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The abundance of soil bacteria applied with compost and *Trichoderma sp*. in tangerine orchard

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ABSTRACT

Introduction: The purpose of this study was to evaluate the abundance of soil bacteria in the orchard. Bacteria that are abundant and symbiotic in the roots of tangerine plants are thought to affect the growth of tangerine plants. This study combined the application of compost and *Trichoderma sp*., as an inducing agent for the resistance of Siamese tangerine plants by ISR. **Methods:** The research was conducted June -October 2022 at the Innovation Garden and Plant Protection Laboratory, Politeknik Negeri Jember. The research design consisted of a combination of organic fertilizer and *Trichoderma sp*, namely control, 10 kg of compost, 20 kg of compost, *Trichoderma sp*, 10 kg of compost and *Trichoderma sp*., 20 kg of compost and *Trichoderma sp*. **Results:** Bacterial exploration in treatments yielded bacterial abundance values between $9x10^{10}$ - $24x10^{12}$ CFU/g. Found 34 bacterial isolates with different morphological characteristics. **Conclusion:** Treatment of 10 kg compost and *Trichoderma sp*. resulted in the highest bacterial population, namely 24 x 10^{12} CFU/g.

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INTRODUCTION

Tangerines are a horticultural commodity that can grow and produce in the lowlands and highlands, besides that this type of plant can also grow in rice fields and moors (Aluhariandu *et al.*, 2016). Tangerines are plants that can have high economic value. This is because tangerine plants are a fruit that is liked by everyone. Apart from that, tangerines are one of the fruits that produce vitamin C. According to the (Badan Pusat Statistik, 2022), tangerine fruit production in 2019 was 2,444,518 tons. In 2020 it was 2,593,384 tons and last year it fell again to 2,401,064 tons. The high increase in demand for tangerine fruit, which is still not sufficient on a national scale, especially for the types of tangerines and the excellent quality of the fruit, has caused an increase in tangerine imports which continues to increase and has made Indonesia the second largest tangerine importing country in ASEAN after Malaysia (Saphira, 2017).

Improvement efforts are needed to make tangerine cultivation more optimal. Improving vegetative growth is the first step that can be taken immediately through optimizing plant nutrition. Providing nutrients through the application of organic fertilizer such as compost. Compost is a product resulting from the partial or full composting process of organic material by decomposing microbes (Dinata, 2022). The benefits of compost in orchards include increasing soil fertility physically and chemically, and providing an energy source for soil microorganisms so that the population of microorganisms increases plant tolerance to various abiotic stresses such as drought, and high temperatures, and can streamline the use of chemical fertilizers (Ullah *et al.*, 2019). Increasing plant resistance is a preventive measure to reduce the incidence of disease. In general, there are two ways SAR mechanism (*Systemic Acquired Resistance*) and ISR (*Induced Systemic Resistance*) (Vallad & Goodman, 2004).

ISR is a plant resistance mechanism that is starting to be widely used by researchers because it is easy to implement, one of which is the use of beneficial microbes such as *Trichoderma sp*. (Yu *et al.*, 2022). Soil health in orchards is an important factor from a biological aspect. One indicator of soil health is assessed through the abundance of bacteria in the soil (Turmuktini & Simarmata, 2011). Many previous studies have revealed the exploration and role of soil bacteria one of which is research on the isolation and enumeration of soil bacteria in Selorejo tangerine picking tourism, Malang Regency (Maula, 2021). Previous research found that indigenous bacteria from exploration in nature were able to control several diseases, both as single antagonists and in consortiums (Dinata, 2018; Dinata, Aini, *et al.*, 2023). The application of biological agents and organic materials is not only to assess plant growth but also to measure the level of diversity of bacterial populations in cultivated soil. *Trichoderma* is a beneficial fungus that can control several plant diseases (Dinata, 2023; Dinata, Mahanani, Soelistijono, *et al.*, 2023; Fatma *et al.*, 2023). *Trichoderma* can resist tangerine plants by inducing plant resistance by controlling plant diseases either directly or indirectly (Tyśkiewicz *et al.*, 2022). Using compost like manure is an important component of the organic farming concept in orchards. Organic fertilizer is more effective in increasing plant growth and plant tolerance to plant pests (Ullah *et al.*, 2019). This research aimed to evaluate the population of soil bacteria in orchards treated with compost and fertilizer *Trichoderma sp*.

