, significantly reduced the disease index compared to the diseased control at a 5% probability level. The highest disease inhibition was observed in Meal + Verm treatment, with 83% reduction in disease index compared to the diseased control. This was followed by F. mos + Verm, F. mos + Meal + Verm, Verm, F. mos + Meal, and F.mos, which reduced the disease index by 64, 57, 45, 40, and 33 percent, respectively (Figure 1, A). Except for Verm, other treatments applying biofertilizer separately or in combination significantly reduced the disease incidence compared to the diseased control. The highest reduction in disease incidence was observed in Meal + Verm (55%), and then in F. mos + Meal + Verm and F.mos + Verm (both 44 %), F. mos + Meal (33%), F. mos and Meal (both 22%) treatments. There was a significant difference among the treatments for their effect on disease incidence and severity (Figure 1, B).

Effect of biofertilizers on growth parameters of aerial parts in bean plants

By evaluating the effect of biofertilizers on the growth traits of bean plants in the presence of *R. solani* in greenhouse conditions, it was found that two combined treatments, namely Meal + Verm and F. mos + Verm, significantly increased the length of the aerial parts. The highest effect on this index was related to Meal + Verm (31% more than the diseased control); this trait was even more than the healthy control (16%). Compared to the diseased control, the fresh weight of the aerial parts was significantly increased by all separate and combined treatments of biofertilizers. The highest and lowest effects on this growth trait were observed in Meal + Verm (63%) and Meal (20%) treatments, respectively. A significant increase in the dry weight of aerial parts compared to the diseased control was observed in combined treatments (F. mos + Verm, F.mos + Meal + Verm, F. mos + Meal, and Meal + Verm), and the highest effect on this index was related to Meal + Verm (50%) (Table 1).

Effect of biofertilizers on growth indices

Only combined treatments of biofertilizers caused a significant increase in root length, and the highest effect on this index was related to F.mos + Verm (43% more than diseased control and 12% more than healthy control). Meal + Verm and F. mos + Meal + Verm treatments were placed in the same statistical group as the healthy control. All separate and combined biofertilizer treatments significantly increased root wet weight; the most significant effect on this growth index was F. mos + Meal (57% more than the diseased control). Regarding the effect on this index, other biofertilizer treatments were placed together in the same statistical group. Also, except for Verm and F.mos + Meal + Verm treatments, other biofertilizer treatments increased the dry root weight significantly. The most observed effect on this index was F. mos + Meal (60% more than the diseased control) (Table 1).

