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Growth response of red chilli plants to flowering phase against the application of *Trichoderma* and *Pseudomonas fluorescens* and P fertilizers

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Abstract. This study aims to determine the effect of the application of *Trichoderma sp*. Fungi. and *Pseudomonas fluorescens* bacteria as biofertilizer agents and P fertilizer doses as well as interactions between microbial biofertilizer agents and P fertilizer doses on vegetative growth and early appearance of red chilli flower flowers to the initial flowering period. This greenhouse experiment was arranged factorially in a completely randomized design. The first factor was the application of microbial biofertilizer agents consisting of no microbes, Trichoderma sp., And P. fluorescens, while the second factor was the dosage of P fertilizer per 5,000 g of soil planting medium consisting of 0, 0.5, and 1.0 g SP-36. Nine combinations of treatments repeated three times to obtain 27 experimental units. The variables observed were plant height, stem diameter, leaf area, wet weight and stover dry weight at 42 days after planting (DAP) and the beginning of flower emergence. The data were analyzed by 5% ANOVA followed by a 5% HSD Test to determine the difference in influence between treatments. The results showed a very significant interaction effect between the application of microbial agents and the dose of phosphate fertilizer to height, stem diameter, leaf area, wet weight and dry weight of stover and the initial flowering time at 42 DAP. The application of P. fluorescens bacteria as a biofertilizer agent combined with 0.5 g SP-36 fertilizer dose per 5,000 g of planting media showed the highest growth effect of red chili plants at age 42 DAP in terms of plant height, stem diameter, leaf area, stover wet weight, stover dry weight, and earlier flower appearance.

1. Introduction

Red chili is a strategic one that often causes social compilation turmoil that occurs between the demand and supply imbalances. The development of chilli plantations on dry land requires efficient cultivation applications and is based on the utilization of local resources. However, the physiology of chili plants is highly dependent on soil fertility, therefore massive fertilization is carried out on each tanana chilli cultivation [1].

The facts on the ground show that the seedling phase until the plant enters the flowering phase is a period prone to plants in the face of environmental stress both biotic and abiotic. Drought greatly affects the ability to produce phenolic compounds, morphological responses, and gene expression of chilli plants [2]. Manipulation of plant rhizosphere environment to be dominated by effective microbes is expected to help plant health and resistance to environmental stress.

The use of effective microbial microbes as biofertilizer agents for the cultivation of local chilli plants or utilized as exotic effective microbes in chilli plantations in various areas far from the habitat of the intended microbial origin is a challenge that must be answered by researchers and practitioners of plant cultivation.

Trichoderma and *Psuedomonas fluorenses* are effective microbes which have been isolated from various locations, collected, and developed as biofertilizer agents because of their various abilities to help plant growth and help improve soil fertility physically by improving soil aggregates [3].

Trichoderma is a fungus that is capable of producing secondary metabolites which act as regulators of plant growth for young sprouts and plants [4, 5]. These fungi also produce various enzymes that work extracellularly [6] degrade organic matter that produces nutrients for plants [7]. Bacteria *P. fluorenses* play a role in increasing plant growth and production as well as increasing available P in the soil while reducing the use of phosphate fertilizers [8].

There are many dry land lands in Indonesia that experience degradation in fertility which among them is characterized by low organic matter (<2%) and low P available [9]. *P. fluorescens* bacteria have one role, which is able to produce acid phosphatase enzymes that can facilitate the release of P so that it will increase availability for plants [10].

Giving organic in fertilizer is no guarantee for the restoration of the status of soil organic matter that can sustain plant life. Enriching organic fertilizers with effective microbes is one of the efforts to improve soil fertility both chemically, physically and biologically. The *Trichoderma* formulation in organic fertilizers not only increases the supply of nutrients as a consequence of degradation of organic matter, but also encourages other microbial activities including various types of bacteria that benefit plants while inhibiting pathogenic microorganisms and other harmful ones [11].