METHODS

Tools and materials

The tools used in this research were 1000 and 100 µl micropipettes, 9 cm Petri dishes, tube Eppendorf 15 ml, autoclave, loop needle, L triangle stick, preparation glass, knapsack sprayer, and GPS camera. The materials used in this research were soil samples about 20 cm deep from 3 years old tangerine plants with the same growth, organic compost fertilizer from cow manure, *Trichoderma sp*. which has been propagated in corn rice, instant Nutrient Agar media, sterile distilled water, alcohol, spirits, KOH 3%, and NaOCl.

Time and place

The research was carried out in June – October 2022. Treatment applications and soil sampling were carried out at the Innovation Garden and bacterial isolation was carried out at the Plant Protection Laboratory, Politeknik Negeri Jember. The location of the research garden is between the coordinates -8.157136,113.725722 and -8.156591,113.725476. Innovation Garden in Politeknik Negeri Jember which has long been known to the public as a fruit and vegetable education tourism area.

Research methods

This research refers to previous research using a randomized block design with two factors (Siswadi *et al.*, 2023). The first factor is the addition of compost manure with three levels, control, given once the recommended dose, and twice the recommended dose. The second factor is giving *Trichoderma sp*. which has been propagated on corn rice with a spore density of 109. The total combination consisted of 6 treatments and 3 replications so there were 18 experimental units. Each experimental unit consisted of two plants with a total of 36 tangerine plants. KT1: Control, KT2: 10 kg compost per plant, KT3: 20 kg compost per plant, KT4: *Trichoderma sp*., KT5: 10 kg compost per plant and *Trichoderma sp*., TR6: 20 kg compost per plant and *Trichoderma sp*. The compost fertilizer treatment was applied once at the start of the research, and *Trichoderma sp*. was applied once a month for four months of research. The criteria for tangerine plants used as samples are healthy plants that are more than 150 cm tall, have a rootstock diameter of 10 cm, have a crown diameter of at least 150 cm, the plant has started to bear fruit and the fruit size is adjusted to the Indonesian National Standard (SNI).

Soil samples were taken from 3 plot points of the orchard, at each plot 5 points were taken for systematic sampling. Root samples were taken from healthy tangerine plants using the method according to Gams. Soil samples are taken near the roots (rhizosphere) or the part attached to the roots of tangerine plants. Soil samples were taken at a depth of 5-10 cm. The samples are then mixed and placed in a plastic bag and stored at a cool temperature.

Bacterial isolation was carried out using the dilution plate method or graded dilution (Madigan & Martinko, 2006). For the isolation of rhizosphere bacteria, samples of rhizosphere soil that had been homogenized were taken and weighed at 1 gram and given 10 ml of sterile distilled water in an Erlenmeyer tube. After that, 1 ml was taken for dilution of 10-2 up to 10-9. Next, bacterial cultivation is carried out using the spread method plate, by planting bacterial suspensions in NA media on plates. Planting by taking 100 µl of solution from each 10 dilution-5, 10-6, 10-7, 10-8, 10-9. Then flattened using stick L. After that, it was incubated at room temperature for 24 hours at a temperature of ±28OC. Bacterial colonies that grow on NA media are then purified. Purification of rhizosphere bacteria is carried out by purifying new NA media and incubating for 24 - 48 hours at room temperature. Each colony that grows with different characteristics is counted and purified until a single, pure culture is obtained. Macroscopic characterization of bacterial colonies was carried out based on the method of (Dinata, Aini, & Kusuma, 2021).

