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Fungus Applications on Growth and Yield of Dena-1 Soybean Varieties

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Abstract. This study aims to determine the response of black soybean variety Dena-1 that has been inoculated with Rhyzobium indigenous bacteria to the activity of Trichoderma and Glomus spp fungi given as soil treatment. The experiment was arranged in a completely randomized design with effective fungal application treatment consisting of without application, T. esperellum, Glomus spp., and both types of fungi. All treatments were repeated five times. Observations were made on plant stem height and diameter, dry weight of the stover, number of pods, number of grains, grain weight, and weight of 100 grains. Data were analyzed by ANOVA and HSD test with a significance level of 5%. Trichoderma and Glomus application had no effect in increasing plant growth and production. The simultaneous application of these two fungi resulted in a decrease in dry weight of stover, number of pods, number of grains, grain weight, and weight of 100 soybeans respectively 15.41, 22.50, 30.87, 69.95, and 49.03%. This indicates that there is competition in the use of resources between the root nodule bacteria and the two biological agent fungi.

1 Introduction

All stakeholders continue to make efforts to free dependence on imports to fulfill national soybean demand. Studies which discuss the production of varieties that are tolerant on various environmental stresses in Indonesia are become extremely required, including the aspect of low daily light intensity. The Dena-1 variety is one of the varieties prepared for soybean cultivation on land that is often under canopy conditions with a light intensity of up to 60%.

Organic matter and low soil pH and the threat of plant-disturbing organisms have always been an important obstacle in utilizing dry land with low light intensity for crop production [1]. This environmental stress will be an obstacle for plants to go through the vegetative phase which is a critical phase [2-3] and raises the problem of important nutrient deficiencies that often occur in this marginal dry land.

The use of effective microorganisms to improve soil fertility and provide protection for plant growth is one of the answers to the challenge of using marginal dry land for crop production [4-5], in addition to using varieties that are resistant to drought and low light intensity. Effective beneficial microbes from the well-known group of fungi are Trichoderma spp. and endomycorrhizal fungi which have the potential to be used as biological agents for

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biofertilizers and biocontrol agents that can suppress various pathogens that cause soil borne diseases so that they can support plant growth while protecting health and increasing plant resistance [6-7]. Thus, these two effective fungi can be relied upon to be used simultaneously in marginal dry land soybean cultivation in an effort to increase national soybean production.

Root nodule bacteria play an important role in the N cycle in nature, namely in the form of nitrogen fixation from the air and converting it into the form needed for plants and [8]. *Rhizobium sp.* bacteria benefit from plants that supply organic compounds and contribute precursors to the process of forming legume plant acid amines [9]. The abundance of substrates released by plants in the form of various metabolites, hormones, and enzymes that break down organic compounds is necessary for various soil microbes in the rhizosphere [10-11].

The activity of Trichoderma fungi in addition to producing various enzymes that can degrade organic matter which releases ionic compounds that can be exchanged by plant roots to meet their nutritional needs [12-13], also produces secondary metabolites [14-15] and various growth regulating compounds [16] which can help plant vegetative growth. *V*arious arbuscular mycorrhizal fungi play an important role to improving soil structure and increasing plant nutrition [17]. Fungi *Glomus etunicatum* plays a role in promoting plant growth through a significant supply of various essential nutrients into plant tissues [18-19].

The joint use of rhizoplane fungi and rhizosphere fungi is expected to provide protection to the plant rhizosphere system and stimulate the vegetative growth of soybean plants. Considering *Trichoderma* and endomycoirza fungi and nodule bacteria *Rhizobium* sp. has an almost overlapping habitat in the roots and rhizosphere [20], so the response of soybean plants needs to be observed to reveal the extent of the effect of activity between the two types of effective fungi on soybean plant life. To what extent the possible interactions arising from such conditions need to be studied further, especially in terms of their effects on plant growth and production.

This study aims to determine the response of growth and production of soybean plants that have been inoculated with root nodule bacteria to the application of *Trichoderma esperellum* and endomycorrhizal *Glomus* spp. as biological fertilizers.

