

## Research Article

# Effects of *Trichoderma* and Foliar Fertilizer on the Vegetative Growth of Black Pepper (*Piper nigrum* L.) Seedlings

Netty Syam , Hidrawati, St. Sabahannur, and Akbar Nurdin

Agrotechnology Program, Faculty of Agriculture, Universitas Muslim Indonesia, Makassar, South Sulawesi 90231, Indonesia

Correspondence should be addressed to Netty Syam; netty.said@umi.ac.id

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Black pepper is a high nutrient-demanding crop; however, high application rates of the chemical fertilizer may give a negative impact on the environment and human health. Coapplication of the chemical fertilizer with biological fertilizers or biological agents is very important to support growth and high yield. This field experiment was carried out to study the effect of *Trichoderma harzianum* and foliar fertilization on the vegetative growth of black pepper. The study was conducted for 4 months. The research method was arranged in a factorial based on randomized block design (RBD). The first factor was the application of *Trichoderma* (50 g, 75 g, and 100 g per plant), and the second one was foliar fertilizers (1.0%, 1.5%, 2.0%, and without foliar fertilizer as the control). The treatments were repeated three times. The results showed that the application of both *Trichoderma* and foliar fertilizer affected the rate of shoot growth, the number of leaves, and the emergence time on black pepper seedlings under the nursery conditions. The application of 50 g/plant of *Trichoderma* and 1.5% of the foliar fertilizer resulted in 26% longer shoot length and 54% more leaves and accelerated the appearance of shoots 10 days earlier compared to without *Trichoderma* and the foliar fertilizer.

## 1. Introduction

Black pepper (*Piper nigrum* L.) is one of the most economically important crops in Indonesia. It has an increasing role in many diets and medicinal applications and also plays an important role in the rural and farming systems of the country. Black pepper can be applied in the manufacture of traditional and modern medicines, nutraceuticals, perfumes, and other products. Also, pepper is used in many different forms. Several value-added products that have been developed include pepper oil, oleoresin pepper, green pepper brine, and other products [1, 2]. With the development of modern science and technologies, greater awareness, and demand among the public for the use of natural products [3, 4], especially in food and medicine, pepper has indeed secured a better position and has better prospect in the coming years [5].

These benefits trigger the high demand for pepper from both domestic and world markets. This encourages farmers in Indonesia to cultivate crops more intensively. According

to the data, the pepper planting area in 2019 reached 189,662 ha with a production of 89,902 tons [6]. Despite the extensive development of plantations, the productivity of Indonesian pepper in 2019 reached only 798 kg/ha. The production is not optimal, and there is even a declining trend. One of the factors causing the low pepper production is the infection of several pathogens such as *Phytophthora* spp. that significantly reduce plant growth and production. Root rot disease caused by a soilborne plant parasitic fungus *Phytophthora capsici* is a serious constraint in black pepper cultivation in Indonesia [7]. Contaminated planting materials or infected black pepper seedlings are important means of disease distribution in the region. The disease has been found in several important pepper-producing areas of Indonesia. The attack on the leaves will cause patches that extend to the entire leaf surface, while attacks on the base of the stem and roots can cause the plant to die [7].

Conventional crop protection is largely based on the use of chemical pesticides to control plant diseases and pests [8, 9], a practice that can have negative effects on end users

and agroecosystem, including inhibition of pollinators, useful predators/parasitoid, and beneficial microbial communities [10]. Current trends in pepper cultivation and the applications of broad-spectrum organophosphate pesticides and high chemical fertilizers can contribute to environmental degradation [3, 8, 11, 12], besides shortening the life of the vines [13]. Different strategies have been raised to improve the efficiency of fertilization in crops [11, 14–16].

Soil fertility and the application of mineral nutrients in the form of chemical or organic fertilizers have an inevitable role in the overall growth of a plant, its performance, productivity, and its tolerance to stresses such as diseases [17–19]. Efforts are made to increase soil fertility in supporting plant growth, namely, by utilizing *Trichoderma* spp. as a biological agent that helps degrade organic matter so that more nutrients are available for plant growth [20–22]. *Trichoderma* spp., which have been studied extensively, are among the most commonly used microorganisms as biological control agents and are currently marketed as the active ingredients of biopesticides, biofertilizers, growth enhancers, and natural resistance stimulants. Applications of biological agents such as *Trichoderma* spp. are a useful and sustainable approach in improving soil fertility systems [22].