Calculation of bacterial abundance uses CFU/g or mg units. CFU is the abbreviation of Colony Form Unit. For example, the abundance of microbes in soil uses wet-weight or dry-weight units so the units used are CFU/g. Calculation of the abundance of bacteria associated with tangerine roots was calculated using the formula:

$$\text{Bacterial abundance (CFU/g)} = (\sum \text{number of colonies x Y}) / (\text{soil weight (g)})$$

Note: Y is the dilution factor times the sample volume

Next, the bacterial isolates were identified by characterization. Morphological characterization of bacteria is carried out to determine the initial identification of bacterial types based on colony morphology including shape, edges, color, and surface of bacterial colonies. Then the bacterial isolates were characterized based on Gram type based on KOH (Schaad *et al.*, 2001). Bacterial isolates aged 48 hours were made into a suspension by taking one tube of bacteria placed on a glass object and adding 1 drop of 3% KOH. The suspension is leveled using a loop needle, quickly and repeatedly. Gram-negative bacteria are characterized by sticky bacterial suspension-like threads. Meanwhile, if the bacterial suspension is dilute and not sticky or does not stick to the needle, then the bacteria are classified as Gram-positive bacteria (Schaad *et al.*, 2001).

Experimental design

This research was carried out using a Randomized Block Design (RBD), 6 treatments, and each treatment was repeated 3 times, resulting in 18 experimental units. Each experimental unit consists of 2 plants, and in one research plot, there are 36 samples of Siamese tangerine plants. The treatment combinations include: A1: Control, A2: 10 kg compost fertilizer, A3: 20 kg compost fertilizer, A4: *Trichoderma sp*., A5: 10 kg + compost *Trichoderma sp*., A6: 20 kg + compost *Trichoderma sp*. This treatment is given to the experimental unit or per plant sample.

RESULTS AND DISCUSSION

The number of rhizosphere bacterial populations in the given treatments gives different values. The population of soil bacteria that are in symbiosis with tangerine roots is presented in Table 1. The results of soil bacteria exploration give a population value of 9x1010 - 24x1012 CFU/g. These results are by research that states that soil organic matter content influences biological properties which can increase microbiological activity and population in the soil, especially related to organic matter decomposition activity (Schnecker *et al.*, 2014). Soil bacteria are soil microorganisms that are very abundant in the soil. Some of these function in mobilizing or facilitating the absorption of various nutrient factors in the soil, not only that, soil bacteria can synthesize and change the concentration of various phytohormones that stimulate plant development (Yunus *et al.*, 2017).

Table 1. Soil bacterial population in orchard

Treatments	Bacterial population (CFU/g)
Control	$24x10^{10}$
Compost 10 kg	$24x10^{10}$
Compost 20 kg	$9x10^{10}$
Trichoderma sp.	$3x10^{10}$
Compost 10 kg and *Trichoderma sp*.	$24x10^{12}$
Compost 20 kg and *Trichoderma sp*.	$16x10^{10}$

Notes: CFU: Colony form unit/gr soil samples

Compost treatment of 10 kg per plant and *Trichoderma sp*. gave the highest bacterial population, namely 24x1012 CFU/g. The compost used is composted cow manure, a type of organic fertilizer that is important for agriculture because the application of manure can increase the chemical fertility of the soil and improve the physical and biological properties of the soil (Lingga & Marsono, 2004). *Trichoderma sp* is a beneficial fungus that has an important role as a biological agent and plant growth promoter. Several studies reveal the use of mushrooms *Trichoderma sp*. in plant disease resistance, among other things, can inhibit the growth of Diplodia on tangerines, and is also able to inhibit the growth of pathogens *Phytophthora infestans*, *Fusarium oxysporum*, *Ganoderma sp*., and other diseases (Bunbury-Blanchette & Walker, 2019; Dwiastuti *et al.*, 2015)

Other treatments give almost the same value 1010 CFU/g. This result is thought to be caused by compost and Trichoderma provide the same effect on improving the biological condition of the soil. The temperature and pH in the soil provide the availability of oxygen and nutrients which are determinants for some microorganisms. Not only that, the dominant nature of certain microorganisms gives rise to competition between other microorganisms to obtain nutrients in the soil (Saraswati *et al.*, 2007).

