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RESEARCH PAPER

Potential use of *Terasi*, an Indonesian traditional fermented seafood paste, to provide healthy lettuce (*Lactuca sativa* L.) seedling

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ABSTRACT

Terasi paste consists of plant nutrients and microorganisms that potentially improve the health of plant seedlings. We evaluated the effects of *terasi* paste on seed germination, seedling growth, and root rot disease severity caused by *Fusarium oxysporum* f.sp. *lactucae* (FOL) in lettuce. *Terasi* paste was tested at four different weights (i.e., 0.00 g [T0], 1.00 g [T1], 2.50 g [T2], and 5.00 g [T3]) in the 100 g nursery soil. The results showed that lettuce seeds from T1 and T2 treatments were fully germinated, as observed for the T0 treatment. However, 40.0% of lettuce seeds from T3 treatment failed to germinate. Thus, 1.00 g and 2.50 g terasi applications in the 100 g soil did not affect lettuce seed germination. Moreover, T1 and T2 treatments tended to improve the shoot and root biomass of lettuce seedlings over T0 treatment, while T3 treatment showed the opposite effects. Following FOL infection, the lettuce seedlings from T2 and T3 treatments had 31.1% significantly lower disease severity than those from the T0 treatment. Therefore, T2 treatment (i.e., 2.50 g terasi application in the 100 g soil) imparts maximum health-promoting benefits to lettuce seedlings.

Key words: Biological agent, fusarium root rot disease, plant growth-promoting microorganism, seedling, *terasi*

INTRODUCTION

In agriculture, the application of organic materials is recommended to enhance plant health (Nwe et al., 2001; Anwar et al., 2008; Marpaung et al., 2014) and sustain soil and environmental health (Anwar et al., 2008; Kasmawan et al., 2018). A healthy plant shows characteristics of vigorous growth, high production yield, and resistance to plant diseases. Healthy soil has high concentrations of plant nutrients and promotes beneficial activities of soil microorganisms. As the plant and soil health are strongly linked with each other, organic materials can significantly influence this association (Tahat et al., 2020). The significance of organic materials can be identified from the seedling preparation of the plant.

Lettuce has been cultivated worldwide and has emerged as a popular and an important diet because of its vitamin and mineral contents (Kim et al., 2016). In Japan, the market demand for lettuce has risen up to 7.40% from 2006 to 2018 (https://japancrops.com/en/crops/lettuce). Therefore, there is a progressive increase in the demand for healthy lettuce seedlings. However, fusarium root rot disease of lettuce caused by *Fusarium oxysporum* f.sp. *lactucae* Matuo et Motohashi (FOL) has posed a serious concern in lettuce cultivation because the infection occurs during seedling preparation and plant growth. The outer leaves of the infected plants or seedlings turn yellowish-brown, which significantly affects the plant growth and production (Matuo & Motohashi, 1967). Therefore, it is crucial to develop a method to control fusarium root rot disease during the seedling preparation to provide healthy lettuce seedlings.

Terasi is a popular fermented Indonesian product derived from seafood and served as a condiment-like agent, rich in glutamate which imparts a specific flavor (Prihanto & Muyasyaroh, 2021). The *terasi* paste contains 25.4–42.5% proteins (Surono & Hosono, 1994a; Ukhty et al., 2017; Romadhon et al., 2018), 23.2–46.4% amino acids (Ali et al., 2020), and 1.51–2.41% total nitrogen (Ukhty et al., 2017; Ali et al., 2020). Nitrogen sources (i.e. ammonium and nitrate) are essentially absorbable nutrients for vegetables such as lettuce (Andriolo et al., 2006; Savvas et al., 2006). Nitrogen can also be supplied from proteins and amino acids through the complex biochemical reactions involving enzymes (e.g. leucine, arylamidase, α-glucosidase, and β-glucosidase) present in the *terasi* paste (Surono & Hosono, 1994b). Amino

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acids, such as glutamate, cysteine, phenylalanine, and glycine, can promote plant growth and defense against abiotic stresses (Teixeira et al., 2017).

The *terasi* paste is enriched with several beneficial microorganisms such as *Bacillus* sp., *Pseudomonas* sp. (Surono & Hosono, 1994a; Surono & Hosono, 1994b) and lactic acid bacteria (LAB) (e.g. *Tetragenococcus* sp. (Kobayashi et al., 2003), *Lactobacillus* sp. (Amalia et al., 2018), and other LAB (Romadhon et al., 2018)). Some of the identified microorganisms have been known as plant growth-promoting (PGP) microorganisms and biological control agents (BCAs). Some microbes can function both as PGPs and BCAs. For instance, *Bacillus* sp. and *Pseudomonas* sp. produce plant growth regulators and compete with plant pathogens (Bhattacharyya & Jha, 2012; Brahmaprakash et al., 2017). LAB can act as BCAs by producing lactic acid, which exerts an antibacterial activity and inhibits growth of pathogenic bacteria (Romadhon et al., 2018). Thus, the *terasi* paste not only serve as a source of bacteria but also as a reservoir with different bioactivities (Prihanto & Muyasyaroh, 2021).

