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# Utilization of *trichoderma* sp. and *pseudomonas fluorescens* as biofertilizer in shade-resistant soybean

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**Abstract**. This study aims to obtain *Trichoderma* fungi and *Pseudomonas fluorescens* bacteria obtained from soybean planting fields and test their ability as biofertilizer agents to assist soybean growth until the end of the vegetative phase. From the soybean crop field, *Trichoderma* fungi and *P. fluorescens* (Pf) were isolated. After purification, observations were made to determine morphological physical characteristics both macroscopically and microscopically. Pf bacterial isolates were tested for their performance characteristics to ensure that bacteria were not pathogenic. For work power testing, as a biofertilizer agent, a factorial experiment was prepared in a completely randomized design (CRD). As the first factor, *Trichoderma* consists of without and with *Trichoderma*, while the second factor is Pf bacteria consisting of without and with Pf. The experiment was repeated four times so that 16 experimental units were obtained. The variables observed were plant height, number of leaves, stem diameter, and time of initial average flower appearance. Data were analyzed by ANOVA followed by HSD testing on a 5% test tarf. The results showed that the fungi *Trichoderma* sp. obtained by having single-celled, smooth-walled conidia measuring 3.2 x 2.0 $\mu$m, green with irregular upright hyaline conidiophores. As for P. fluorensence does not cause foul symptoms in test potatoes which are non-pathogenic. Fungi *Trichoderma* sp. TC-Jro-02 isolates and *Pseudomonas fluorescens* bacteria which were applied as biofertilizer agents were each increasing and inhibiting growth in plant height, leaf number, stover dry weight, and dry weight of soybean root of Dena varieties grown in shade up to 30 days after planting. Interactions between them affect the dry weight of stover and the dry weight of plant roots. The bacterial activity of *Pseudomonas fluorescens* inhibits the effect of the performance of *Trichoderma* fungi in supporting the growth of soybean plants in the shade.

## 1. Introduction

Efforts to increase the production of Indonesian soybeans in order to be able to be independent in fulfilling national needs are carried out not only to increase the genetic capabilities of varieties and cultivation techniques, but also to use dry land that is still widely available. The use of land which is as large as the lower vegetation strata through shading has begun to be tricked by researchers by producing shade resistant varieties. However, the performance of varieties that have been proven to be shade-resistant remains prone to environmental stress due to the lack of soil organic matter and the weak role of the supporting microflora of plant growth.

*Trichoderma* fungi and *Pseudomonas fluorenses* bacteria which are soil borne microorganisms have the potential to be used to help plant growth on dry land. *Trichoderma*, besides having a role in protecting plants from pathogenic disorders in rhizofer [1], has the ability as a biofertilizer agent because of some of its superior characteristics, namely degrading organic matter to produce nutrients

and produce growth regulating compounds for plants [2-4]. *P.fluorescens* bacteria is one type of bacteria that has the ability to produce growth regulating compounds such as auxin [5], producing enzymes that are capable of working in the P-organic mineralization process into P-inorgnaik which is available to plants [6]. Both types of microbes can be used together to help the growth of vegetative plants and maintain plant health so that plants are able to produce well [7-8].

On dry land with limited production resources such as water, nutrition, light intensity that is often very low, good vegetative growth greatly determines the success of growing plants until the production phase. Since sprouts to the final vefetative phase of soybean plants require environmental support both biotic and abiotic. The existence and good performance of microbes in dry land rhizosphere can support plant growth to pass the critical phase at the beginning of growth,

In addition, a test is needed that can ensure that between the two potential microbes the biofertilizer biological agents can synergize with soybean plants, especially in shade resistant varieats. The success of this test can be input into the development of a biofertlizer application technology package on soybean plants on land, especially those under the auspices of the upper strata vegetation canopy such as plantations and plantations.

The aim of this study was to obtain *Trichoderma* fungi and *P. fluorescens* bacteria which were obtained from soybean planting fields and tested their ability as biofertilizer agents to help grow soybean plants until the end of the vegetative phase.

