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Effect of Using *Tithonia diversifolia* Green Manure and *Trichoderma* sp. Secondary Metabolites on the Growth and Yield of Tomato Plants (*Solanum lycopersicum* L.) on Alluvial Soil

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ABSTRACT

The primary challenge in utilising alluvial land for tomato cultivation was the low soil fertility. *Tithonia diversifolia* can be used as an ameliorant to address this issue. Additionally, secondary metabolites from *Trichoderma* sp. can be employed to control plant diseases. This research aimed to assess the impact of using *Tithonia diversifolia* green manure and *Trichoderma* sp. secondary metabolites on the growth and yield of tomato plants in alluvial soil. The research was conducted in the experimental field of the Faculty of Agriculture, Science, and Technology, Panca Bhakti University Pontianak, from December 2023 to March 2024. A factorial completely randomised design (CRD) was implemented, involving two factors: the first factor was *T. diversifolia* green manure (p) with three treatment levels: p1 = 200 g per polybag, p2 = 250 g per polybag, p3 = 300 g per polybag. The second factor was *Trichoderma* sp. secondary metabolites (m) with three treatment levels: m1 = 100 mL per plant, m2 = 150 mL per plant, m3 = 200 mL per plant. Observations focused on plant growth and yield. Data were analysed using the F-test and Tukey test at the 5% significance level. The application of 300 g of *Tithonia diversifolia* green manure and 200 mL of *Trichoderma* sp. secondary metabolites can enhance the growth and

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yield of tomato plants optimally in alluvial soil, although no significant interaction was observed ($p > 0.05$). Further research is needed to identify other factors that influenced the interaction so that the use of *Tithonia diversifolia* green manure and *Trichoderma* sp. could be recommended to increase tomato productivity in alluvial soil.

1 INTRODUCTION

The tomato plant (*Solanum lycopersicum* L.) is a significant horticultural crop with development potential in Indonesia. Tomatoes are rich in protein, carbohydrates, fats, minerals, and vitamins, making them a staple and complementary ingredient in the Indonesian diet. As tomato consumption rises, market demand increases, but production has not kept pace. To address this, evaluating planting media that do not support root growth is crucial, as roots need to absorb nutrients efficiently, necessitating alternative fertilisation methods[1]. According to the Central Bureau of Statistics of West Kalimantan[2], the average tomato production in West Kalimantan was 1,857 tonnes. Nationally, tomato productivity was 74,558 tonnes in 2019, increasing by 9,352 tonnes in 2020[3]. This highlights the importance of tomatoes as both a staple food and a raw material for processing industries in Indonesia. However, productivity in regions like West Kalimantan remains low and below expectations.

West Kalimantan has substantial potential for tomato cultivation due to its extensive alluvial soils, which cover 24.42 percent of the region's area or 3.59 million hectares[4]. However, alluvial soils have physical, chemical, and biological properties that are less supportive of plant growth. These soils are predominantly dusty (90.34%), moist, shallow, low in pH, and have limited nutrients (N, P, K). Poor soil aeration also hampers microorganism development, reducing soil organism populations[5]. The use of organic ameliorants was a method that could be employed to improve the properties of alluvial soil[6]. Utilisation of wild plant biomass as a source of soil organic materials can be done as an optimisation of local resource utilisation and to minimise environmental damage. One of the wild plants that can be used as organic soil material is *Tithonia diversifolia*[7]. *T. diversifolia*, which grows naturally, produces a high biomass of around 5.6-8.1 tonnes per hectare per year, making it a valuable source of organic material for green manure or compost. The nutrient content in *T. diversifolia* organic manure is substantial and can enhance soil fertility, indirectly boosting plant growth and yield. Liquid organic fertiliser from *T. diversifolia* contains 2.7-3.59% nitrogen (N), 0.14-0.47% phosphorus (P), and 0.25-4.10% potassium (K)[8]. Applying *T. diversifolia* organic manure has been shown to increase total soil nitrogen content, NO_3^-, exchangeable nitrogen (N) and potassium (K), soil pH, phosphorus (P), calcium (Ca), cation exchange capacity (CEC), and soil organic matter[9]. According to research by Farni et al.[10], *T. diversifolia* organic manure improves soil chemical quality, nitrogen absorption, and maize growth in degraded sandy soils in Malang, East Java.