*Trichoderma harzianum*, a filamentous fungus, is used as a successful biological control agent to control different soilborne plant pathogens such as *Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp., and *Sclerotium rolfsii*. It was demonstrated that *T. harzianum* induced defense responses and systemic resistance in addition to the control of plant pathogens [23]. In addition, several *Trichoderma* species promoted the growth and development of seedlings of vegetable and nonvegetable crops [24]. Moreover, yields of cucumber, bell pepper, and strawberry were significantly increased with the application of *Trichoderma* spp. [25].

As global demand for organic agricultural products increases, there is a need to develop healthy and sustainable agricultural practices for the improvement of the environment and the people who live in it [26, 27]. At present, the organic approach is highly preferred in increasing the growth and productivity of black pepper plants [5]. In addition, the application of chemical fertilizers to the leaves shows several benefits over the application of the soil, especially in poor soil conditions or adverse climates [17, 28, 29]. Many studies had proven that the application of the foliar fertilizer was able to enhance the growth of spice crops by supplying secondary nutrients and micronutrients (zinc, manganese, iron, copper, boron, and molybdenum) and supplementing N-P-K needs for short and/or critical growth stage periods [14]. The previous study indicated that the application of the liquid fertilizer was able to increase the vegetative growth of black pepper in the field, and furthermore, there was a significant interaction when applied with *Trichoderma* [30]. The development of a liquid or foliar fertilizer represents a suitable method for effective minerals' delivery to plant tissues [29, 31]. Therefore, the present study was undertaken to determine the efficiency of *Trichoderma* on the growth of black pepper seedlings when used in combination with foliar fertilizers under nursery conditions.

## 2. Materials and Methods

The research was carried out at a screenhouse in the Field Laboratory of Agriculture, Faculty of Universitas Muslim Indonesia, Padang Lampe, Pangkep Regency, South Sulawesi (04° 44' 0" S–119° 37'0" E), from May to October 2019. This location has a daily temperature varying from 25° to 30°C, has rainfall 3174 mm/year, mainly occurring from October to May (Climatology Station in Maros, South Sulawesi, 2019), and is at an altitude of 15–20 m above sea level.

The research was designed in the randomized block design (RBD) factorial with two factors. The first factor was the application of *Trichoderma* consisting of 3 levels, i.e., 25 g/plant, 50 g/plant, and 75 g/plant, and without *Trichoderma* as the control. *Trichoderma* used was a solid formulation of *Trichoderma harzianum*. The second factor was the use of foliar/liquid fertilizers with a concentration that was 1.0%, 1.5%, and 2.0% and without foliar fertilization as the control. Foliar fertilizer (trademark: Lestari Green) was applied once in two weeks; the nutrients' content of the fertilizer is shown in Table 1. The highest number of leaves was recorded at 3.53 leaves in seedlings treated with 50 g/plant of *Trichoderma* which was statistically different from other doses of *Trichoderma* and control.

There were 16 treatment combinations and 3 replications. Each treatment consisted of 5 polybags (plants), and there were 240 plants used. Pepper vines were obtained from Natar 1 variety pepper plants which were  $\pm 36$  months old. The cuttings consisted of 2 segments, and 2 leaves (Figure 1) were obtained from a healthy pepper vine, a 3-year-old Natar 1 variety, which was prepared as the parent tree through the *Trichoderma* application one year before [13].

The potting mixture consisted of soil : sand : compost (1 : 1 : 1 w/w) and had the nutrient composition of 0.20% N, 20.04 ppm P and O, 0.78 mg/L K<sub>2</sub>O, and pH 5.98 (Table 1). The compost (*Gliricidia sepium* leaves) was used as a potting mixture (not sterilized) mixed with *Trichoderma* as per the treatment doses and filled into polybags (17 cm × 25 cm size) with 1.5 kg/polybag. The data collected were analyzed by the analysis of variance (ANOVA) and post hoc comparison (Tukey's HSD test) at the significance level set at  $\leq 0.05$ .

## 3. Results and Discussion

**3.1. Effect of *Trichoderma* on the Growth of Pepper Seedlings.** *Trichoderma* treatment application has a significant effect on the growth properties of pepper plants. Data regarding shoot appearance time, number of shoots, shoot length, number of leaves, leaf area, and root volume are presented in Figure 2. The result showed that *Trichoderma* had a significant effect on shoot length at  $p \leq 0.01$  and the number of leaves at  $p \leq 0.05$ , but there was no significant effect on shoot emergence time, shoot number, leaf area, and root volume. The highest shoot length was recorded at 12.35 cm in seedlings treated with *Trichoderma* 50 g/plant which was statistically similar to *Trichoderma* 75 g/plant, i.e., 11.78 cm. However, *Trichoderma* 50 g/plant was statistically different with *Trichoderma* 25 g/plant (9.38 cm) and control (9.81 cm).