## 2. Experimental method

### *2.1. Biofertlizer agent*

To get a microbial biofertilizer agent for soybean plants, the location of soil sampling in an area that has a history is often used for planting soybeans with a satisfying appearance of growth and production. The activity of isolating the biofertilizer microbial agent was carried out in the Microbiology laboratory, followed by application testing in the green house of the Universitas Muhammadiyah Sidoarjo from January to May 2019.

Soil samples from the land commonly planted with soybeans were used to obtain *Trichoderma* isolates and *P. fluorescens* (Pf) bacteria. The land is in Ngembat Village, Gondang District, Mojokerto Regency with a lower vegetation strata which is partially covered by pine canopy with an intensity of canopy cover equivalent to 50-80%. The suspension produced from mixing 5 grams of soil that has been diluted in 1,000 ml of distillate water is sprayed into a medium of Potato Dextrose Agar-chloramphenicol (PDA-c) in a petri dish. This PDA-c media is made of 200 g of potatoes extracted through heating in a one liter volume which has been mixed with 20 g of dextrose and 20 g of agar. After 90 hours the incubation period appears various fungal colonies. Carefully the colonies that show the characteristics of the Trichodema colony are touched with the tip of the needle and transferred to the new media. Then after 7 days the incubation period of the *Trichoderma* isolate colonies can be observed. Condiospora is observed under a microscope and the determination of the *Trichoderma* type refers to Gams and Bissett (2002) [9].

The soil suspension from soybean cropping land (in Katerungan Village, Krian District, Sidoarjo Regency, East Java) resulted in a dilution of $10^3$ ml of 1 ml sprayed onto the surface of Kelman's media. This medium is a mixture of 10 g peptone, 10 g glucose, 1 g casamino acid, and 20 g agar white bar dissolved in distilled water to a volume of 1000 ml which is stirred until the mixture appears homogeneous. Before being used as a bacterial growth medium, the Kelman media was sterilized in an autoclave for 0.5 hours at 121 ºC, 1 atm. After an incubation period of 36 hours of colony that corresponds to the characteristics of Pf, using the tip of the ose needle touched by the center of the colony that appears and is scratched onto the surface of the new media, all activities are carried out in aseptic conditions. The next stage is to ensure that the bacterial colonies that have been isolated do not have pathogenic characters, namely by inoculating them aseptically on sterile potato pieces by soaking them in 50% alcohol for 5 seconds and rinsing them in distlat water 3 times. Non-pathogenic bacteria will not show symptoms of decay in the potato pieces after 72 hours of incubation.

### 2.2. *Biofertilizer application test*

For the manufacture of biofertilizers, *Trichoderma* and Pf bacteria as a result of this isolation must be formulated with sterile compost as a carrier agent. Each mature culture is harvested and mixed with sterile compost until evenly distributed and incubated for 14 days. Thus obtained two kinds of biological fertilizers. Before each biofertilizer was used, the contents of conidiospora (for *Trichoderma*) and bacterial cells were examined in each gram of biofertilizer. The examination was carried out using a dilution method to obtain the conidiospora *Trichoderma* content of $10^7$ cfu/g, while the content of Pf bacterial cells was $10^9$ cfu/g.

Sterile soil that is used as a planting medium is placed on a polybag with a capacity of five kg. In accordance with the treatment designed, the treatment of *Trichoderma* biofertilizer application and application of biofertilizer Pf were each given at a dose of 200 g per polybag. Thus the average content of conidiospora *Trichoderma* and Pf bacterial cells in the soil growing media reached 4 x $10^5$ and 4 x $10^7$ cfu/g, respectively. Furthermore, the soybean seeds of Dena varieties are planted into each polybag. The position of polybag that has been planted with soybeans is arranged randomly and placed under a paranet shade of 60% or with the intensity of sunlight entering 40%.