Aside from low soil fertility, tomato production is also hindered by plant diseases, notably *Fusarium* wilt caused by the fungus *Fusarium oxysporum*[11], which can significantly reduce yields. An effective biocontrol method for *Fusarium* wilt is using *Trichoderma* sp., a soil saprophytic fungus known to contain active compounds that inhibit and kill plant pathogens[12]. *Trichoderma* sp. produces antibiotic secondary metabolites such as viridin and trichomycin[13]. Research by Suyanto et al.[14] indicates that using *Trichoderma* sp. and its secondary metabolites can suppress *Fusarium* wilt in tomatoes by up to 100%. According to Vinale et al.[15], secondary metabolites from *Trichoderma* sp. act as elicitors, enhancing plant resistance to diseases. These metabolites include antibiotics, enzymes, hormones, and toxins that can be transported with water and nutrients to reach plant tissues. Antibiotic compounds produced by *Trichoderma* sp. include viridin, kininginin, cytosperone, trichodermol, mannitol, and 2-hydroxymalonate acid[16,17]. Enzymes such as proteases, cellulases, cellobiases, chitinases, and 1,3-ß-glucanases in *Trichoderma* sp. secondary metabolites play crucial roles in controlling plant diseases[18]. These metabolites inhibit pathogens by denaturing structural and functional proteins in pathogen cells, causing cell lysis and death[19].

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The use of a combination of fertilisers and organic biological control is very promising because it can reduce synthetic fertilisers and pesticides, which can be harmful if used excessively and continuously. Determining the optimal application dosage needs to be done to provide recommendations for organic tomato cultivation in alluvial soil. This research aimed to determine the extent to which the use of green manure *T. diversifolia* and secondary metabolites of *Trichoderma* sp. can enhance the growth and yield of tomato plants in alluvial soil using polybags.

2 MATERIALS AND METHOD

This research was conducted in the experimental field of the Faculty of Agriculture, Science, and Technology at Panca Bhakti University, Pontianak, West Kalimantan Province. The research was carried out from December 2023 to March 2024. The materials used consisted of tomato seeds of the Timoty F1 variety, crude *Trichoderma* sp. secondary metabolites, *T. diversifolia* green manure, alluvial soil, and agricultural lime. The equipment used included polybags, hoes, machetes, regular scales, analytical scales, buckets, writing tools, bags, stoves, pots, sieves, shakers, jerrycans, laminar air flow, measuring cups, and funnels. The production of secondary metabolites of *Trichoderma* sp. and green manure *T. diversifolia* was carried out in the Laboratory of the Faculty of Agriculture, Science, and Technology, Panca Bhakti University, Pontianak.

The experimental design used in this research was a factorial completely randomised design (CRD). The treatments consisted of two factors: the first was *T. diversfolia* green manure with three treatment levels, and the second was *Trichoderma* sp. secondary metabolites (m) with three treatment levels. Each treatment was replicated three times, with each replication consisting of three plants, resulting in a total of 81 plants used in this research. The treatment levels were as follows:

(i) The first factor was *T. diversfolia* green manure with the following treatment levels: p1 = *T. diversifolia* green manure at a dose of 20,000 kg ha^{-1}, p2 = *T. diversifolia* green manure at a dose of 30,000 kg ha^{-1}, p3 = *T. diversifolia* green manure at a dose of 40,000 kg ha^{-1}.

(ii) The second factor was the dose of secondary metabolites (m), consisting of 3 treatment levels: m1 = secondary metabolites at a dose of 100 mL per plant with 7 applications at weekly intervals, m2 = secondary metabolites at a dose of 150 mL per plant with 7 applications at weekly intervals, m3 = secondary metabolites at a dose of 200 mL per plant with 7 applications at weekly intervals.

2.1 Stages of Research

Preparation of growing media

The alluvial soil, collected from a depth of 0-20 cm below the surface, was cleaned of root debris, grass, and other litter. The soil was then sieved using a sand sieve, and 10 kg of soil was weighed and placed into each polybag. The soil was taken from the Sungai Kakap District, Kubu Raya Regency, West Kalimantan Province. The soil was made up of a clay-loam texture, a pH of 4.42 (very acidic), 2.97% organic carbon (medium), 0.42% total nitrogen (medium), 4.89 ppm P_2O_5 (very low), 2.42 cmol kg^{-1} calcium (low), 0.95 cmol kg^{-1} magnesium (low), 0.77 cmol kg^{-1} potassium (high), 0.59 cmol kg^{-1} sodium (medium), a cation exchange capacity of 32.17 cmol kg^{-1} (high), and a base saturation of 14.70% (very low).

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Seeding

Before sowing, tomato seeds were soaked in cold water for 1 hour to stimulate germination. Seeding was done using a mixture of soil and manure prepared one week before sowing at a ratio of 1:1. Regular watering was conducted twice a day, in the morning and afternoon.