On the other hand, the low availability of P requires the provision of inorganic P fertilizer which is ready to be absorbed by plants for optimal crop production purposes. In large-scale plant production activities, P applications require a large additional cost; while the efficiency of production costs while increasing the capacity of indigenous resource support needs to be improved. The application of *Trichoderma* and the bacterium *P. fluorenses* is expected to be able to answer these challenges both applied individually or simultaneously as a form of fertilization.

This study aims to determine the effect of microbial biofertilizer agents especially *Trichoderma* and P. *fluorenses* as well as the application of organic fertilizers in "economical" and "optimal" ways as well as the possible interaction between the two treatment factors on the growth of red chillies until the beginning of flowering phase.

2. Experimental method

Psuedomonas fluorescens (Pf) bacteria used in this experiment were Pf-Kat-01 isolates and *Trichodema sp*. Fungi were Tc-Clkt-01 isolates which are collections of the Microbiology Laboratory of the Universitas Muhammadiyah Sidoarjo (UMSIDA). The experiment was conducted at UMSIDA green house in February-May 2019.

The initial stage is to prepare the seedlings for the red pepper variety Gada. Chilli seeds are placed in the seed tray which contains sterile soil and incubated for 20 days. After that the seeds are ready to be moved into the polybag capacity of 5,000 gr of planting media. On the other hand phosphate fertilizers are prepared, which in this experiment are divided into three types, namely 0, 0.5, and 1.0 g TSP, each of which is given as a mixture in 5,000 g of soil of the planting medium.

The main step is to multiply the microbial biofertilizer agents. To prepare Pf biofertilizer, we do a multiplication of Pf-Kat-01 isolates on Kelman's media with a composition of 10 g peptone, 10 g glucose, 1 g casamino acid, 20 g bar gelatin, and distillate water which is heated and stirred evenly with the total volume 1000 ml before sterilizing in the autoclave. We inoculated bacterial isolates by scraping inoculants using a needle on the surface of the petri dish containing Kelman's media. We harvested the culture after an incubation period of seven days, then crushed it with a blender and diluted it with distilled water up to 103 times, then the suspension was poured into a vessel containing sterile compost and stirred evenly. This bacterial biofertilizer mixture is then incubated for 10 days.

For *Trichoderma* fungi, we propagated using PDA-c media with an incubation period of 10 days and carried out in the same way as preparing a bacterial biofertilizer with the same incubation period.

Before applying as fertilizer, each biofertilizer was examined for spore content through a dilution method to obtain 10^7 cfu / g of conidiospora *Trichoderma* and 10^9 cfu / g of Pf bacterial cells. As a biofertilizer with a sterile compost carrier each prepared to be applied as fertilization at the beginning of planting with a dose of 200 g per polybag capacity of 5,000 g of planting media. The process of examining the biofertilizer propagul agent is carried out for a maximum of 5 days, so that it takes 15 days from the beginning of the incubation of the biofertilizer to be applied as fertilization. At that time the red chilli seeds were ready to be moved and the experiment was ready to be implemented.

The soil used as a planting medium in this experiment came from the village of Randegan, Sidoarjo Regency, East Java, which is known to have low P availability with the following chemical analysis results: pH (H_2O) 5.7, pH (KCl 1N) 5.4, C inorganic 0.64%, N-total 0.09, P (Bray 1) 6.29 mg.kg^{-1}, and CEC 34.02 me / 100 mg.

This factorial experiment was arranged in a completely randomized design with the first factor being the application of microbial biofertilizer agents consisting of: without microbes (M0), *Trichoderma sp*. (M1), and *P. fluorescens* (M2); as the second factor, the dosage of P fertilizer consisted of: 0, 0.5, and 1.0 g SP-36 per 5,000 gr of growing media. Repeat three times, to obtain 27 units of experiment. Variables observed were: plant height, stem diameter, leaf area, stover wet weight, and stover dry weight at 42 days after planting (DAP) and the time of flower appearance until the plant was 42 DAP. Data analysis was performed on each observation variable by using variance analysis (ANOVA) at the level of 5 and 1% followed by an Honest 5% Real Difference Test to determine the differences between treatments.