Exploration of bacteria in orchards provided a variety of soil bacterial morphologies (Figure 1). Found 34 isolates of soil bacteria with different morphologies. This bacterium was found in all treatments using the multilevel isolation method (dilution plate). Bacterial morphology was observed through pigmentation, form, margin, elevation, size, and Gram-bacteria which are presented in Table 2. Soil bacteria in orchards are circular, irregular, and filamentous in shape. The bacteria found have entire, undulate, filiform margins or edges. The bacteria found had raised, flat, umbonate, and crateriform elevations.

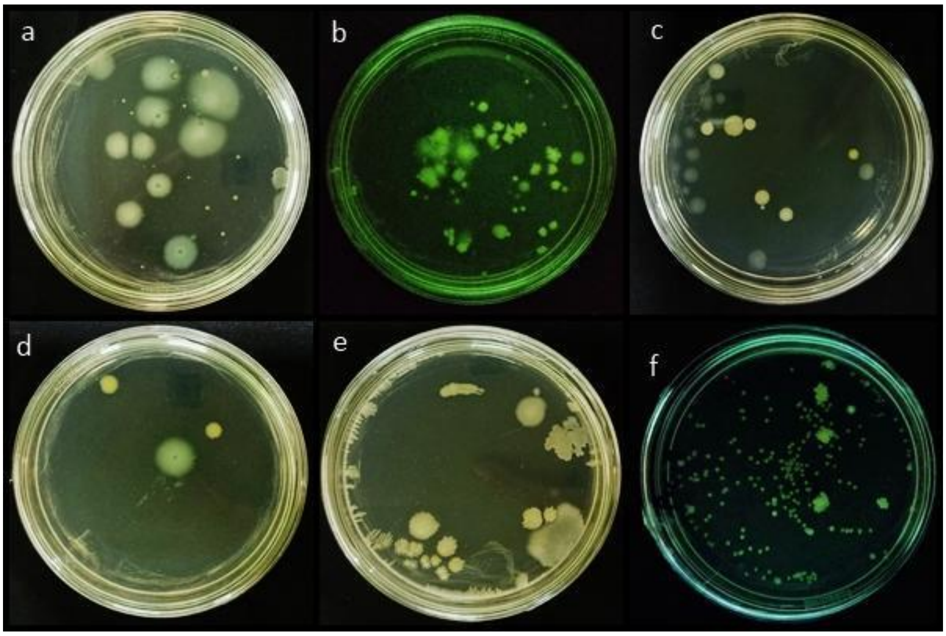

Figure 1. Results of 48 hours bacterial exploration on Nutrient Agar media. (a) control. (b) 10 kg compost. (c) 20 kg compost. (d)*Trichoderma sp*. (e) 10 kg compost and *Trichoderma sp*. (f) 20 kg compost and *Trichoderma sp*.