Liming

Liming was done 2 weeks before planting. Dolomite lime with a neutralising power of 104% was used to raise the soil pH. The dose of lime given was 1,923 kg ha^{-1}.

Application of T. diversifolia green manure

T. diversifolia green manure was applied two weeks before planting by mixing it with the growing media.

Application of secondary metabolites

Secondary metabolite was applied two weeks after planting by infusing the plants using a drip tool placed inside the polybag.

Planting

Planting was done after the seeded seeds were 3 weeks old and had 5-6 leaves. Then, the planting was done by transferring the seedlings from the seedling tray into the growing media in polybags.

Maintenance

Watering: Watering was done twice a day, about 0.5 L each time, in the morning and afternoon. It was adjusted according to the weather, and watering was skipped during rainfall.

Weed control: Weed control or weeding was performed immediately when weeds appeared on or around the growing media.

Pest and disease control: Pest control was conducted manually by removing pests found on plant leaves, and botanical pesticides were used according to the type of pests attacking the plants.

Harvesting: Tomato harvesting took place 50-60 days after planting (DAP), targeting ripe, orange-coloured fruits. Harvesting was done by picking the fruits from the stem.

Observation parameters

The parameters observed in this research included plant height (from the base of the stem to the tip of the highest leaf), leaf count, branch count, fresh fruit count per plant, and fresh fruit weight per plant. A F-test at the 5% significance level was used to assess the effects of treatments on these variables. If the F-test indicated significant effects at the 5% level, further analysis was conducted using the Tukey test, also at the 5% level. The Tukey test was employed to compare the different treatment levels. Data analysis was performed using the R Studio application.

3 RESULTS AND DISCUSSION

Prior to utilising *T. diversifolia* as a source of organic material, a nutrient analysis was performed on *T. diversifolia* green manure. The analysis revealed that the plants contained high levels of nutrients, including carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), with a low carbon-to-nitrogen (C/N) ratio (Table 1). This nutrient-rich profile suggests that *T. diversifolia's* green manure has significant potential to serve as an organic material, enhancing soil chemical properties by increasing nutrient availability.

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Based on the analysis of variance results (Table 1), the interaction between *T. diversifolia* green manure and *Trichoderma* sp. secondary metabolites did not significantly affect all parameters ($p > 0.05$). However, both *T. diversifolia* green manure and *Trichoderma* sp. secondary metabolite treatments individually had a significant effect on all parameters.

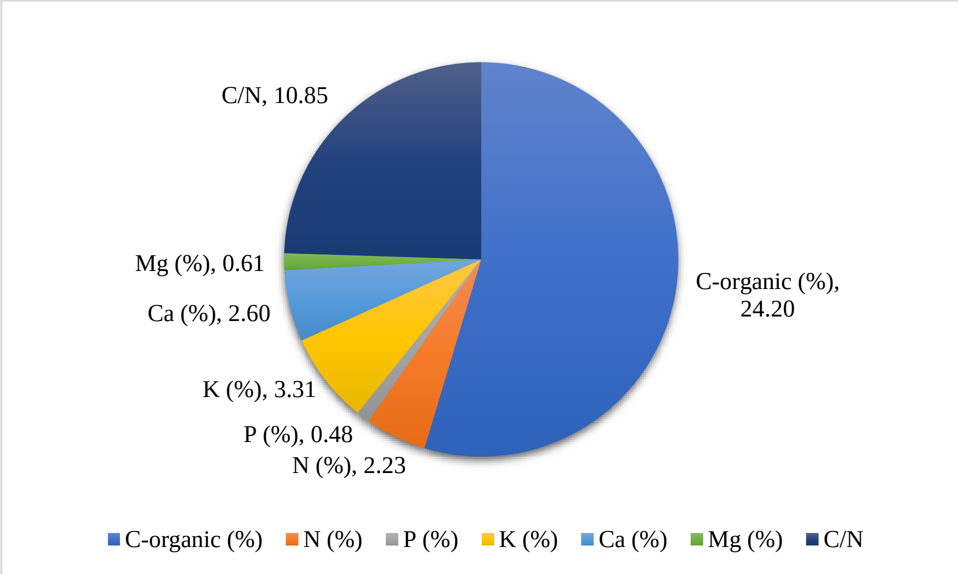

Fig. 1. Nutrient content of *Tithonia diversifolia* green manure.