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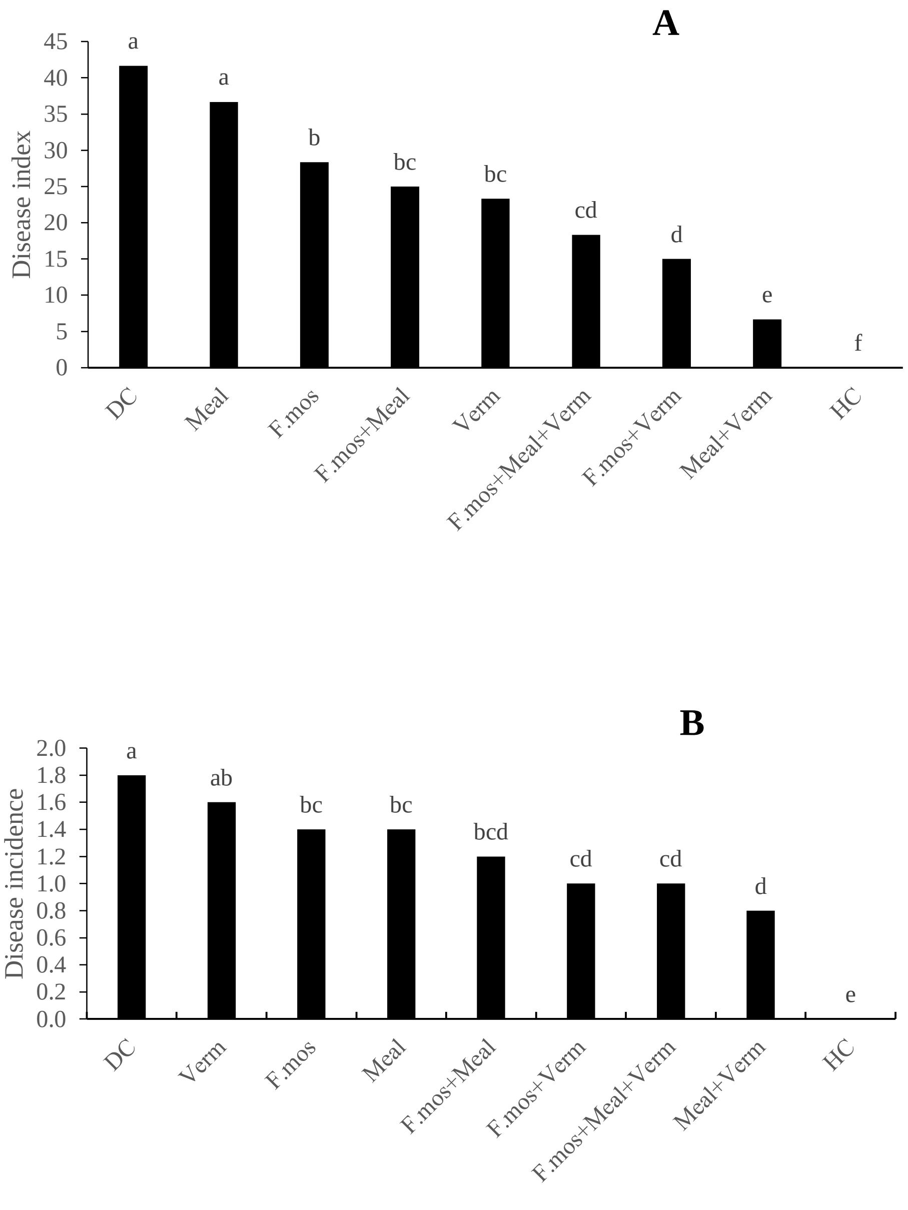

Figure 1 Effect of separate and combined application of biofertilizers on disease index (A) and incidence (B) of Rhizoctonia root rot of bean plants caused by *R. solani* in greenhouse conditions.
DC = Diseased control (infected by *Rhizoctonia solani*), Verm = Vermicompost, F.mos = *Funneliformis mosseae*, Meal = Mealworm frass and HC = Healthy control.

Root colonization rate by mycorrhizal treatments

The highest rate of mycorrhizal root colonization was observed in F. mos (59%) and F. mos + Verm (56%) treatments, with no significant difference between these two treatments. Mycorrhizal root colonization in F. mos + Meal and F. mos + Meal + Verm treatments (47% and 13% roots, respectively) was significantly lower than *F. mosseae* (Figure 2).

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Table 1 Effect of separate and combined application of biofertilizers on growth traits of bean plants in the presence of R. *solani*, the causal agent of Rhizoctonia root rot of bean.

Treatment	Stem length (cm)	Foliage wet weight (g)	Foliage dry weight (g)	Root length (cm)	Root wet weight (g)	Root dry weight (g)
DC	15.8 c	4.28 e	0.474 d	12.4 e	1.128 d	0.236 d
F.mos	16.4 c	5.44 d	0.556 cd	13.2 de	1.478 c	0.330 bc
F .mos + Meal	15.3 c	6.38 c	0.680 ab	14.2 d	1.776 b	0.386 b
F. mos + Meal + Verm	16.0 c	6.16 c	0.592 c	15.8 b	1.408 c	0.292 cd
F. mos + Verm	17.8 b	6.06 c	0.680 ab	17.8 a	1.506 c	0.320 bc
HC	17.9 b	7.56 a	0.760 a	15.8 b	2,004 a	0.474 a
Meal	15.9 c	5.16 d	0.546 cd	13.3 de	1.422 c	0.320 bc
Meal + Verm	20.7 a	6,99 b	0.730 a	15.4 bc	1.588 c	0.362 bc
Verm	15.0 c	5.43 d	0.492 d	13.0 de	1.452 c	0.290 cd

The numbers in the table are the averages of five replications. The non-common letters next to the numbers of each column indicate a statistically significant difference in treatments based on Duncan's test at 5% probability level.
DC = Diseased control (infected by *Rhizoctonia solani*), Verm = Vermicompost, F.mos = *Funneliformis mosseae*, Meal = Mealworm frass, and HC = Healthy control.