3. Results and discussion

The results of the variance analysis on plant height data showed that there was a very significant interaction effect (P <0.01) between the microbial application of biofertilizer agents and phosphate fertilizer doses at the end of the vegetative phase of red chilli plants (42 DAP). The mean plant height in response to the interaction of the two factors is shown in Table 1. Growth in plant height for all combinations of treatments from 7 DAPs to 42 DAPs is shown in Figure 1.

Table 1.The mean effect of the interaction of application of biofertilizer agents and fertilizer dosage P on plant height of the red chilli plant42 DAP (cm)*)

P fertilizer dosage per 5,000 g of planting media soil	Biofertilizer microorganism agents			HSD 5%
	Without m.o.	*Trichoderma*	*P. fluorescens*	
0 g	33,67 a A	33,50 ab A	36,17 b A	
0.5 g	31,33 a B	34,57 a AB	35,93 a A	2,70
1.0 g	32,50 a AB	30,17 b B	33,73 a A	
HSD 5%		2,70		

*)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

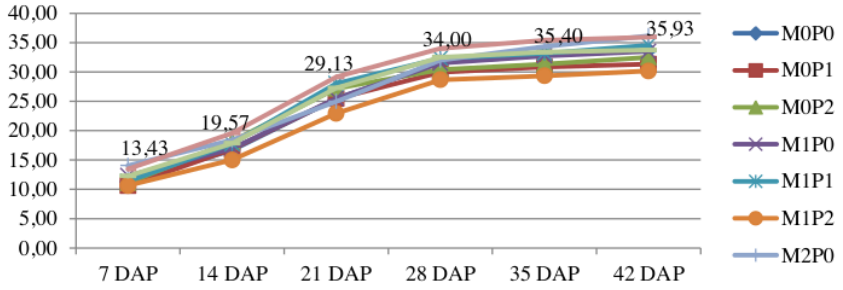

Figure 1. Graph of plant height growth in all treatment combinations 7-42 DAP (cm)

The microbial application of biofertilizer agents and phosphate fertilizer application each had a significant effect ($p < 0.05$) and was very significant ($p < 0.01$) on stem diameter, as well as the interaction of both had a very significant effect on the stem diameter of chili meram plants 0.01). The average stem diameter of the plant in response to the interaction of the two factors is shown in Table 2. Growth of plant stem diameters for all combinations of treatments from 7 DAPs to 42 DAPs is shown in Figure 2.

Table 2. The mean of the interaction effect of the application of biofertilizer agents and P fertilization doses on the stem diameter of the red chilli plant 42 DAP (mm)*)

P fertilizer dosage per 5,000 g of planting media soil	Biofertilizer microorganism agents			HSD 5%
	Without m.o.	*Trichoderma*	*P. fluorescens*	
0 g	1.57 b A	1.58 ab A	1,58 b A	
0.5 g	1.63 ab B	1.82 a A	1,85 a A	0.15
1.0 g	1.70 a A	1.55 b B	1,80 a A	
HSD 5%		0.15		

*)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

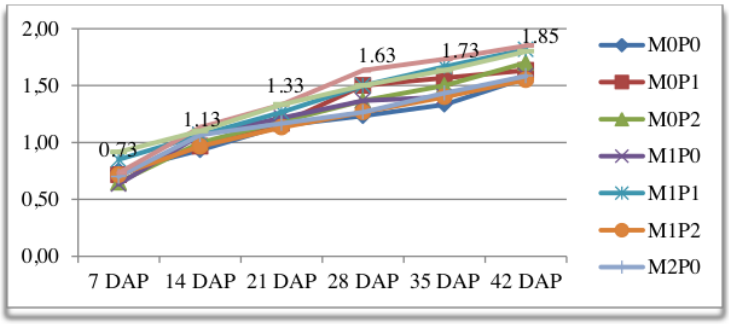

Figure 2. Growth chart of red chili plant stem diameter in all treatment combinations 7-42 DAP (mm)

The application of biofertilizer agents and phosphate fertilizer application had no significant effect (p > 0.05) but the interaction had a very significant effect (p <0.01) on the area and red chilli plants. The mean leaf area response to the interaction of application of biofertilizer agents and phosphate fertilizer application is shown in Table 3.