TABLE 1: Chemical characteristics of the potting mixture and nutrient content of the fertilizer.

Potting mixture	Values	Nutrient content of the fertilizer	Values
pH	5.98	pH	6.57
C-organic (%)	0.77	C-organic (%)	5.17
N-total (%)	0.20	N (%)	5.25
C/N ratio	3.85	C/N ratio	0.99
P <sub>2</sub> O <sub>5</sub> (ppm)	20.04	P <sub>2</sub> O <sub>5</sub> (%)	2.12
Exchangeable K <sup>+</sup> (cmol(+)/kg)	0.3	K <sub>2</sub> O (%)	7.94
		SO <sub>4</sub> (%)	3.67
		Ca (%)	2.25
		Mg (%)	0.20
Cation exchangeable capacity	13.06	Cl (%)	1.68
		Fe (%)	0.10
		Na (%)	0.27
		Trace elements	Zn, Mn, B, Mo, Co



FIGURE 1: The cuttings used consisted of 2 segments and 2 leaves of Natar 1 variety.

Application of *Trichoderma* with doses more than 50 g/plant tended to result in a smaller increase in shoot length. Length of the shoot of seedlings treated with 50 g/plant produced a longer shoot length of 31.73% compared with *Trichoderma* 25 g/plant and 25.91% compared with the control. Figure 3 shows that there was a significant relationship between the application of *Trichoderma* with the shoot length ( $R^2 = 0.620$ ,  $r = 0.787$ ) with regression ( $Y = -0.0354x^2 + 1.0646x + 8.4312$ ). This is due to its ability to protect plants, enhance vegetative growth, and contain pathogen populations under numerous agricultural conditions, as well as to act as soil amendments/inoculants for the improvement of nutrient ability, decomposition, and biodegradation. Otherwise, the application of *Trichoderma* did not significantly affect the time of shoot emerging, but at a dose of 50 g/plant of *Trichoderma*, it was able to accelerate the emergence (35.47 days after planting) of pepper cuttings compared to other doses (Figure 2). There was a significant relationship between the application of *Trichoderma* with the emergence time of pepper shoots ( $R^2 = 0.945$ ,  $r = 0.972$ ) with regression ( $Y = 0.9771x^2 - 4.7046x + 44.625$ ) as shown in Figure 3.

The highest number of leaves was recorded in seedlings treated with 50 g/plant of *Trichoderma* which was statistically different from other doses of *Trichoderma* and control. The number of leaves produced from seedlings treated with 50 g/plant of *Trichoderma* was 34.60% more than the control,

37.66% more than seedlings treated with 75 g/plant of *Trichoderma*, and even 63.08% more than seedlings treated with 25 g/plant of *Trichoderma* (Figure 2). Statistically, the application of *Trichoderma* did not have a significant effect on the emergence time of shoots, but at a dose of 50 g/plant of *Trichoderma*, it was able to accelerate the emergence (35.47 days after planting) of pepper cuttings compared to other doses (Figure 2). Increasing the *Trichoderma* dose higher than 50 g/plant resulted in a delay in the emergence of pepper cuttings. There was a significant relationship between giving of *Trichoderma* with the emergence time of pepper shoots ( $R^2 = 0.945$ ,  $r = 0.972$ ) with regression ( $Y = 0.9771x^2 - 4.7046x + 44.625$ ) as shown in Figure 3. The effect of *Trichoderma* on length of a shoot of pepper seedlings indicates a fairly close relationship, which can be seen in Figure 3.

The highest length of shoot and number of leaves were recorded in seedlings treated with 50 g/plant of *Trichoderma*, and this could be due to better provision of soil minerals and hormones to plant roots [8, 32, 33]. Several researchers have found similar evidence for a significantly higher increase in the number of leaves, tiller number, branches, flowers, fruits, and plant height by *Trichoderma* spp. which are available [34, 35]. It has been shown that the rhizosphere isolates produce some metabolites that actively influence the growth of *Trichoderma*-colonized plants due to their action as plant growth regulators [19, 36]. These compounds have optimum activity at low concentrations, while they have an inhibitory effect at high doses [37]. This condition may justify the observed inhibitory effect of the *Trichoderma* spp. in high doses.