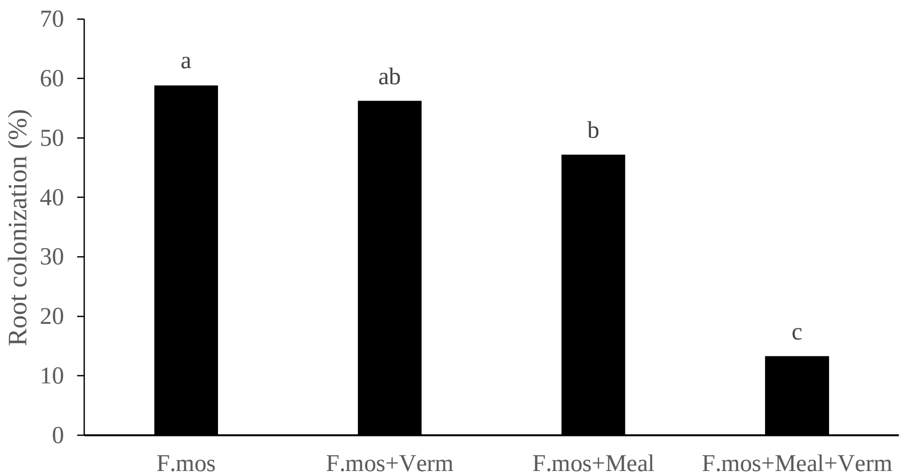

Figure 2 Mycorrhizal roots colonization of bean plants (*Phaseolus vulgaris* L.) by *Funneliformis mosseae* alone or in the presence of other biofertilizers
F. mos = *Funneliformis mosseae*, Meal = Mealworm frass, Verm = Vermicompost

Discussion

Sustainable agriculture aims to increase soil fertility, biocontrol of plant pests and diseases, and reduce or eliminate the use of chemical fertilizers and pesticides in the long term (Fathi *et al.*, 2017). *R. solani* is an important soilborne plant pathogen that reduces the yield and quality of beans worldwide (Donmez *et al.*, 2015). Using fungicides to control soil-borne plant pathogens, including *R. solani* is impractical due to numerous environmental problems and high costs (Kumar *et al.*, 2017; Kala *et al.*, 2015). Therefore, using compounds that can act as biocontrol agents against plant pathogens and, simultaneously, as biofertilizers for improving plant growth, will be a fundamental step in realizing sustainable agriculture and producing healthy food. In the present study, the effect of separate and combined application of three

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biofertilizers for reducing the damages of Rhizoctonia root rot disease and enhancing the growth traits of bean plants in the presence of pathogen was investigated in greenhouse conditions.

Different treatments by *F. mosseae*, not only reduced disease incidence (22.22%) and severity (33.33%) in the presence of *R. solani*, but also enhanced some growth traits of bean plants (wet weight of foliage and wet and dry weight of roots). There are other reports where *F. mosseae* alone (Moarrefzadeh *et al.*, 2023) or in combination with some other mycorrhizae species (Hafez *et al.*, 2013) has decreased the severity and incidence of Rhizoctonia root rot, and enhanced growth in bean plants. In cucumber plants (*Cucumis sativus* cv. Cevher), *F. mosseae* increased the dry weight of aerial and root parts and decreased the severity of damping-off caused by *R. solani* (Aljawasim *et al.*, 2020). AMF can increase the growth of their host plants by improving nutrient uptake (Jeffries *et al.*, 2003), and this improvement in growth can play a vital role in compensating for damages caused by plant pathogens (Wu *et al.*, 2021). In addition to improving the nutrition status of the plant (Kareem and Hassan, 2014), the increased resistance of mycorrhizal plants to soil-born pathogens has been mainly attributed to the morphological changes in roots (Liu *et al.*, 2019; Bever *et al.*, 2009), direct competition with pathogens in soil and plant roots for colonization sites and host photosynthetic products (Aljawasim *et al.*, 2020; Azcón-Aguilar and Barea, 1996), activation of defense mechanisms against soil-borne pathogens through biochemical, physiological, and structural changes in plants (Miozzi *et al.*, 2019; Shasmita *et al.*, 2019; Hafez *et al.*, 2013; Amer and Abou El, 2008).