Table 2. Morphology of soil bacteria

No	Pigmentation	Form	Margin	Elevation	Size	Gram
BT1	yellowish white	circular	entire	Raised	small	-
BT2	yellow transparent edge	irregular	undulate	flat	big	-
BT3	white milk	circular	Entire	umbonate	small	-
BT4	yellow	circular	entire	raised	small	-
BT5	yellow	circular	entire	raised	small	+
BT6	white	circular	entire	raised	small	+
BT7	yellow	irregular	filiform	flat	big	+
BT8	white	irregular	filiform	flat	big	+
BT9	white milk	circular	entire	raised	big	+
BT10	yellow transparent edge	irregular	undulate	flat	big	+
BT11	yellowish white	circular	entire	umbonate	small	+
BT12	white	irregular	undulate	flat	small	+
BT13	White milk	filamentous	undulate	flat	big	+
BT14	yellow	circular	entire	raised	small	+
BT15	white	circular	entire	raised	small	+
BT16	yellow	circular	entire	raised	small	-
BT17	transparent	circular	entire	raised	small	-
BT18	white	circular	entire	raised	small	-
BT19	white	irregular	undulate	flat	big	-
BT20	transparent	irregular	undulate	flat	big	-
BT21	yellow	circular	entire	raised	small	-
BT22	white	circular	entire	raised	big	-
BT23	white	circular	umbonate	flat	big	-
BT24	shiny yellow	circular	entire	raised	small	+
BT25	transparent yellow	circular	entire	crateriform	small	-
BT26	Shiny yellow	circular	entire	crateriform	small	-
BT27	White milk	irregular	lobate	umbonate	big	+
BT28	White milk	circular	entire	umbonate	big	+
BT29	White milk	irregular	undulate	flat	big	+
BT30	White milk	irregular	entire	flat	big	+
BT31	White milk	circular	entire	umbonate	big	+
BT32	yellow	circular	entire	Raised	small	-
BT33	yellow	irregular	undulate	flat	small	+
BT34	white	circular	entire	Raised	small	+

Table 3. Isolate codes and bacterial abundance

Exploration of Bacteria in Tangerine Soil			
Isolation Code	Bacterial Abundance (CFU/g)	Isolation Code	Bacterial Abundance (CFU/g)
BT1	$10X10^{10}$	BT18	$6x10^{10}$
BT2	$10X10^{10}$	BT19	$1x10^{10}$
BT3	$2x10^{10}$	BT20	$1x10^{10}$
BT4	$2x10^{10}$	BT21	$6x10^{10}$
BT5	$4x10^{9}$	BT22	$1x10^{10}$
BT6	$20x10^{9}$	BT23	$10x10^{10}$
BT7	$15x10^{9}$	BT24	$1x10^{11}$
BT8	$2x10^{9}$	BT25	$90x10^{11}$
BT9	$3x10^{9}$	BT26	$13x10^{11}$
BT10	$2x10^{9}$	BT27	$2x10^{10}$
BT11	$1x10^{9}$	BT28	$1x10^{10}$
BT12	$3x10^{10}$	BT29	$1x10^{11}$
BT13	$3x10^{10}$	BT30	$18x10^{12}$
BT14	$1x10^{10}$	BT31	$3x10^{12}$
BT15	$10x10^{10}$	BT32	$1x10^{12}$
BT16	$10x10^{10}$	BT33	$1x10^{12}$
BT17	$2x10^{10}$	BT34	$1x10^{12}$