Terasi has been used in agriculture as one of the ingredients of home-made liquid organic fertilizer (Anwar et al., 2008; Marpaung et al., 2014; Kasmawan et al., 2018). Chinese cabbage (Anwar et al., 2008) and potato (Marpaung et al., 2014) plants are vigorously grown under fertilization with the liquid organic fertilizers. A fermented shrimp paste and sauce from Myanmar similar to *terasi* was mixed with agricultural waste (i.e. straw, beda, compost, animal waste, and night soil) and positively tested as an organic fertilizer that could improve the growth and yield of rice plant (Nwe et al., 2001). These studies demonstrate the potential of fermented seafood products to improve plant health. Nonetheless, there are no studies about the potential of *terasi* alone in a nursery phase of vegetables to provide healthy seedlings.

The present study was performed to evaluate the potential of *terasi* to provide healthy lettuce seedlings by promoting seedling growth and suppressing fusarium root rot disease. We tested the *terasi* paste in four different weights (i.e. 0.00; 1.00; 2.50; and 5.00 g) by mixing in the soil and hypothesized that the application of 2.50 g of *terasi* paste would provide maximum benefits for lettuce seedlings. The results of this study will be applicable in the agriculture sector, especially for vegetable cultivation system.

MATERIALS AND METHODS

Research Site. The research was carried out in The Center for International Field Agriculture Research and Education, College of Agriculture, Ibaraki University, Japan.

Treatment And Research Design. We used 0.00 g (T0), 1.00 g (T1), 2.50 g (T2), and 5.00 g (T3) *terasi* paste mixed in 100 g commercial soil to cultivate vegetable seedlings. *Terasi* paste was purchased from a local market in Yogyakarta, Indonesia. The treated soils were incubated for two weeks to allow for adjustment (Nwe et al., 2001; Marpaung et al., 2014) prior to cultivation of seedlings. The ready treated soil was filled in a L57 × W57 × H50 mm^3 pot.

The research was set in a randomized complete block design with three replicates. Each replicate comprised 20 pots, including 10 pots for seed germination and seedling growth evaluation and 10 pots for FOL inoculation test.

Seed Germination and Seedling Growth. Seeds of lettuce (*Lactuca sativa* L. var. *capiata* 'summer green') were sown in the treated soil and maintained in an incubator at 25 °C and 60% relative humidity. The number of germinated seeds was counted at four days after sowing (DAS).

In each replicate, five lettuce seedlings with average growth were selected for growth evaluation. The seedling growth was evaluated as leaf number, shoot biomass, and root biomass at 25 DAS. Fully opened leaves were counted per seedling. The shoot and root of lettuce seedling were separated. The root was gently washed to removed any attached soils. The shoot and root were then oven-dried at 60 °C for four days and weighted.

FOL Inoculation and Disease Severity Evaluation. The FOL strain H111 (Yamauchi et al., 2005) was grown in potato dextrose agar for a week. FOL conidia were harvested and used to prepare a suspension in sterile distilled water. The conidia density was counted using a haemocytometer (Fukae Kasei Co., Ltd., Kobe, Japan) and adjusted to 1×10^6 conidia mL^{-1} water. For each replicate, five lettuce seedlings with average growth at 25 DAS were selected and inoculated with 5 mL FOL suspension.

A week later, yellowish-brown leaves were observed as the disease symptom. The disease severity was analyzed following three steps. First, the number of yellowish-brown leaves per total number of leaves was recorded as a percentage. Second, the score of disease per seedling was determined based on the percentage of yellowish-brown leaves listed in Table 1. Third, the disease severity was calculated using the following formula:

$$\text{Disease severity}(\%) = \frac{\Sigma\{Z \times N\}}{\{z \times n\}}$$

Z = the specific score;
N = the number of seedling with the specific score;
z = the highest score;
n = total number of seedling.

Data Analysis. One-way analysis of variace (ANOVA) followed by Tukey's honestly significant difference (HSD) test at $P < 0.05$ was performed using R v.3.4.1 (R Core Team, Vienna, Austria) to determine the effects of different treatments on the lettuce seedling growth and disease severity. The data in percent of lettuce disease severity were arcsine transformed prior to statistical analysis.