The application of *T. diversifolia* green manure at the highest dose of 300 g per polybag (p3) and *Trichoderma* sp. secondary metabolites at 200 mL per plant (m3) showed relatively significant improvement in plant growth results compared to the application of *T. diversifolia* green manure at 200 g per polybag (p1) and *Trichoderma* sp. secondary metabolites at 100 mL per plant (m1). The treatments of p3 and m3 showed slightly better results compared to the treatments of p2 and m2. This suggests that higher doses provide more available nutrients to the plants, resulting in more optimal growth processes and thus higher plant growth and yield compared to the lowest dose treatments. There were no significant differences in all results between p1 and p2 treatments, except for the fresh fruit weight per plant in Table 2. Meanwhile, in the *Trichoderma* sp. secondary metabolites treatment (Table 3), plant height for m1 was the only parameter that showed a significant difference compared to the m2 treatment.

Table 1. Analysis of variance recapitulation results on all observed parameters

F value	Plant height (cm)	Leaf count	Branch count	Fresh fruit count per plant	Fresh fruit weight per plant (g)	F table α = 5%
T. diversfolia green manure	12.38*	8.10*	3.91*	11.24*	8.70*	3.55
Secondary metabolites	14.64*	11.22*	10.86*	10.11*	9.63*	3.55
Interaction F value	1.47ns	1.35ns	0.94ns	0.47ns	1.07ns	2.93

Note: *=significant; ns=not significant

The independent effects observed in this research attributed to *T. diversifolia* green manure and *Trichoderma* sp. secondary metabolites are believed to stem from the specific functions of each treatment. *T. diversifolia* green manure is recognised for its ability to enhance soil organic matter content and improve soil nutrient levels, thus enhancing soil physical, chemical, and biological properties. This, in turn, leads to increased soil productivity and resistance to erosion. A key advantage of *T. diversifolia* green manure lies

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in its ability to improve soil physical and chemical properties, manage weeds, and protect against nematodes[20]. Meanwhile, the secondary metabolites of *Trichoderma* sp. are known to function as biological agents, thus protecting tomato plants from diseases, and enabling optimal growth[21].

Table 2. Mean values of all observed parameters in the *Tithonia diversifolia* green manure treatment

Treatments	Plant height (cm)	Leaf count	Branch count	Fresh fruit count per plant	Fresh fruit weight per plant (g)
p1	$80.00^{a} \pm 3.14$	$27.00^{a} \pm 1.28$	$4.26^{a} \pm 0.46$	$15.48^{a} \pm 1.29$	$363.85^{a} \pm 48.85$
p2	$81.35^{a} \pm 2.07$	$28.67^{ab} \pm 2.77$	$4.48^{ab} \pm 0.64$	$16.26^{ab} \pm 1.60$	$428.93^{b} \pm 43.78$
p3	$84.37^{b} \pm 3.17$	$30.56^{b} \pm 3.28$	$4.89^{b} \pm 0.79$	$18.19^{b} \pm 1.87$	$435.04^{b} \pm 63.69$

Note: Numbers followed by the different letter in the same column are significant in the 5% Tukey Test

Table 3. Mean values of all observed parameters in the *Trichoderma* sp. secondary metabolites treatment

Treatments	Plant height (cm)	Leaf count	Branch count	Fresh fruit count per plant	Fresh fruit weight per plant (g)
m1	$79.17^{x} \pm 2.78$	$26.63^{x} \pm 1.52$	$4.15^{x} \pm 0.50$	$15.56^{x} \pm 1.20$	$377.85^{x} \pm 65.30$
m2	$82.74^{y} \pm 2.86$	$28.78^{xy} \pm 1.62$	$4.33^{x} \pm 0.40$	$16.26^{x} \pm 1.81$	$393.63^{x} \pm 41.39$
m3	$83.81^{y} \pm 2.48$	$30.81^{y} \pm 3.52$	$5.15^{y} \pm 0.66$	$18.11^{y} \pm 1.86$	$456.33^{y} \pm 44.81$

Note: Numbers followed by the different letter in the same column are significant in the 5% Tukey Test

Moreover, *T. diversifolia* green manure provides essential macronutrients (N, P, K, Ca, Mg) and micronutrients[22]. Its application in the soil can substitute for nitrogen and potassium from conventional fertilisers, elevate soil pH, decrease aluminium solubility, and preserve crucial mineral reserves like phosphorus, calcium, and magnesium[7]. Plant height is influenced by the levels of nitrogen (N) and phosphorus (P) in the soil. Nitrogen is required by plants in the highest amounts and significantly impacts the biomass distribution between roots and stems. Phosphorus is crucial for plant growth and is necessary for achieving optimal crop production and quality. This nutrient is vital for cell division, reproduction, and plant metabolism. Additionally, phosphorus plays a role in energy acquisition, storage, and utilization. While potassium maintains vital turgor pressure for heightened photosynthesis and metabolic processes[23].