**3.2. Effect of the Foliar Fertilizer on the Growth of Pepper Seedlings.** Application of chemical or organic fertilizers plays an important role in improving soil biochemical characteristics such as bulk density and increasing microbial activities, water absorption, and nutrient availability to the plant [15, 16, 38]. The analysis of variance of the data showed that the application of foliar fertilizer has a significant effect on emergence time of the shoot at  $p \leq 0.01$ , shoot length at  $p \leq 0.05$ , and the number of leaves at  $p \leq 0.05$  compared to the control (without the fertilizer). However, fertilization

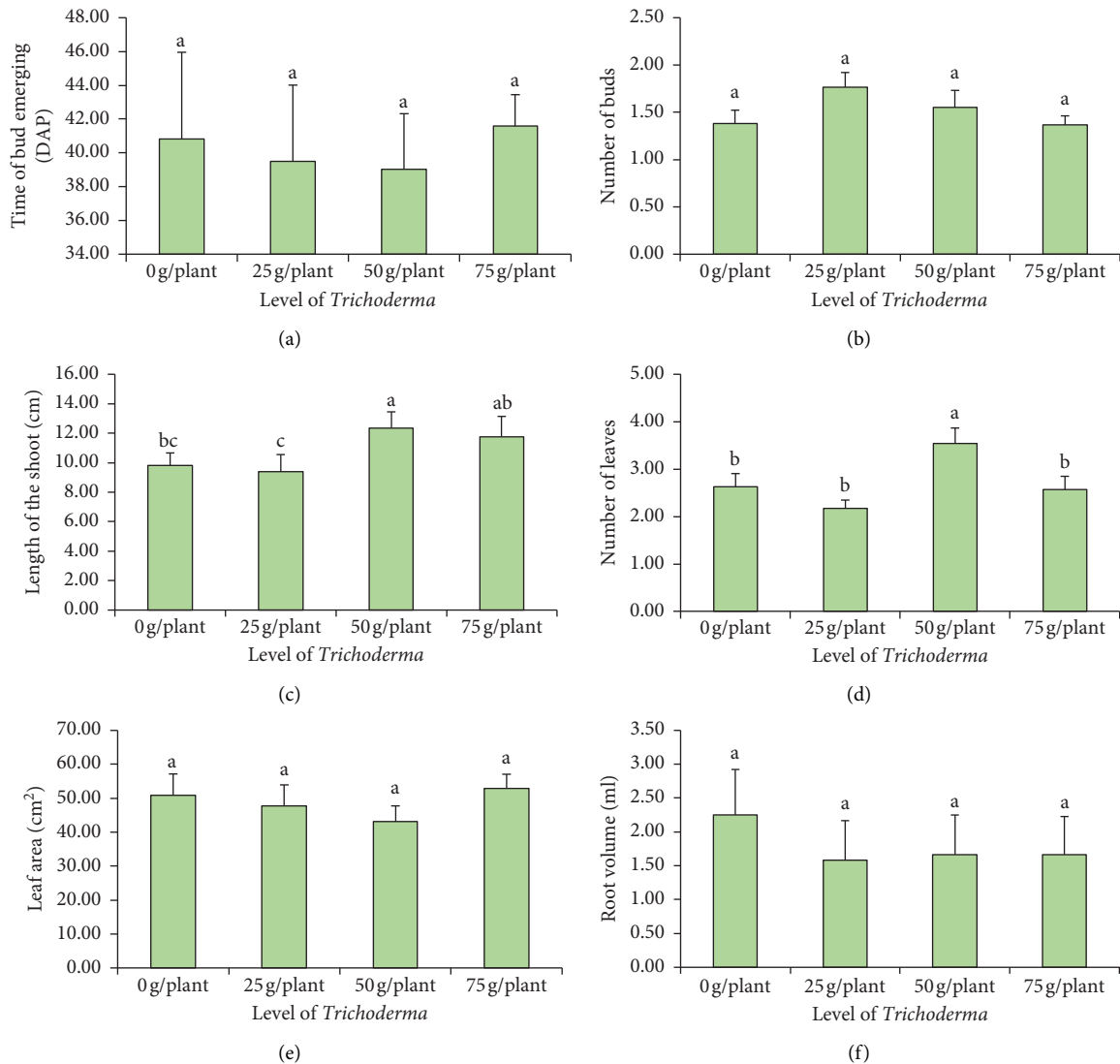


FIGURE 2: Effect of *Trichoderma* on (a) shoot emergence time, (b) shoot number, (c) shoot length, (d) the number of leaves, (e) leaf area, and (f) root volume of black pepper seedlings (after 8 weeks of planting). Different letters indicate significant differences among treatments as determined by Tukey's HSD test at the  $p < 0.05$  level. Vertical bars represent SE.

treatment had no significant effect on the number of shoots, leaf area, and root volume (Figure 4). Figure 4 shows that foliar fertilizer application at the concentration of 1.0%–1.5% accelerated the emergence time of shoot, shoot length, and the number of leaves, which was significantly different from the control plant.