In this study, vermicompost treatment reduced the severity of Rhizoctonia root rot (45%) in bean plants and improved some growth traits (the weight of root and aerial parts). In other pot studies, vermicompost obtained from agricultural waste (Simsek Ersahin *et al.*, 2009) and bamboo (*Phyllostachys edulis*) (You *et al.*, 2019), improved plant growth, and effectively controlled damping-off caused by *R. solani* in cucumber plants. Due to the activity of the intestinal microflora of earthworms, vermicompost is rich in microbial communities, macro and micronutrients, vitamins, growth hormones, and enzymes such as protease, amylase, lipase, cellulose, and chitinase (Olle, 2019). Earthworms swallow plant growth-promoting rhizobacteria (PGPR) such as *Azotobacteria* sp., *Azospirillum* sp., and *Pseudomonas* sp. (Pathma and Sakthivel, 2012). Because of the favorable micro-environment in their intestines, they can activate or increase the number of such bacteria (Sinha *et al.*, 2010). Subsequently, these diverse and active PGPR would promote plant growth directly by dissolving soil nutrients and making them available to plants (Yatoo *et al.*, 2021; Jangra *et al.*, 2019). Also, they, produce growth hormones and 1-aminocyclopropane-1- carboxylate (ACC) deaminase (Donate-Correa *et al.*, 2005), nitrogen fixation in the soil (Han *et al.*, 2005) and indirectly, by creating undesirable conditions for the growth of plant pathogens (Pathma and Sakthivel, 2012) through mechanisms such as competition, antibiosis, hyperparasitism and systemic induced resistance (Hoitink and Grebus, 1994), and play a role in the biological control of plant diseases and increasing the yield of plants (Simsek-Ersahin, 2011). Vermicompost can also enhance the growth of plants by increasing humic acid concentration, soil porosity, and water retention capacity, as well as changing the pH and soil mass density (Simsek-Ersahin, 2011; Sarma *et al.*, 2010; Datta *et al.*, 2016).

In this study, mealworm frass treatment alone reduced the incidence of Rhizoctonia root rot disease and improved some growth traits (foliage wet weight and wet and dry weight of roots) in bean plants. Poveda *et al.* (2019) reported that compared to negative unfertilized control, mealworm frass derived from different diets increased the growth of leaf beet (*Beta vulgaris* var. *cicla*) and increased the tolerance of bean seedlings to abiotic stresses (drought, flooding, and salinity). By examining the microbial population in mealworm frass, they found that most of them had at least one plant growth-promoting trait, such as nitrogen fixation, phosphorus, and potassium solubilization, and production of growth-promoting compounds such as auxin, gibberellin, siderophore, and ACC-deaminase, which play important roles in promoting plant growth and increasing plant tolerance to stresses (Poveda *et al*., 2019). It has also been reported that mealworm frass has effectively increased the biomass and the amounts of nutrients absorbed in barley (*Hordeum vulgare*). Similar to mineral NPK fertilizer, these effects have been attributed to the rapid mineralization of mealworm frass, providing nutrients as an available form for the plant and increasing the soil microbial diversity and activity (Houben *et al*., 2020). Despite the few studies on the effect of mealworm frass on plant growth, no report was found regarding its efficiency in inhibiting plant diseases. However, some promising results have been achieved with the frass of other insects. The frass of black soldier flies (*Hermetia illucens*) reduced the wilt disease caused by *Fusarium oxysporum* in cowpea (*Vigna unguiculata*) plants (Quilliam *et al.*, 2020). Several studies have been conducted on the disease reduction mechanisms by insect frass, and various results have been reported; Xuan *et al.* (2022) stated that the frass from *Protaetia brevitarsis* contained high amounts of humic acid and microorganisms that not only improve plant growth and accelerate the degradation of organic matter but also help to suppress the fungal pathogens *Sclerotium rolfsii*, *F. oxysporum* and *Sclerotinia sclerotiorum*. Poveda (2021) stated that detecting microorganisms and biomolecules in frass by plant roots may activate the plant's systemic resistance through salicylic acid or jasmonic acid pathways, so frass can act as priming stimuli in plants (Sørsdal, 2021). The strongest argument for such defensive stimuli is the presence of chitin in insect waste that originates from the exoskeleton of insect larvae during the molting process or is excreted from the chitin content of peritrophic membranes of the intestines of insects (Blakstad, 2021). This chitin benefits plant growth and disease resistance (Chavez and Uchanski, 2021). Quilliam *et al.* (2020) reported that frass from black soldier flies activated defensive responses to Fusarium wilting disease in cowpea plants and increased the resistance, also attributed these to the chitin content of the frass. Some researchers have also suggested that the increased resistance of plants fertilized by insect frass to biological stresses may be associated with increased plant growth (Temple *et al.*, 2013). In addition, frass may contain volatile organic compounds. Volatile compounds, may have adverse effects on plant pests (Zhang *et al.*, 2019) and increase the resistance to plant pathogens (Veselova *et al.*, 2019; Moarrefzadeh *et al.*, 2021a).