2 Method

2.1 Research preparation and implementation

This study used isolates of Trichoderma asperellum Tkd-Sd-01 and endomycorrhizal fungi, Glomus spp., which are a consortium of three isolates. The bacteria Rhizobium sp. isolated from root nodules of Mimosa pudica on agricultural land in Jiken (Tulangan, Sidoarjo). *Glomus* spp. was also obtained from the same location as the origin of *Rhizobium* sp. isolates. As for *Trichoderma* sp. obtained from Ngembat village, Jatirejo District, Mojokerto Regency, East Java Province.

Trichoderma fungal isolates were propagated in PDA-chloramphenicol media. The isolate cultures were harvested after 14 days and made into a distillate water-soluble suspension with a spore population density of 10^8 CFU.ml^{-1}. Glomus spp. are propagated by growing them on maize plants harvested after 8 weeks of planting. Retrieval of endomycorrhizal spores from the soil of the dismantled corn planting medium is modified in several ways [21], including: (i) mixing 2 g of soil sample into distilled water and stirring it for 30 seconds, then poured into a filter with a level of 250 µm and 50 µm with continuous flow of water, (ii) pouring the particles that are held in the 50 µm filter into a beaker and given a sucrose solution and stirring evenly, (iii) the suspension is put into a vial and then in centrifugation at 1000 rpm for 5 minutes, pouring out the liquid and its sediment containing

endomycorrhizal spores. The next step is to count the number of spores observed under a binocular microscope at a magnification of 100 times. Furthermore, the spore requirements were determined according to the treatment. All microbial preparation, propagation, and inoculation activities, as well as experiments were carried out at the Microbiology and Biotechnology Laboratory and the UMSIDA greenhouse in February-May 2022.

Pure isolates of Rhizobium sp. cultured for seven days on NA medium. For the purpose of experimental application in plants, these two biological agents' fungi are formulated in sterile husk powder as carrier agents. *T. asperellum* and *Glomus* sp. formulated, and each had a spore density of 10^8 CFU.g^{-1} and 200 spores 100 g^{-1}. The population density of Rhizobium sp. in the suspension was 10^{10} CFU.ml^{-1}.

The soil prepared was from the same soil where the Rhizobium bacteria used in this experiment were isolated. The soil texture is dusty clay with 0.56% organic C content, C to N ratio 14, cation exchange capacity 29.64 me/100 gram of soil, and pH (H_2O) 7.05. Before being placed into polybags, the soil was freed from microbes through sterilization using autoclave (120^o C, 1 atm, 30 minutes). Six hours before planting, all the prepared soil was inoculated with *Rhizobium* sp. by spraying the suspension evenly so that it can be ascertained that the average number of bacterial cells is 10^9 CFU.g^{-1}. Husk compost containing *Trichoderma* and *endomycorrhizal* propagules was given at a dose of 200 g per plant and ensured that it was evenly mixed. Thus, the polybag that already contained the growing media contained spores of *T. esperellum* and *Glomus* spp. respectively 10^7 CFU.g^{-1} and 400 per polybag.

The black soybean seeds of the Dena-1 variety used in this study, were soaked in a 50% alcohol solution for 3 seconds to kill microbes on the surface of the seeds. After rinsing with distilled water 3 times and drained, the seeds were put in a container containing Rhizobium bacterial spores formulated in rice husk flour and stirred evenly so that the entire surface of the seeds was covered by bacterial propagules and incubated for six hours. Furthermore, as many as three seeds were placed on the surface of the polybag where the planting medium was 5 cm thick from the surface which already contained a mixture of sterile soil and endomycorrhizae that had been formulated as biological fertilizer. The condition of the media in the polybag is always kept moist so that the germination process takes place properly. One week after laying the seeds, it was determined that the best sprouts were to be maintained until the end of the experiment. After that, one week later, the Trichoderma which has been formulated as a biofertilizer is carefully put into polybags until it is evenly distributed around the roots of young plants.

2.2 Experimental design and statistical analysis

The treatments in this experiment consisted of no application of biological agents or only 200 g of compost per polybag, application of Trichoderma fungus, application of endomycorrhizal fungi, and application of Trichoderma and endomycorrhizal fungi formulated in compost. husk 200 gr. Each treatment was repeated five times and all of them were arranged in a Completely Randomized Design (CRD). In this experiment, it was observed: plant height and stem diameter at the end of the vegetative phase (cm), dry weight of the plant stove at harvest, number of pods per plant, number of seeds per plant, weight of seeds per plant, and weight of 100 seeds. The data obtained were analyzed using analysis of variance followed by the HSD test at the 5% level. Then the mean of each treatment was compared with the mean of treatment without both biological agents (control) which was symbolized as Δx with a percentage value of (+) and a value of (-) which respectively meant an increase and a decrease compared to the control.