RESULTS AND DISCUSSION

The T1 and T2 treatments resulted in the complete germination of lettuce seeds, consistent with the observations reported in the T0 treatment (Table 2). Thus, the application of 1.00 g and 2.50 g *terasi* paste in the 100 g nursery soil did not affect lettuce seed germination. However, T3 treatment led to a 40.0% decrease in the number of germinated lettuce seeds. This result may be attributed to the higher levels of amino acids from *terasi* paste in the T3 treatment soil than in T1 and T2 treatments soil that could negatively affect seed germination. As per the study of Surono & Hosono (1994a), 1.00–5.00 g *terasi* paste may contain 0.01–0.18% amino acids. We expect that the soil with 5.00 g *terasi* paste had the highest amino acid levels. Teixeira et al. (2017) similarly reported that the accumulation of different types of amino acids (i.e. glutamate, cysteine, phenylalanine, and glycine) at > 0.12% concentrations either separately or in combination could disturb the cell metabolic regulation and subsequently seed germination. This finding may explain the observation reported for the T3 treatment in our study.

Consistent with the effects of *terasi* on lettuce seed germination, the number of lettuce leaves in T3 treatment was lower than that in other treatments (Table 2). Thus, the development of lettuce leaves was suppressed by the application of 5.00 g *terasi* paste in the 100 g nursery soil. Contrarily, the application of 1.00 g and 2.50 g *terasi* paste in the nursery soil resulted in the vigorous development of lettuce leaves, as observed in seedlings without *terasi* paste (T0 treatment). The development of lettuce leaves depends on the enlargement of cortical cells of the lettuce radicle during the germination phase, which is affected by plant growth regulators including indole-3-acetic acid [IAA] and gibberellins [GAs] (Mangmang et al., 2014). The IAA and GAs are produced by PGPs, such as *Bacillus* sp. and *Pseudomonas* sp. (Bhattacharyya & Jha, 2012; Brahmaprakash et al., 2017), which are present in the *terasi* paste (Surono & Hosono, 1994a; Surono & Hosono, 1994b). Therefore, based on our results, we assume that substantial IAA and GAs levels may be produced in the soil supplemented with 1.00 g and 2.50 g *terasi* paste that supported the growth of lettuce leaves. However, quantity of IAA and GAs may be over expressed in the soil with 5.00 g *terasi* paste, thereby inhibiting the lettuce radicle development and consequently leading to fewer leaves.

The shoot and root biomass of lettuce seedlings from T1 and T2 treatments tended to be higher than those of the seedlings from the T0 treatment and significantly higher than those of the seedlings from the T3 treatment

Table 1. Score of disease based on the percentage of yellowish-brown leaves of lettuce seedling caused by FOL

Yellowish-brown leaves (%)	0.00	< 10.0	10.1–20.0	20.1–30.0	30.1–50.0	50.1–70.0	> 70.1
Score of disease	0	1	2	3	4	5	6

Table 2. Effects of *terasi* paste on lettuce seed germination and lettuce leaf number

Treatment	Number of germinated seed	Number of leaves per seedling
T0	10.00	6.27 a
T1	10.00	7.53 a
T2	10.00	6.80 a
T3	6.00	3.33 b

T0, T1, T2, and T3 are treatments using 0.00; 1.00; 2.50 g; and 5.00 g *terasi* paste mixed in 100 g soil, respectively. Different letters in the column for number of leaves show the significant difference among the treatments based on Tukey's HSD at $P < 0.05$ ($n = 3$).

(Figure 1). Thus, the health of lettuce seedlings in terms of above-and below-ground biomass was positively influenced by the treatments with 1.00 g and 2.50 g *terasi* paste. One of the reasons underlying this effect may be the adequate concentration of ammonium (Caliskan et al., 2014) in range of 21.1–63.3 mg $NH4^+$-N per kg of soil (Savvas et al., 2006), which can be obtained from *terasi* paste that has 1.51–2.41% nitrogen content (Ukhty et al., 2017; Ali et al., 2020). This assumption is also based on the result of Marpaung et al. (2014) who demonstrated that 0.05 g *terasi* paste in an intermediate dose (6 mL L^{-1} water) of liquid organic fertilizer supplied sufficient amount of nitrogen to increase the height of potato plant. Anwar et al. (2008) also showed that, 3.52 g *terasi* paste in a 100 mL liquid organic fertilizer supplied 0.35% nitrogen, which was sufficient to promote Chinese cabbage growth.