Information: CFU=Colony Form Unit, BT=Bacteria Tangerine

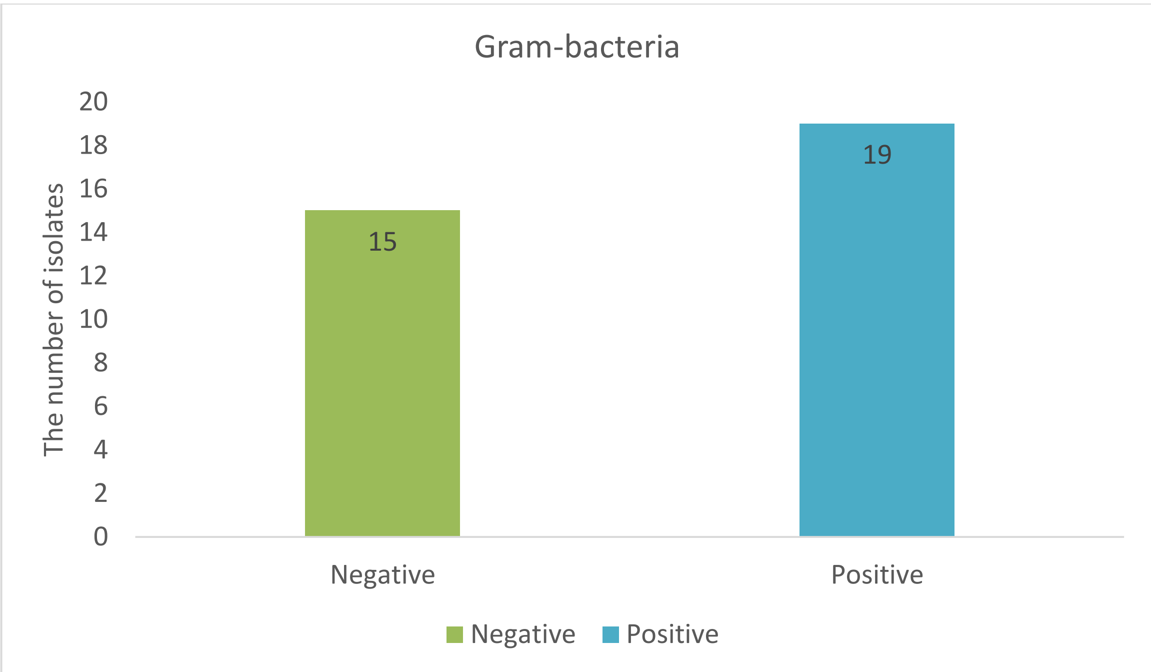

Figure 2. Number of isolates based on characteristics of Gram-bacteria in all treatments *Trichoderma sp*.

The abundance of soil bacteria in each treatment produced diverse bacterial isolates. The bacterial abundance in each bacterial isolate is presented in Table 3. The bacterial isolates were coded BT1-BT34. The bacterial abundance is between 1x109 – 18x1012 CFU/g. Bacterial populations are influenced by different land uses and types of management because each management produces organic material input in different amounts and quality (Rinady *et al.*, 2023). Microbes interact with each other both synergistically and antagonistically, this is to survive extreme conditions and can coexist with other microorganisms (Hibbing *et al.*, 2010).

Soil bacteria in the orchard are Gram-positive and Gram-negative based on the KOH 3 test (Figure 2). The gram characterization aims to initially identify the soil bacteria found. The results of the characterization of bacteria isolated on tangerine roots showed that there were 15 Gram-negative bacteria and 19 Gram-positive bacteria. This research produced many positive gram bacteria. Gram-positive bacteria retain crystal violet and appear purple when observed under a microscope. Meanwhile, Gram-positive cell walls consist of thicker peptidoglycan with a thickness of 20-80 µm (Kaiser, 2021). Gram-negative bacteria produce pure osmolytes in response to drought, while Gram-positive bacteria produce osmolytes for substance exchange (Schimel *et al.*, 2007).

Many studies have been reported on bacterial isolates from the rhizosphere that have the potential to promote plant growth (Dinata, Ariani, Purnomo, *et al.*, 2021; Safriani *et al.*, 2020). Several researchers discovered the potential of several bacterial isolates that could be used as PGPB (Plant Growth Promoting Bacteria) from shallot plants (Dinata, Aini, & Abadi, 2021; Wandita *et al.*, 2018). PGPR application can also increase plant growth and reduce the incidence of plant diseases infected by viruses (Taufik *et al.*, 2010) and fusarium pathogenic fungi (Zunairoh *et al.*, 2019). Exploration of the soil yields diverse numbers of bacteria. The bacteria obtained have their characteristics and benefits. Bacterial life in nature can live to form communities and form symbiotic relationships in roots to provide benefits to plants, but if isolated and developed there are bacteria whose roles cannot be combined or consortiumed (Dinata, Aini, & Abadi, 2021).

CONCLUSION

Exploration of soil bacteria given application of compost and *Trichoderma sp*. in tangerine orchard provides an abundance of soil bacteria 9x1010 - 24x1012 CFU/g. In this study, 34 isolates of soil bacteria with different morphologies were found that were symbiosis in tangerine plants.

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