3 Results and discussion

3.1 Plant growth

Black soybean plant Dena-1 variety gave a different response ($p<0.05$) in the form of plant height at the end of its vegetative growth to the application of effective fungi. The average plant height and percentage Δx (%) (Table 1) show the different plant responses.

Table 1. The mean height of Dena-1 soybean.

Effective fungal application treatment	Plant height (cm)	Δx (%)
Without effective fungi (control)	160.8 b	-
Trichoderma	176.8 a	9.95
Glomus spp	159.6 b	-0.75
Trichoderma and *Glomus* spp.	165.2 b	2.74

Numbers followed by different letters show different effects based on the HSD test at the 5% level, Δx is the percentage increase or decrease (-) against the control

The response of soybean plants in the form of stem diameter at the end of the vegetative phase to the application of effective fungi was significantly different ($p<0.05$). The average stem diameter and the value of Δx (%) are presented in Table 2.

Table 2. The mean of stem diameter of soybean varieties Dena-1.

Effective fungal application treatment	stem diameter (mm)	Δx (%)
Without effective fungal (control)	22.00 a	-
Trichoderma	19.80 b	-10.00
Glomus spp.	21.80 a	-0.91
Trichoderma and *Glomus* spp.	21.20 a	-3.64

Numbers followed by different letters show different effects based on the HSD test at the 5% level, Δx is the percentage increase or decrease (-) against the control

Effective function application elicited soybean plant responses in terms of different dry weight of the stover ($p<0.05$). The dry weight and Δx (%) (Table 3) show the difference in the mean.

Table 3. The mean of dry weight of Dena-1 soybean stover at harvest.

Effective fungal application treatment	Stover dry weight (g)	Δx (%)
Without effective fungal (control)	3.14 a	-
Trichoderma	3.12 a	-0.51
Glomus spp.	4.32 a	37.58
Trichoderma and *Glomus* spp.	2.66 b	-15.41

Numbers followed by different letters show different effects based on the HSD test at the 5% level, Δx is the percentage increase or decrease (-) against the control

Soybean is responsive to light [22], However, in this experiment, Dena-1 soybean variety was shown to be tolerant of low light intensity. This is shown by its intrinsic ability to symbiosis with *Rhizobium sp.* in optimizing existing resources. This is shown by being manifested in high growth as a representation of its ability to produce optimal sugar, which is very important for the formation and growth of young tissues [23], so that the vegetative growth phase of plants can be well exceeded [24-25].

The response of soybean plants to the activity of *Rhizobium* sp., *Trichoderma*, *Glomus* spp., and the combination of the two fungi in terms of vegetative growth showed differences (Tables 1 and 2). The plant height given by *Trichoderma* sp was higher than the other treatments. Conversely, in terms of stem diameter, it shows the smallest size in *Trichoderma* treatment compared to other treatments.

The activity of *Trichoderma* utilizes organics as a substrate for its activity [26-27] to produce various secondary metabolites including compounds that act as plant growth regulators such as auxins [28-29] which absorbed by plants to spur growth in height. The role of endomycorrhizae in its activity is to help plants absorb nutrients and the bacteria *Rhizobium* sp. which provides amino acid precursors for the formation of proteins that are useful for plant growth; in this experiment was shown in particular in terms of stem diameter and dry weight of the stover.

3.2 Yield

The response of soybean plants to the application of effective fungi in terms of the number of pods per plant showed a significant difference ($p < 0.05$) with the average and Δx (%) presented in Table 4.

Table 4. The mean number of pods of Dena-1 soybean

Effective fungal application treatment	Number of pods per plant	Δx (%)
Without effective fungal (control)	16.00 a	-
Trichoderma	15.00 a	-6.25
Glomus spp.	16.20 a	1.25
Trichoderma and *Glomus* spp.	12.40 b	-22.50

Numbers followed by different letters show different effects based on the HSD test at the 5% level, Δx is the percentage increase or decrease (-) against the control

Application of biological agent fungi significantly affected the number of seeds ($p<0.05$). Table 5 shows the mean number of seeds per plant and Δx (%).