Foliar spray of the liquid fertilizer significantly increased the shoot length and the number of leaves compared to the control plant. The highest shoot length and leaf numbers were obtained from foliar fertilizer concentration 1.5%. Application of the foliar fertilizer with a concentration higher than 1.5% resulted in the inhibition of the enhancement on shoot length and leaf numbers of pepper seedlings as shown in Figure 4. The results of the study show that the level of fertilizer application was highly significant to determine the emergence of shoots, the leaf numbers, and the shoot length of pepper seedlings using the regression

models. It was found that the level of fertilizer application is well correlated to the emergence of shoots ( $R^2 = 0.868$ ,  $r = 0.931$ ), the number of leaves ( $R^2 = 0.991$ ,  $r = 0.995$ ), and the shoot length ( $R^2 = 0.994$ ,  $r = 0.997$ ) as shown in Figure 5.

These findings indicated that the application of foliar fertilizers could better supply minerals to plant leaves, resulting in better growth conditions [12, 39–41]. This might probably be due to the better utilization of the nutrients supplied. The growth rates recorded from pepper seedlings from fertilization treatment indicated that fertilization through leaves on pepper cuttings plays an important role in the early growth of the plant. Ann reported [5] that the application of the organic fertilizer through leaves greatly affected the growth of pepper vines. Furthermore, Ann's [42] explanation for this condition is that, in the early stages of growth, especially when pepper cuttings are moved from the field, the root system is not fully developed, so absorption of

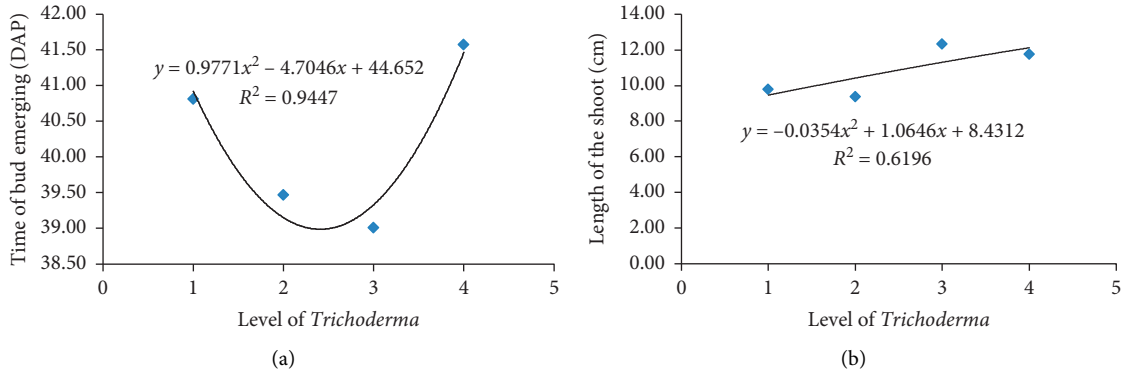


FIGURE 3: Regression analysis between the time of bud emerging (a) and length of the shoot (b) of pepper seedlings and doses of *Trichoderma* on pepper seedlings after 8 weeks of planting.