Considering the suppressive effects of T3 treatment on the shoot and root biomass of lettuce seedlings, the application of 5.00 g *terasi* paste in the 100 g nursery soil continuously and negatively influenced the health of lettuce seedlings. The soil with 5.00 g *terasi* paste may have higher nitrogen concentration than other treated soils. However, the high nitrogen concentration in T3 treatment may be toxic to lettuce seedlings, as concluded by Andriolo et al. (2006) who noted that high ammonium concentration (45.1–180 mg $NH4^+$-N kg^{-1} soil) suppressed lettuce growth. In potato cultivation, high dose (9 mL L^{-1} water) of liquid organic fertilizer from *terasi* paste caused wilting to the plant owing to nitrogen toxicity (Marpaung et al., 2014). Given the importance of nitrogen to the health of lettuce seedlings, evaluation of its concentration in the *terasi*-treated soil is necessary in the future.

Soil pH is another factor affecting lettuce seed germination and seedling growth, which is not investigated in this study. The pH of *terasi* paste is 5.67–7.53, which increases with changes in nitrogen forms (Surono & Hosono, 1994a; Ukhty et al., 2017). Thus, the application of *terasi* paste in the nursery soil may change the pH levels of the treated soil. In lettuce cultivation system using organic materials, lettuce plant requires the soil pH to be maintained at approximately 7.80 (Caliskan et al., 2014). However, in the T3 treatment, the change in the pH of the nursery soil may be unsuitable for lettuce seed germination and seedling development. It is necessary to evaluate the changes in the soil pH and their effects on the health of plant

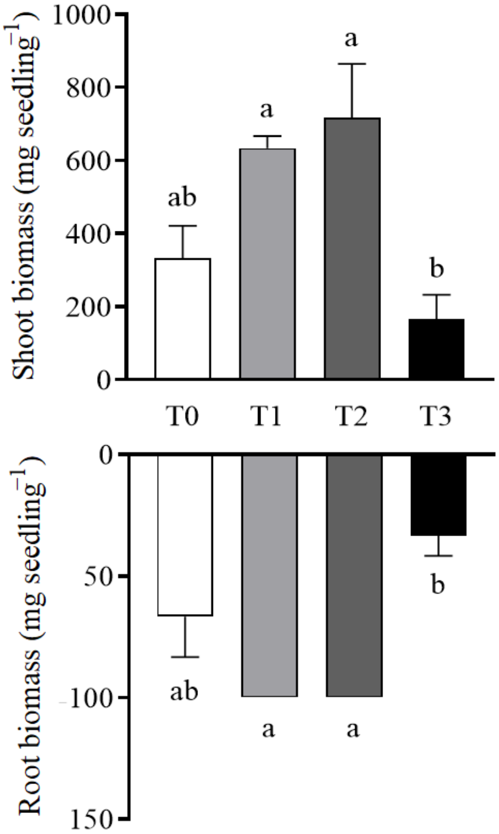

Figure 1. Effects of *terasi* paste on above-and below-ground biomass of lettuce seedlings. T0, T1, T2, and T3 were the treatments using 0.00; 1.00; 2.50; and 5.00 g *terasi* paste in 100 g soil, respectively. Different letters in each bar of each parameter show the significant difference among the treatments based on Tukey's HSD at P< 0.05 (n= 3). Vertical bars show the standard error.

seedlings following *terasi* paste application in the future.

The increase in the lettuce biomass observed in T1 and T2 treatments may be associated with the biological activities of PGPs. PGPs, such as *Bacillus* sp. and *Pseudomonas* sp., occupy plant roots and mutually interact with the plant by receiving carbon source from the plant root exudates and producing IAA and GAs, which induce root formation and regulation (Bhattacharyya & Jha, 2012; Brahmaprakash et al., 2017; Tahat et al., 2020). Therefore, IAA and GAs indirectly stimulate the absorption of inorganic nitrogen from the soil into lettuce seedlings and improve the shoot biomass (Mangmang et al., 2014). We suggest that *Bacillus* sp. and *Pseudomonas* sp. from *terasi* paste (Surono & Hosono, 1994a; Surono & Hosono, 1994b) inhabited the roots of lettuce seedlings and contributed to the improvement in the lettuce root and shoot biomass, especially in the case of T1 and T2 treatments (Figure 1). Future studies should investigate the amount of nitrogen absorbed by lettuce seedlings and clarify the potential of *terasi* as an organic fertilizer in promoting lettuce shoot biomass at the final harvest time, as reported by Nwe et al. (2001) in rice plant, Anwar et al. (2008) in Chinese cabbage, and Marpaung et al. (2014) in potato plant.