Based on the results of the analysis, the green manure *T. diversifolia* showed a low C/N ratio, indicating rapid decomposition, allowing the manure to supply nutrients to the soil and plants quickly. With the availability of nutrients in the soil, the plants absorbed nutrients optimally, which increased their growth, as seen in the height of the plants, the number of leaves, and the number of branches. A greater number of leaves was also related to light absorption and the rate of photosynthesis in the leaves. When the rate of photosynthesis activity increased, it also increased the growth of the plant's fruit[24]. Additionally, high concentrations of organic matter from *T. diversifolia* green manure reduced bulk density, enhanced soil moisture retention, soil structure and aeration, and increased water holding capacity, which are indicative of productive soil[25]. Furthermore, organic fertilisers can enhance soil cation exchange capacity and bind harmful iron, aluminium, and manganese cations, besides enhancing soil physical, chemical, and biological properties. They also mitigate excessive reliance on inorganic fertilisers and augment production productivity[25].

Trichoderma sp. is a soil saprophytic fungus that contains active compounds capable of inhibiting and eliminating plant pathogenic pathogens. Additionally, *Trichoderma* sp. can produce antibiotic secondary metabolites like viridin and trichomycin[26]. Research conducted by Suyanto et al.[7] has demonstrated that *Trichoderma* sp. and its secondary metabolites can effectively suppress *Fusarium* wilt attacks on tomato plants by up to 100%. Although *Fusarium* was not introduced to the plants in this study, it is suspected that the secondary metabolites of *Trichoderma* sp. can enhance the immunity of tomato plants, allowing them to grow optimally.

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According to Vinale et al.[15], secondary metabolites can serve as elicitors, bolstering plant resistance to pest incursions. *Trichoderma* sp. employs antagonistic mechanisms against pathogenic fungi, including competition, mycoparasitism, and antibiosis. The antibiosis mechanism is exemplified by the production of secondary metabolite compounds such as peptiabol, pyron, viridin, koningin, gliotoxin, gliovirin, and viriodol[27]. Enzymes found in Trichoderma sp. secondary metabolites are protease, cellulase, cellobiase, chitinase, and 1,3-*ß*-glucanase. They play pivotal roles in controlling plant diseases[18].

The mechanism of action by which secondary metabolites inhibit pathogen development involves denaturing structural and functional proteins in pathogen cells. Active compounds in secondary metabolites penetrate cells, causing them to lyse and perish[19]. Apart from their role as plant disease controllers, *Trichoderma* sp. secondary metabolites also stimulate plant growth and development. They achieve this by fostering interactions with plants to promote growth hormones[28] and producing growth-promoting enzymes for both plants and roots[29].

Trichoderma sp. secondary metabolites can enhance tomato growth and yield through several mechanisms, such as plant protection against pathogens, root growth promotion, improved nutrient absorption, and increased photosynthetic capacity[30]. *Trichoderma* also boosts photosynthetic efficiency and reduces plant photorespiration activity while maximising the re-assimilation of photorespiration products through the synthesis of new amides[31]. According to Pascale et al.[32] and Carillo et al.[31], *Trichoderma* produces secondary metabolites with auxin-like properties, such as 6-pentyl-α-pyrone (6PP) and harzianic acid (HA), which enhance plant growth and development as well as disease resistance. Mazzei et al.[21] discovered that *Trichoderma harzianum* significantly increased tomato plant growth due to the influence of phytohormone-like compounds, specifically the auxin harzianolide.

4 CONCLUSION

Based on the research results, the application of *Tithonia diversifolia* green manure and *Trichoderma* sp. secondary metabolites can enhance the growth and yield of tomato plants in alluvial soil. However, no significant interactions were observed ($p > 0.05$). The treatments utilising 300 g of *Tithonia diversifolia* green manure (p3) and 200 mL of *Trichoderma* sp. secondary metabolites (m3) gave the best results and significantly differed from the low-dose treatments p1 and m1. Further research was needed to identify other factors that influenced the interaction so that the use of *Tithonia diversifolia* green manure and Trichoderma sp. could be recommended to increase tomato productivity in alluvial soil.

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CONFLICT OF INTEREST

The authors report there is no conflict of interest in this work.

AUTHORS' CONTRIBUTIONS

Conceptualization: A. Suyanto
Data curation: I. Astar
Methodology: Setiawan
Formal analysis: A. Suyanto
Visualisation: A. Suyanto
Software: I. Astar
Writing (original draft): A. Suyanto

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Writing (review and editing): Setiawan
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