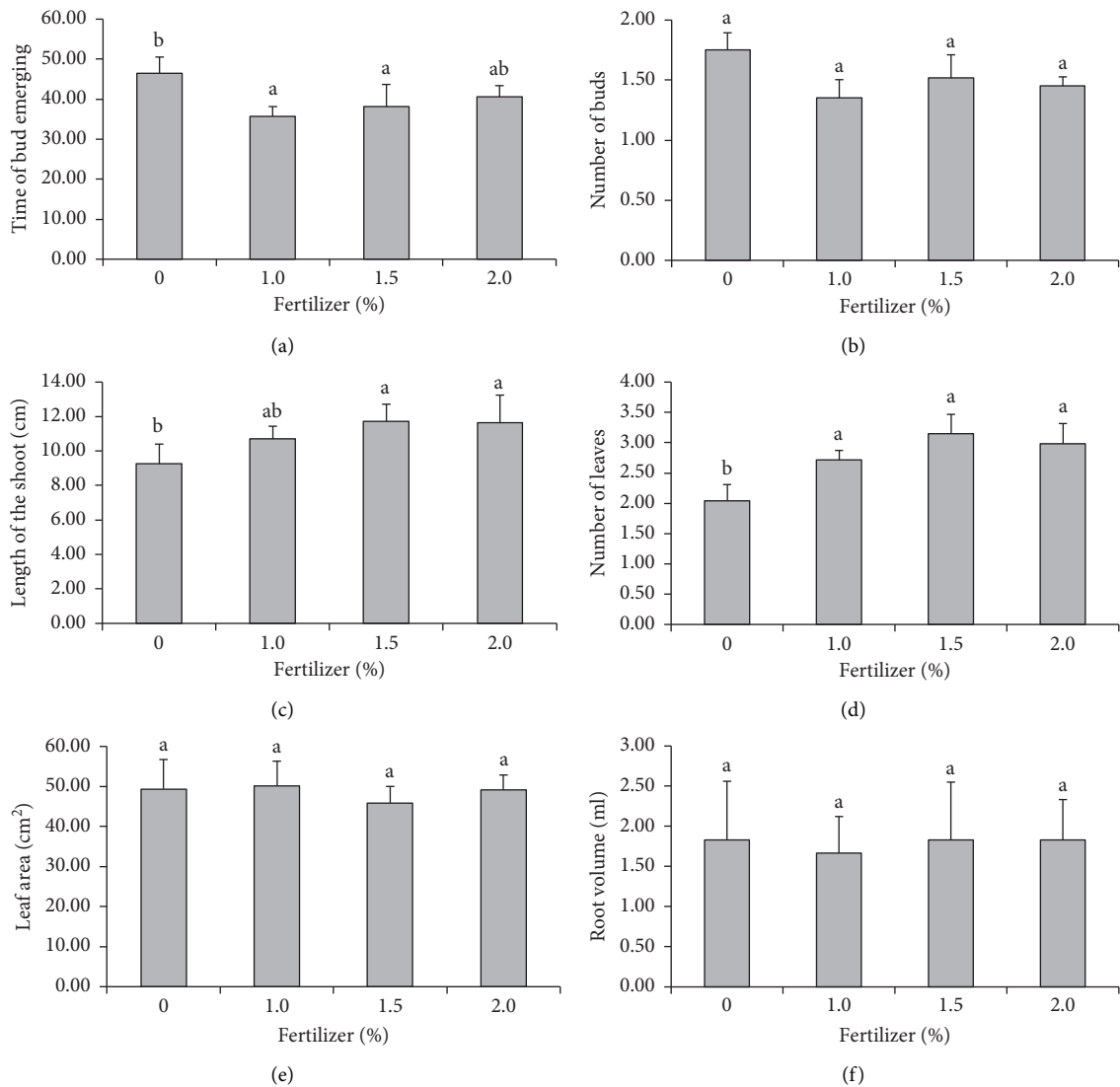


FIGURE 4: Effect of foliar fertilizer (FF) application on (a) emergence time of the shoot, (b) number of shoots, (c) shoot length, (d) the number of leaves, (e) leaf area, and (f) root volume of black pepper seedlings (after 8 weeks of planting). Different letters indicate significant differences among treatments as determined by Tukey's HSD test at the  $p < 0.05$  level. Vertical bars represent SE.