The other parameter related to the seedling's health is the resistance to plant disease. In comparison with the T0 treatment, the T1 treatment showed relatively lower disease severity and T2 and T3 treatments showed clear suppression of the fusarium root rot severity (Figure 2). This result indicates that fusarium root rot disease in lettuce seedlings could be controlled by prior application of 2.50 g and 5.00 g *terasi* paste into the 100 g nursery soil. However, lettuce seedlings from the T2 treatment (Figure 3C) were healthier than those from the T3 treatment (Figure 3D) and other treatments (Figure 3A–B), as evident from their greener color. This result was in accordance with the result of Marpaung et al. (2014) who demonstrated that 6 mL L^{-1} water of liquid organic fertilizer consisted of *terasi* paste showed the lowest rate of *Phytophthora infestans* disease infection in potato plant among all of the tested doses (i.e. 9, 6, and 3 mL L^{-1} water).

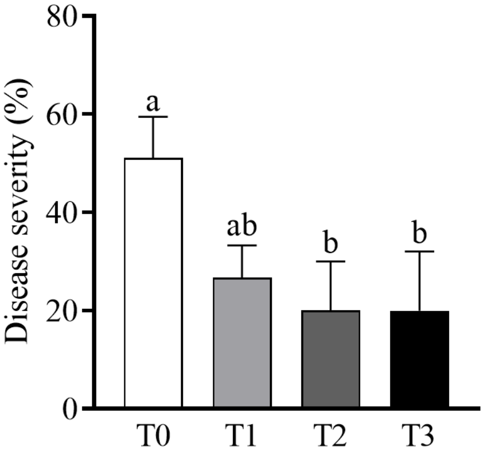

Figure 2. Effects of *terasi* paste on fusarium root rot disease severity in lettuce seedlings. T0, T1, T2, and T3 are treatments using 0.00; 1.00; 2.50; and 5.00 g *terasi* paste in 100 g soil, respectively. Different letters in each bar show the significant difference among the treatments based on Tukey's HSD at $P < 0.05$ (n = 3). Vertical bars show the standard error.

Figure 3. Yellowish-brown leaf symptom of fusarium root rot disease in lettuce seedlings cultivated in soil. (A) 0.00 g; (B) 1.00 g; (C) 2.50 g; and (D) 5.00 g *terasi* paste.

The suppression of fusarium root rot disease in lettuce seedlings from the T2 and T3 treatments may be associated with the anti-pathogenic activities of BCAs from the 2.50 g and 5.00 g *terasi* paste. In particular, *Pseudomonas* sp. may produce lytic enzymes to hydrolyze cells (Brahmaprakash et al., 2017) of FOL and reduce the number of FOL conidia. *Bacillus* sp. (Brahmaprakash et al., 2017) and LAB (Romadhon et al., 2018) can antibiotically poison or kill FOL, and consequently ameliorate its pathogenicity. This assumption is strongly linked to the study by Chinta et al. (2014), where lactic acid from corn steep liquor, an organic fertilizer, was suggested to be toxic against FOL. These phenomena of anti-pathogenic activities of beneficial microorganisms in the *terasi* paste may have occurred in the present study. In terms of the unclear effect of T1 treatment on the disease severity over other treatments (Figure 2), we assume that the lower amount of the *terasi* paste applied to the nursery soil led to a lower activity of BCAs and the produced antibacterial compounds such as lactic acid. Therefore, the application of 1.00 g *terasi* paste in the 100 g nursery soil was ineffective against FOL. Further research is warranted to evaluate the density of FOL conidia in the soil and the anti-pathogenic activity of *terasi* paste and its beneficial bacteria.

CONCLUSION

The application of 2.50 g *terasi* paste to the 100 g nursery soil is the most potent method to provide the healthy lettuce seedlings owing to the positive effects on seed germination and leaf development, relative improvement in the lettuce shoot and root biomass, and suppression of the lettuce fusarium root rot disease. The application of 1.00 g and 5.00 g *terasi* paste per 100 g nursery soil resulted in inconsistent effects on the health of lettuce seedlings. Further studies are necessary to clarify the mechanisms (e.g. appropriate nitrogen concentration and activities of PGPs and BCAs) underlying the effects of *terasi* paste.

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AUTHORS' CONTRIBUTIONS

YDC considered, planned, and carried out the experiment and performed the data analysis. TS provided the research funding source, supervised the experiment, and validated the data analysis. All the authors have prepared the manuscript and have read and approved the final manuscript.

COMPETING INTERESTS

We declare there are no relevant financial or non-financial competing interests to report.

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