This factorial experiment was arranged in a completely randomized design (CRD) with the first factor *Trichoderma* biofertilizer consisting of two levels, namely without *Trichoderma* and with *Trichoderma*, while the second factor was biological fertilizer *P. fluorescens* (Pf) consisting without Pf and with Pf; the experiment was repeated four times. The variables observed in this experiment were plant height, number of leaves, dry weight of plant stover, and dry weight of plant roots at the end of the vegetative growth phase. All data were analyzed using variance analysis (ANOVA) at the level of 5% and 1% to determine the effect of each treatment factor and its interactions. Regarding the observation variable which shows the real effect, then the Honest Real Difference (HSD) test is carried out at the level of 5% to determine the difference between treatments.

## 3. Results and discussion

### 3.1. *Bioferilizer agent*

The results of the isolation of *Trichoderma* fungi from the soil from the land which is usually shaded by pine canopy showed *Trichoderma* sp isolates with the appearance of colonies and microscopic structures as shown in Figure 1.

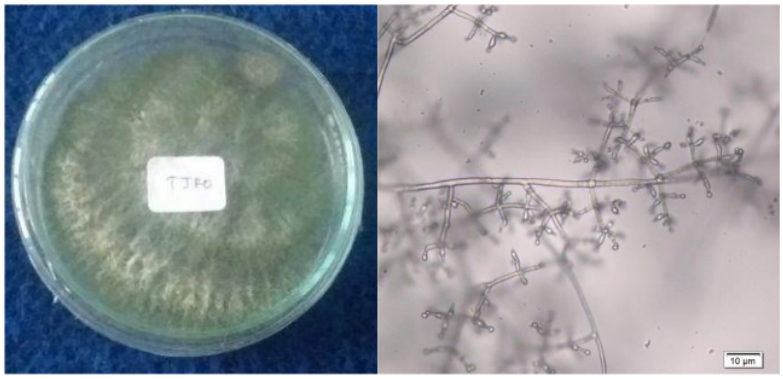


**Figure 1**. Appearance of colonies (left) and microscopic structures (right) *Trichoderma* isolates Tc-Jro-02

The fungi colony on PDA-c media was originally woven with white hyphae which was immediately followed by the presence of green which was the result of thickening of the ends of

hyphae which thickened to hyidiic conidiophores, erect irregular, smooth walls. Single-celled conidia, smooth-walled, and measuring 3.2 x 2.0 $\mu$m. When referring to the description shown by Gams and Bissett (2002) [9], the microscopic appearance of this isolate is similar to *T, koningii*. However, the final results of determination should be carried out using molecular markers. For practical purposes in the application experiment, the findings of this finding are referred to as *Trichoderma* sp. Tc-Jro-02 isolate, a code of isolates which later became the collection of the Agricultural Microbiology Laboratory of Muhammadiyah University, Sidoarjo.

The observation of colonies of *P. fluorescens* isolates showed that gram negative bacteria had transparent pink colored colonies. The color formed is the result of pigment production by *P. fluorescens* [10]. Curved or straight stem-shaped cells measuring 0.5-1.0 x 1.5-5.0 µm. The results of testing the inoculation on the surface of fresh potato pieces show no discoloration that indicates the decay process. This shows that *P. fluorescens* isolates are non-pathogenic bacteria and are suitable for use as biofertilizer agents. For practical purposes for application experiments, these bacteria are referred to as *P. fluorescens* Pf-Kat-01 isolates and later become a collection of Microbiology laboratories at the Universitas Muhammadiyah Sidoarjo.

### *3.2. Biofertilizer application test*

The results of variance analysis on plant height data showed no interaction effect between the application of *Trichoderma* and the application of *P. fluorescens* to the height of soybean plants of Dena varieties at the end of the vegetative phase, but each factor had a significant effect ($p < 0.01$). The mean height of soybean plants is shown in Table 1. The *Trichoderma* application increased the growth of plant height by 13.95%, but the application of *P. fluorescens* was 13.09% lower than without *P. fluorescens*.