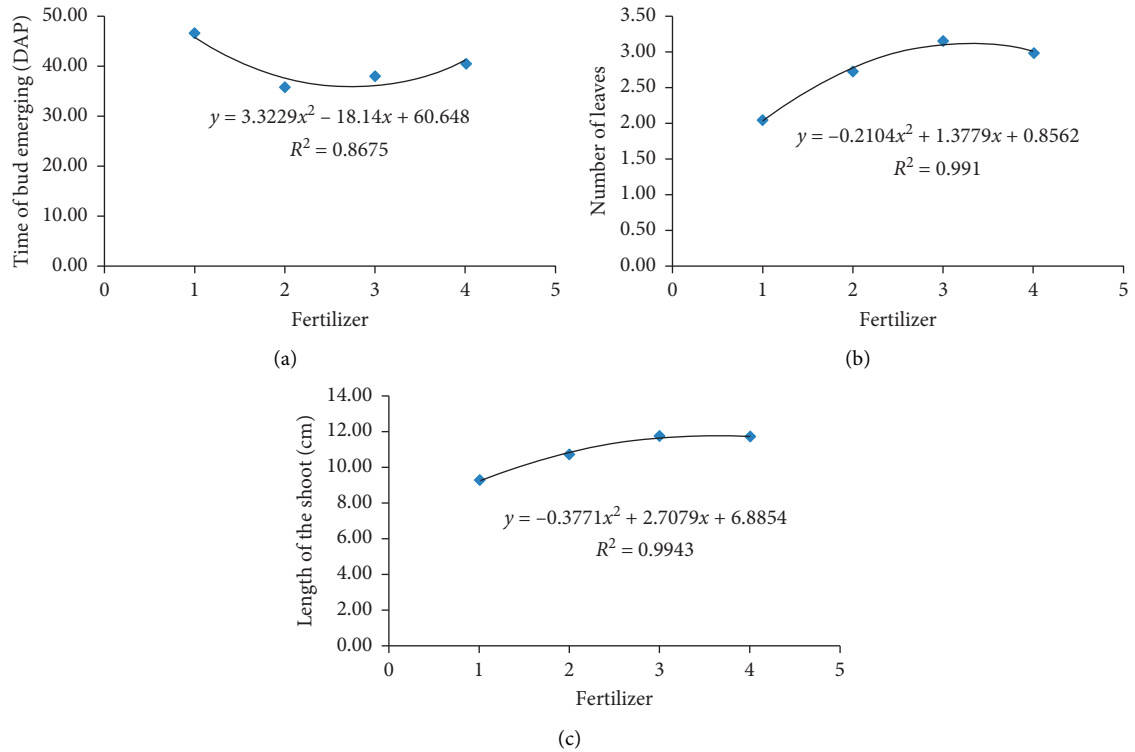


FIGURE 5: Regression analysis between foliar fertilizer application and time of bud emerging, number of leaves, and length of the shoot on pepper seedlings after 8 weeks of planting.

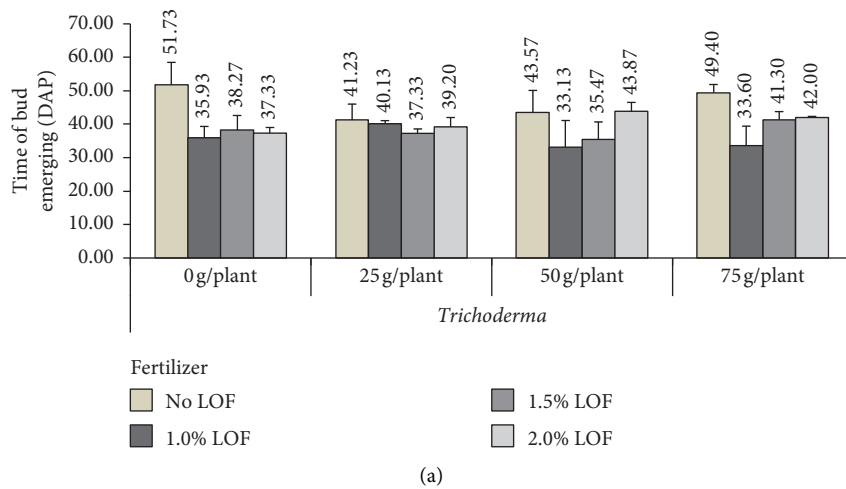


FIGURE 6: Continued.