Table 3. The mean of the interaction effect of the application of biofertilizer agents and P fertilization

P fertilizer dosage per 5,000 g of planting media soil	Biofertilizer microorganism agents			HSD 5%
	Without m.o.	*Trichoderma*	*P. fluorescens*	
0 g	25.93 a A	25.20 a AB	22.93 b B	
0.5 g	25.53 a A	25.47 a A	29.13 a B	2.81
1.0 g	25.47 a AB	21.93 b A	28.00 a A	
HSD 5%		2.81		

doses on the leaf area of red chilli plants 42 DAP (cm^2)*)

*)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

The microbial application of biofertilizer agents, phosphate fertilizer application, and the interaction of both had a very significant effect (p <0.01) on the wet weight of red chili plant stover 42 DAP. The mean wet weight of the interaction effect is shown in Table 4.

Table 4. The mean interaction effect of application of biofertilizer agents and fertilizer dosage P on the wet weight of red chili plant stover 42 DAP (g)*)

P fertilizer dosage per 5,000 g of planting media soil	Biofertilizer microorganism agents			HSD 5%
	Without m.o.	*Trichoderma*	*P. fluorescens*	
0 g	29.67 a A	33.33 a A	34.57 b A	
0.5 g	23.67b B	33.83 a B	46.93 a A	4.14
1.0 g	28.17 a A	23.57 b B	35.33 b A	
HSD 5%		4.14		

*)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

The microbial application of biofertilizer agents has a very significant effect (p <0.01) but phosphate fertilizer application has no significant effect (p> 0.05), and both interactions have a very significant effect (p <0.01) on the dry weight of red chili plant stover 42 DAP. The mean dry weight of the interaction effect of the stover is shown in Table 5.

Table 5. The mean interaction effect of application of biofertilizer agents and fertilizer dosage P on the dry weight of red chili plant stover 42 DAP (g)*)

P fertilizer dosage per 5,000 g of planting media soil	Biofertilizer microorganism agents			HSD 5%
	Without m.o.	*Trichoderma*	*P. fluorescens*	
0 g	2.67 a A	2.46 a A	2.51 b A	
0.5 g	2.26 a A	2.57 a A	3.56 a B	0.45
1.0 g	2.68 a	2.31 a	3.26 a	

	A	A	A
HSD 5%		0.45	

*)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

The interaction between microbial application of biofertilizer agents and phosphate fertilizer application at the end of the vegetatif phase of red chilli plants (42 DAP) had a very significant effect (P <0.01) on the time of flower appearance. The application of microbes and phosphates as a single factor has no significant effect (p> 0.05) on the time of flower appearance. The average time for the appearance of red chilli plants in response to the interaction of the two factors is shown in Table 6.

Table 6. The mean of the interaction effect of the application of biofertilizer agents and P fertilizer dosage on the initial flowering time of red chilli plants up to 42 DAP (days)*)

P fertilizer dosage per 5,000 g of planting media soil	Biofertilizer microorganism agents			HSD 5%
	Without m.o.	*Trichoderma*	*P. fluorescens*	
0 g	35.2 ab	34.2 a	37.8 a	
	AB	A	A	
0.5 g	38.0 a	33.6 a	33.8 b	3.1
	A	B	B	
1.0 g	34.7 a	36.2 a	33.8 b	
	A	A	A	
HSD 5%		3.1		

*)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

Effective microbial application of biofertilizer agents and application of P fertilizers both in combination and in a very real effect on all observation variables except for each treatment factor did not significantly affect leaf area and the beginning of flower appearance.