Compared with separate treatments of biofertilizers, their combined treatments had better effects in reducing the disease incidence and severity. In many cases, the combined treatments also had better effects on bean plants' growth traits than the separate treatments. This indicates the synergistic and positive effect of such combinations of biofertilizers in controlling disease and improving the growth of plants. Interestingly, Meal and Verm treatments, which separately had no significant effect on disease incidence and severity, when applied in combination (Meal + Verm), caused the highest reduction in disease severity (83.33%) and incidence (55.55%) and also showed the best growth improvement in the aerial parts of bean plants. Organic matter acts as carbon and energy sources for soil microorganisms, and their addition to the soil is often accompanied by an increase in total microbial activity and biomass (Janvier *et al.*, 2007; Larney and Angers, 2012). Therefore, any treatment that increases the overall activity of microbes in the soil may improve general disease suppression by increasing competition over available resources (Yatoo *et al.*, 2021). Previous studies have also shown that mealworm frass (Poveda *et al*., 2019) and vermicompost (Yatoo *et al.*, 2021) increase the activity of microbes and antagonists in the soil. In Meal + Verm treatment in the present study, mealworm frass and vermicompost may have increased the activity of microorganisms in each other and improved each other's efficiency, both as fertilizers and as biocontrol agents. Other possible reasons for the positive and synergistic effect of biofertilizer combinations compared to their separate application may include the following: effectiveness of microbial mixtures in a broader range of environmental conditions, the occurrence of several disease suppression mechanisms, and several growth-promoting properties, better plant access to the required nutrients and having different patterns for plant root colonization. Therefore, such combinations may improve the growth and health of crop and show higher efficiency for disease control (Srivastava *et al.*, 2010; Alabouvett *et al.*, 2001; Atwa, 2018; Palmieri *et al.*, 2016).

In the present study, the effect of F. mos + Verm on the severity and incidence of disease and growth factors was more than the effect of these biofertilizers alone. Our results are similar to Fekrat *et al.* (2017), who reported that combining *Glomus versiforme* and vermicompost was more effective than their separate treatments. The combined application of AMF and vermicompost has also increased wheat plants' yield (Hussain *et al.*, 2016). The beneficial effects observed by the combined application of AMF and vermicompost in plants have been mainly attributed to improving soil conditions, providing the necessary nutrients from the soil for plants to a greater extent, and consequently increasing the growth, biomass production, and total crop yield (Anwar *et al.*, 2005; Singh *et al.*, 2008; Van Wyk, 2018).

The highest root colonization was found in F.mos (59%) and F. mos + Verm (56%) treatments. Therefore, vermicompost had no negative or positive effect on root colonization by F.mos. This is consistent with the results of Xue *et al.* (2022). In contrast, some studies have reported the positive effect of vermicompost on mycorrhizal root colonization (Fekrat *et al.*, 2017; Hussain *et al.*, 2016). It has been suggested that mycorrhiza may have had more available carbon sources and colonized the roots better (Siddiqui and Akhtar, 2008). The difference between our findings and previous reports may be related to the different characteristics of vermicompost (Xue *et al.*, 2022).