Table 5. The mean number of grains per plant of Dena-1 soybean.

Effective fungal application treatment	Number of grains per plant	Δx (%)
Without effective fungal (control)	29.80 a	-
Trichoderma	27.20 a	-8.72
Glomus spp.	28.40 a	-4.70
Trichoderma and *Glomus* spp.	20.60 b	-30.87

Numbers followed by different letters show different effects based on the HSD test at the 5% level, Δx is the percentage increase or decrease (-) against the control

The response of plants in the form of seed weight to the different treatments showed a significant difference ($p<0.05$). The average seed weight per plant for each treatment and the percentage Δx is shown in Table 6.

Table 6. The mean seed weight per plant of Dena-1 soybean.

Effective fungal application treatment	seed weight per plant (g)	Δx (%)
Without effective fungal (control)	1.85 a	-
Trichoderma	1.39 a	-25.08
Glomus spp.	1.21 a	-34.59
Trichoderma and *Glomus* spp.	0.56 b	-69.95

Numbers followed by different letters show different effects based on the HSD test at the 5% level, Δx is the percentage increase or decrease (-) against the control

The response of Dena-1 soybean varieties to the application of effective fungi in the form of weight of 100 seeds was significantly different ($p<0.05$). Meanwhile, the mean weight of 100 pieces per treatment and Δx (%) can be seen in Table 7.

Table 7. The mean weight of 100 grains of Dena-1 soybean

Effective fungal application treatment	Weights 100 grains (g)	Δx (%)
Without effective fungal (control)	5.61 a	-
Trichoderma	4.93 a	-12.15
Glomus spp.	4.56 a	-18.74
Trichoderma and *Glomus* spp.	2.86 b	-49.03

Numbers followed by different letters show different effects based on the HSD test at the 5% level, Δx is the percentage increase or decrease (-) against the control

The application of the fungus Glomus did not significantly affect all variables observed in production, but like the dry weight of the stover (Table 3), this fungus had an effect on increasing the number of pods per plant (Table 5). This is also a form of response to the contribution of Glomus spp. performance [30]. Endomycorrhizal fungi help provide nutrients so that they can increase plant growth and plant production [31-32]. Giving Glomus spp. can increase the dry weight of the stover by 37.58% against the control. In control plants, root nodule bacteria have no competitors in the rhizosphere which allows them to show their ability to produce compounds that can be utilized by plants to stimulate their growth [33]. The response of Dena-1 soybean varieties in all aspects of their vegetative growth proves that there is a synergistic effect of the interaction of Trichoderma and *Rhizobium* sp. In its activity it produces various extracellular compounds that can induce an increase in the activity of beneficial fungi and bacteria in the rhizosphere [34-35] which in this experiment can support the life of *Rhizobium* sp. which is inoculated. The chitinase enzyme produced by Trichoderma [36-37] usually exerts a suppressive effect and disrupts the stability of the cell wall of pathogenic fungi [38-39]. However, the joint application between the fungi *Trichoderma* and *Glomus* spp. in fact, it does not create synergy between the two. As shown in Table 3-7, the treatment of Trichoderma and Glomus spp. shows all the lowest crop production variables. The allegation of space competition can be proven by the lack of external hyphae of mycorrhizal fungi that stick out from the roots of plants [40]. The loss of the external hyphae structure outside the root cells is also thought to be an internal mechanism of the fungus in increasing the efficiency of respiration, considering that hyphal respiration outside the root is higher than inside the cell, even higher than the respiration rate of fine roots [41-42]. Thus, there has been competition between the indigenous root nodule bacteria and the two fungi in the roots and rhizosphere which reduces the role of the two fungi as biological agents for soybean varieties of Dena-1.

4 Conclusion

The application of *Trichoderma* and Glomus spp. on soybean varieties of Dena-1 that had been inoculated with *Rhizobium* indigenous bacteria had no effect on increasing the dry weight of the stover, the number of pods, the number of grains, the grain weight, and the weight of 100 grains. The application of these two biological agents turned out to cause a decrease in dry weight of plant stover, number of pods, number of grains, grain weight, and weight of 100 soybeans of Dena-1 variety, respectively 15.41%, 22.50%, 30.87%, 69.95%, and 49.03% compared with plants without biological agents. This fact indicates that there is competition in utilizing resources between each biological agent fungus and this indigenous root nodule bacteria.

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