Although leaf area does not show a difference in response to the application of different biofertilizer agents and application of P fertilizer, the various other growth variables show significant differences. This is given that leaves function as a means of producing energy sources for all metabolism which is more represented in the form of plant height, stem diameter, stover wet weight, stover dry weight, and the initial length of appearance.

The more growth of chilli plants is supported by the role of *Trichoderma* as a nutritional contributor to the degradation of organic matter[12], micronutrients and metabolites which act as growth regulator compounds for plants [13]. All donations from Trichoderma activity are needed for the process of plant growth and induction of flower appearance. The application of cucumber plants also shows the effect of very good growth [14]. The same was shown by *P. fluorescens* given its ability to produce various growth-regulating compounds for plants and produce acid phosphatase enzymes which can help provide P for plants, increasing wet weight and dry weight of plants[15].

Increasing the dose of P will increase plant growth. This very significant response is also supported by the results of soil analysis of the planting media used in this study, namely P available 6.29 mg.kg^{-1} including low category. However, the soil chemical status of the planting medium, especially the relatively high value of soil cation exchange capacity used in this experiment (34.02 me / 100 g) seems to support the normal growth of plants, especially in plant height and stem diameter. Inorganic P is absorbed by plants in the form of orthophosphate ($H_2PO_4^-$ and HPO_4^{2-} play an important role in metabolic processes such as photosynthesis, energy transfer, signal transduction [16] and various processes that can guarantee plant resistance to environmental stress. In all combinations of treatments in this experiment, including treatment without fertilizer and without microbial biofertilizer agents showed the same growth pattern (Figures 1 and 2).

The effect of the interaction of the two factors which is very real with the highest value of each variable is indicated by the combination of the application of P. fluorescens bacteria and the application of "economical" doses or 0.5 g / 5000 g of growing media. This is in line with the evidence of the role of these bacteria as a provider of P for plants [17] which guarantees the adequacy of plants for P elements [18] as shown in the form of plant height (Table 1), stem diameter (Table 2), leaf area (Table 3) , wet stover weight (Table 4), stover dry weight (Table 5), and the initial length of flower appearance (Table 6). Although the average value of all observation variables in the treatment of the combination dose of P is "economical" (0.5 g) and the application of *Trichoderma* is below the average value in the combination of P 0.5 g and application of *P. fluorescens*, but statistically (HSD) the 5% test) on some variables of combining the two combinations was not significantly different, namely 34.57 cm and 35.93 cm, respectively (plant height, Table 1), 1.82 mm and 1.85 mm (stem diameter; Table 2).), as well as 33.6 and 33.8 (the duration of the onset of interest; Table 6). Growth in plant height and stem diameter is largely determined by the activity of growth regulating compounds in the context of cell division and the adequacy of nutrients which in this case can be fulfilled with the help of *Trichoderma* activity [19, 20]. Likewise the process of flower appearance is strongly influenced by the availability of nutrients that can be met by the supply given by *Trichoderma* or by *Pseudomonas* [4, 21]. Both types of microbes have been tested for their ability as phosphate solvent agents [10, 22]. Thus the application of *Trichoderma* and the application of *P. fluorescens* is an alternative to be combined with fertilizer application at a dose of 0.5 g / 5000 grams of planting media in the cultivation of red chillies.

4. Conclusions

The microbial application of biofertilizer agents and P fertilization doses had an effect on increasing the growth of plant height, stem diameter, stover wet weight and dry weight of red chili plant stover at 42 days after planting. The interaction of the two influential factors increases all observation variables and speeds up the initial flowering time. The application of *Pseudomonas fluorescens* bacteria combined with 0.5 g / 5000 g fertilizer media planting media resulted in a response of red chili plants in terms of plant height, stem diameter, leaf area and wet weight and the highest dry stover weight. The response of early flower appearance was indicated by a combination of 0.5 g fertilizer dose with the application of *P. fluorescens* bacteria or with the application of *Trichoderma sp*.

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