**Table 1**. The mean effect of the application of *Trichoderma* and *P. fluorescens* on the height of soybean plants of Dena variety 30 days after planting (DAP) (cm) [*)]

| *Trichoderma* application | Average plant height (cm) | Increase (decrease) (%) | *P. fluorescens application* | Average plant height (cm) | Increase (decrease) (%) |
|---|---|---|---|---|---|
| Without *Trichoderma* | 60.50 a | - | Without *P. fluorescens* | 69.25 a | - |
| *Trichoderma* | 68.94 a | 13,95 | *P. fluorescens* | 60.19 b | 13.09 (-) |
| HSD 5% | 7.13 | | HSD 5% | 7.13 | |

[*)]The numbers followed by the same letter in the same column show no different based on the 5% HSD test

The results of the variance analysis on observational data on the number of leaves of soybean plants in Dena variety showed that the application of *Trichoderma* and the application of *P. fluorescens* had a very significant effect ($p < 0.01$) on the leaf leaves at the end of the vegetative phase, but the interaction was not significant ($p > 0, 05$). The average number of leaves of soybean plants is shown in Table 2. The *Trichoderma* application increased the growth of plant leaves by 11.68%, but in the application of *P. fluorescens* the number of plant leaves was 13.09% lower than without *P. fluorescens*.

**Table 2**. The mean effect of the application of *Trichoderma* and *P. fluorescens* on the number of leaves per soybean plant of Dena varieties 30 DAP*)

| *Trichoderma* application | number of leaves | Increase (decrease) (%) | *P. fluorescens application* | number of leaves | Increase (decrease) (%) |
|---|---|---|---|---|---|
| Without *Trichoderma* | 17.13 b | - | Without *P. fluorescens* | 16.38 b | - |
| *Trichoderma* | 19.13 a | 11,68 | *P. fluorescens* | 19.88 a | 21.37 (-) |
| HSD 5% | 1.69 | | HSD 5% | 1.69 | |

*) The numbers followed by the same letter in the same column show no different based on the 5% HSD test

The application of *Trichoderma* and the application of *P. fluorescens* each had a very significant effect on the dry weight of soybean stover of Dena varieties at the end of the vegetative phase ($p < 0.01$), as well as the interaction was very significant. The average dry weight of soybean stover in response to the combination of treatments is shown in Table 3. Rearata effect of the application of *Trichoderma* and *P. fluorescens* are shown in Table 4. *Trichoderma* application produced 36.80% higher dry stover weight than without *Trichoderma*, on the contrary the application of *P. fluorescens* was 45.058% lower than without *P. fluorescens*.

**Table 3**. The mean interaction effect of the application of *Trichoderma* and *P. fluorescens* on the dry weight of soybean stover of Dena 30 varieties DAP (g) *)

| | *Trichoderma* application | | HSD 5% |
|---|---|---|---|
| *P. fluorescens* application | Without *Trichoderma* | *Trichoderma* | |
| Without *P. fluorescens* | 1.82 a<br>B | 3,05 a<br>A | 0,35 |
| *P. fluorescens* | 1.36 b<br>A | 1,29 b<br>A | |
| HSD 5% | 0,35 | | |

*) The numbers followed by the same lowercase letter in the same column and followed by the same capital letters on the same line show no different based on the 5% HSD test

**Table 4**. The mean effect of the application of *Trichoderma* and *P. fluorescens* on the dry weight of soybean stover of Dena varieties 30 DAP (g) *)

| *Trichoderma* application | stover dry weight (g) | Increase (decrease) (%) | *P. fluorescens application* | stover dry weight (g) | Increase (decrease) (%) |
|---|---|---|---|---|---|
| Without *Trichoderma* | 1.59 b | - | Without *P. fluorescens* | 2.43 b | - |
| *Trichoderma* | 2.17 a | 36.80 | *P. fluorescens* | 1.3 a | 45.58 (-) |
| HSD 5% | 1.69 | | HSD 5% | 1.69 | |

*) The numbers followed by the same letter in the same column show no different based on the 5% HSD test

The application of *Trichoderma* and the application of *P. fluorescens* each had a very significant effect on the dry weight of soybean root Dena varieties at the end of the vegetative phase ($p < 0.01$), while the interaction between the two had a significant effect ($p < 0.05$). The mean interaction effects on the dry weight of soybean root as shown in Table 5. The average effect of the application of *Trichoderma* and *P. fluorescens* is shown in Table 6. *Trichoderma* application produced 107.98% root

dry weight higher than without *Trichoderma*, while the application of *P. fluorescens* 59.38% lower than without *P. fluorescens*.