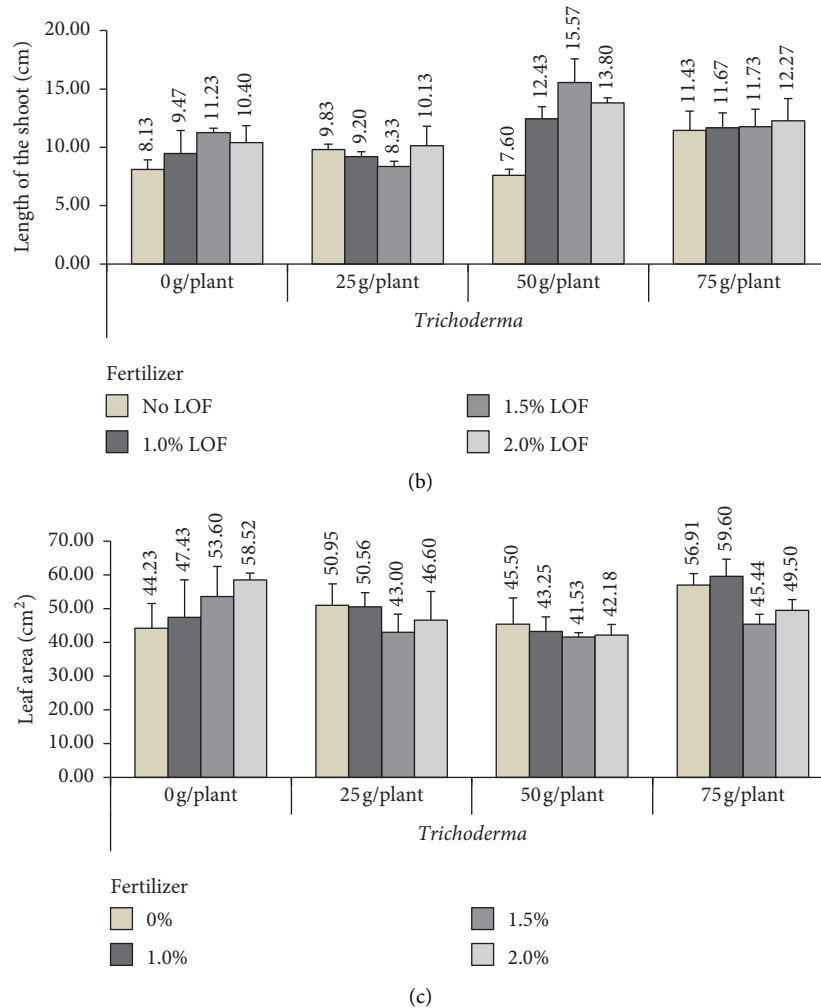


FIGURE 6: Effect of the combination treatment of *Trichoderma* and foliar fertilizer application on emergence time of the shoot (a), length of the shoot (b), and leaf area (c) of black pepper seedlings (after 8 weeks of planting). Vertical bars represent SE.

nutrients through the leaves is considered the right way to absorb nutrients.

The effect of the combination of *Trichoderma* and foliar fertilizer on the vegetative growth of black pepper seedlings can be seen in Figure 6. The combined application of *Trichoderma* and foliar fertilizer to pepper seedlings had a positive effect on shoot length, leaf numbers, and shoot emergence. Application of 50 g/plant of *Trichoderma* and 1.5% of foliar fertilizer resulted in 26% longer shoot length and 54% more leaves and accelerated the emergence time of shoots 10 days earlier compared to without *Trichoderma* and foliar fertilizer. *Trichoderma* 50 g/plant combined with foliar fertilizer at various concentrations is shown in Figure 7. This result was similar to studies reported by other researchers who found that foliar feeding of black pepper can only increase plant growth and production when used in combination with *Trichoderma* [42, 43]. Various mechanisms have been proposed to explain growth promotion including the control of minor pathogens, enhanced nutrient uptake, increased carbohydrate metabolism and photosynthesis, and



FIGURE 7: The length of the shoot of black pepper seedlings (after 8 weeks of planting) in combination with *Trichoderma* 50 g/plant at various concentrations of the foliar fertilizer.

phytohormone synthesis [23, 33, 44]. There is strong evidence for the role for microbe-produced indole acetic acid (IAA), although it is most likely that *Trichoderma* stimulates growth by influencing the balance of hormones such as IAA, gibberellic acid, and ethylene [45, 46]. Furthermore, *Trichoderma* species promote nutrient uptake by secreting organic acids to dissolve minerals and activate nutrients in the soil, leading to the circulation and utilization of nutrients in the soil [33]. At the same time, due to the strong colonization ability of *Trichoderma* species, they expand the contact area between the rhizosphere and soil and increase the secretion of extracellular enzymes such as sucrose, urease, and phosphatase, as well as organic acids in the rhizosphere, to improve nutrient cycling and enzyme activity in the soil [47]. *Trichoderma* inoculation increased the effective nutrient content and the soil enzyme activity to repair soil and promote plant growth [33]. The use of *Trichoderma*-based biological products will have an important role in agricultural production in the future, in light of changing worldwide perspectives by consumers and governing bodies.

#### 4. Conclusions

It was found that the application of *Trichoderma* can only increase plant growth when used in combination with foliar fertilizer application. The sole application of *Trichoderma* or foliar fertilizer led to inferior vegetative growth compared to what was obtained by their combined application. Nursery trial showed that the integrated *Trichoderma* and foliar fertilizer are the best options for the proper nutrient management of pepper seedlings. The application of both *Trichoderma* and foliar fertilizer influenced the growth rate of the length of the shoot, the number of leaves, and the emergence time of the shoot of black pepper seedlings under nursery conditions. There was a positive effect of the pepper seedling for the application of 50 g/plant of *Trichoderma* and 1.5% of foliar fertilizer which resulted in 26% longer shoot length and 54% more leaves and accelerated the emergence time of shoots 10 days earlier compared to without *Trichoderma* and the foliar fertilizer.

#### Data Availability

All data generated or analyzed during this study are included within this article.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Authors' Contributions

Netty Syam designed the field experiment, supervised the project, and wrote the manuscript. Akbar Nurdin assisted in the field data collection and conducted data analysis. Hidrawati helped in the preparation of the manuscript. St. Sabahannur helped in manuscript review.

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