In the present study, mycorrhizal colonization of bean roots had decreased in F. mos + Meal + Verm and F. mos + Meal treatments. The microbial community and rhizobacteria in the plant rhizosphere have promoting or inhibitory effects on the growth of AMF (Ravnskov and Jakobsen, 1999) and influence their germination, spore production, hyphae growth, and finally, their root colonization (Baradar *et al.*, 2015). In the present study, such interactions had likely occurred among microorganisms in a combination of vermicompost and mealworm with mycorrhiza in treatments where reduced mycorrhizal root colonization was observed. Interestingly, this reduction in colonization in F. mos + Meal and F. mos + Meal + Verm treatments had no negative effect on their plant growth stimulating and biocontrol ability. Other researchers have also observed a lack of correlation between mycorrhizal colonization with disease reduction and growth in the host plant (St-Arnaud *et al.*, 1994; Moarrefzadeh *et al.*, 2022; Moarrefzadeh *et al.*, 2023); Dugassa *et al.* (1996). The effect of AMF symbiosis on plant health depends more on the genotype of the host plant and pathogen than the rate of AMF colonization (Dugassa *et al.*, 1996).

Conclusion

In the present study, vermicompost, mealworm frass, and *F. mosseae* biofertilizers showed good potential in suppressing the Rhizoctonia root rot and increasing the growth of bean plants. The beneficial effects were generally more in treatments with combinations of biofertilizers. The efficiency of mealworm frass in reducing the occurrence of an important plant pathogenic fungus was proved for the first time. Mealworm frass and its combination with vermicompost had the best effect on reducing disease incidence and severity and enhancing the growth of bean plants. Therefore, the combination of biofertilizers can be used as an efficient and environmentally friendly method to improve soil fertility, increase the growth and yield of plants, manage Rhizoctonia root rot disease, and develop sustainable agriculture. Nevertheless, further research is required to identify the effective factors in these fertilizers and their mechanisms in promoting plant growth and inhibiting plant diseases. More studies are also needed to be conducted in field conditions and determine the best time and amount of their application for achieving the desired results by these fertilizers.

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چکیده: بیماری پوسیدگی ریزوکتونیایی ریشه ناشی از بیمارگر قارچی *Rhizoctonia solani* از مهم‌ترین عوامل کاهش عملکرد لوبیا است. هدف از پژوهش حاضر، بررسی اثر جداگانه و ترکیبی کودهای زیستی میلورم، میکوریز و ورمی‌کمپوست در کنترل این بیماری و رشد لوبیا در شرایط گلخانه بود. اثر کاربرد جداگانه و ترکیبی کودهای زیستی *Funneliformis mosseae* (F. mos)، ورمی‌کمپوست (Verm) و پسماند میلوُرم (Meal) بر وقوع و شدت بیماری و صفات رشدی لوبیا با حضور *R. solani* در قالب طرح کاملاً تصادفی با نُه تیمار و پنج تکرار در گلخانه بررسی شد. کودهای زیستی به‌کار رفته به‌صورت جداگانه و ترکیبی سبب کاهش شدت (به‌استثنای Meal) و بروز بیماری (به‌جز Verm) شدند و ترکیب Meal + Verm بیش‌ترین تأثیر را در این زمینه داشت. افزایش وزنِ تر ریشه و اندام هوایی و نیز وزن خشک ریشه (به‌جز Verm و F. mos + Meal + Verm) در کلیه تیمارهای کودهای زیستی، طول ریشه و وزن خشک اندام هوایی فقط در تیمارهای ترکیبی، و طول ساقه در Meal + Verm و F. mos + Verm مشاهده شد. بیش‌ترین افزایش صفات رشدی اندام‌های هوایی، طول ریشه و وزنِ تر و خشک ریشه نیز به‌ترتیب در Meal + Verm، F. mos + Verm و F. mos + Meal مشاهده شد. بیش‌ترین کلنیزاسیون میکوریزی ریشه نیز در F. mos و F. mos + Verm وجود داشت. در مقایسه با کاربرد جداگانه، ترکیب کودهای زیستی، اثر هم‌افزا و بهتری در افزایش رشد و مهار پوسیدگی ریزوکتونیایی ریشه داشت. توصیه می‌شود بهترین زمان و مقدار کاربرد این کودها طی مطالعات مزرعه‌ای تعیین شود تا بتوان علاوه بر مدیریت بیماری، به کاهش کاربرد سموم و ترکیبات شیمیایی و توسعه کشاورزی پایدار کمک نمود.

واژگان کلیدی: کلنیزاسیون، کنترل زیستی، کودهای زیستی، *Funneliformis mosseae*، *Rhizoctonia solani*

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