**Table 5**. The average effect of the interaction of the application of *Trichoderma* and *P. fluorescens* on the dry weight of soybean roots of Dena varieties 30 DAP (g) *)

| | *Trichoderma* application | | HSD 5% |
|---|---|---|---|
| *P. fluorescens* application | Without *Trichoderma* | *Trichoderma* | |
| Without *P. fluorescens* | 0.48 a<br>B | 0.96 a<br>A | 0,17 |
| *P. fluorescens* | 0.18 b<br>B | 0.41 b<br>A | |
| HSD 5% | 0,17 | | |

*)The numbers followed by the same lowercase letter in the same column and followed by the same capital letters on the same line show no different based on the 5% HSD test

**Table 6**. The mean effect of the application of *Trichoderma* and *P. fluorescens* on the dry weight of soybean roots of Dena varieties 30 DAP (g) *)

| *Trichoderma* application | dry weight of soybean roots (g) | Increase (decrease) (%) | *P. fluorescens application* | dry weight of soybean roots (g) | Increase (decrease) (%) |
|---|---|---|---|---|---|
| Without *Trichoderma* | 0.33 b | - | Without *P. fluorescens* | 0.72 a | - |
| *Trichoderma* | 0.68 a | 107.98 | *P. fluorescens* | 0.29 b | 59.38 (-) |
| HSD 5% | 0.17 | | HSD 5% | 1.69 | |

*) The numbers followed by the same letter in the same column show no different based on the 5% HSD test

The application of *Trichoderma* and *P. fluorescens* produced an interaction effect on dry weight of stover and root dry weight. This shows that the two biological agents show their role as biofertilizers. The role of both has not given the effect of interactions on plant height and number of leaves until the end of the vegetative phase of the plant,

*Trichoderma* produces several enzymes [11] which degradate organic matter to produce nutrients and produce compounds that play a role in promoting plant growth [12]. *Trichoderma* singly increased plant height (Table 1), number of leaves (Table 2), stover dry weight (Table 4) and root dry weight (Table 6), even under shade conditions; the variety used in this experiment proved to be able to grow well at a shade intensity of 60-80% [13, 14].

*P. fluorescens* is a bacterium that is capable of producing compounds that can facilitate the process of phosphate release in the soil [15]. This bacterium also produces metabolites which act as regulators of plant growth [16]. In this trial, until the vegetative final phase, it appears that bacteria behave as users of the resources produced by *Trichoderma* activity. As shown in Tables 1 to 6, it appears that all growth variables show lower values in the application of these bacteria compared to without the application of *P. fluorescens* bacteria as biofertilizers. Thus in this experiment the activity of *P. fluorescens* bacteria did not provide benefits for plant growth. In the first 8 weeks vermicomposting process, *P. fluorescens* showed a significant decrease in alkaline phosphatase activity [17].

Based on the facts mentioned above, it appears that the rhizosphere of the soybean varieties of Dena varieties that hold this shade does not provide compatibility for the life and activity of the bacteria *P. fluorescens*, on the other hand it is suitable for the activity of *Trichoderma* fungi. The activity of this fungi in the advanced composting process will produce dissolved compounds that

benefit other soil microorganisms [18, 19]. The presence of *P. fluorescens* in soybean rhizosphere in this experiment has suppressed the effect of *Trichoderma* fungi.

## 4. Conclusion

Application of *Trichoderma* sp. Fungi TC-Jro-02 isolates and application of *Pseudomonas fluorescens* bacteria isolate Pf-Kat-01 as biofertilizer agents had a very significant effect on plant height, leaf number, stover dry weight, and root dry weight, while interactions between them affected the dry weight of stover and root dry weight with soybean varieties Dena. The bacterial activity of *Pseudomonas fluorescens* inhibits the effect of the performance of *Trichoderma* fungi in supporting the growth of soybean plants grown in the shade.

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