The effect of method and dosage application of biofungicide extract of Legundi leaf fermented with *Trichoderma harzianum* fungus for control of Fusarium wilt disease on shallots

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Abstract. Legundi weed (*Vitex trifolia*) has the potential to be used as a biofungicide to control Fusarium wilt disease on shallots. For the manufacture of biofungicides, legundi leaf extract was first fermented using the fungus *Trichoderma harzianum*. The aim of this study was to determine the effect of the method and dose of fermented Legundi leaf extract biofungicide on the Fusarium fungus that causes shallots wilt. The research was carried out in Senteluk Village, Batu Layar District, West Lombok Regency using an experimental method with a factorial randomized block design with two factors. As the main effect, namely the application of biofungicide fermented Legundi leaf extract consisting of two levels, namely: seed treatment before planting, and spraying treatment for plants aged 7 days after planting. Meanwhile, the simple effect is the application dose of fermented Legundi leaf extract biofungicide consisting of 5 levels, namely: 0.00 mL, 2.50 mL; 5.00 mL; 7.50 mL; and 10.00 mL. The combination method and dose of fermented Legundi leaf extract biofungicide was repeated three times each, so there were 30 treatment combinations. The results obtained were the application of Legundi leaf extract biofungicides by soaking the seeds before planting at a starting dose of 2.50 mL to effectively control onion wilt disease. The applied biofungicide can increase plant height and increase the dry weight of the harvest.

Keywords: Dosage, biofungicide, Legundi, *Trichoderma*, Fusarium, shallots.

1. Introduction

Legundi (*Vitex trifolia*) is one of the wild plants or weeds that has the potential to be used as a base material for the manufacture of vegetable fungicides. Vegetable fungicides are substances contained in a plant or wild plant or weed that can control diseases caused by fungi. This vegetable fungicide should be fermented first using the fungus *Trichoderma harzianum* so that it becomes a vegetable biofungicide. The results of a preliminary study of the biofungicide of fermented legundi leaf extract can inhibit the development of the fungus *Fusarium oxysporum* f. sp. *cepea* causes wilt in shallots plants [1].

Legundi has pharmacological effects such as antibacterial, antifungal, insecticide, anticancer, analgesic, tracheospasmolytic, antiallergic and antipyretic [2]. These plants contain chemical compounds such as essential oils and can act as antibacterial and antifungal [3]. The use of plant-based fungicides in addition to inhibiting disease development is also safe for consumers and the environment because it is easily biodegradable and does not leave residues on agricultural products [4].

The use of biofungicides from fermented legundi leaf extract is important because of its ability to break dormancy of chlamydospores of Fusarium fungus. According to Sudantha [5] that Fusarium fungi have chlamydospores that can live as saprophytes in the soil for about 3-4 years even without a host plant and are difficult to control using chemical fungicides.

Preliminary tests carried out at home laboratories, greenhouses and in the field showed that the fermented legundi leaf extract of *Trichoderma* can inhibit wilt disease in shallot plants [6] and can stimulate the growth of shallot plant height [7].

However, it is not yet known how the application and dosage of this fermented Legundi leaf extract biofungicide. As an illustration, Sudantha *et al*. [8] (2020) stated that the biofungicide of
fermented coffee leaf extract which was applied to the treatment of shallot bulbs before planting at a
dose of 5 mL/plant was effective in controlling wilting of shallot plants.

Based on the description that has been stated, a study was carried out with the aim of obtaining
the optimal dose of biofungicide from fermented Legundi leaf extract of *T. harzianum* and its application
in inhibiting Fusarium wilt and its effect on plant height and weight of harvested dry shallots.

2. Methodology

2.1. Production of Biofungicide Fermented Legundi Extract *T. harzianum*

Figure 1 shows the colony and morphology of the *Trichoderma* fungus used for biofungicide
fermentation of Legundi leaf extract. The culture and suspension procedures were in accordance with
the Sudantha 2019 procedure [9].

Legundi weeds (Figure 2) were used for the manufacture of biofungicides whose manufacturing
procedures were in accordance with the Sudantha 2020 procedure [10], namely: 10 kg of Legundi leaves
were dried and crushed by blending. The crushed Legundi leaves are put into a container containing 5
liters of water, then kneaded by hand to produce an extract. Furthermore, the Legundi leaf extract was
put into a jiregen and inoculated with a suspension of the fungus *T. harzianum* and covered with opaque
paper or porous paper and tied with rubber. Jiregen that has been tightly closed is stored in a cool place
and not exposed to direct sunlight. Storage time for 7 days for the fermentation process by the fungus
*T. harzianum*. After 7 days, the jiregen was opened and filtered to separate the fermented solution from
the Legundi leaf dregs. The filtered solution has become a biofungicide of Legundi leaf extract and is
ready to be used for experiments.

![Figure 1. Colony and morphology of the fungus *T. harzianum* (scale 1: 10 µm).](image)

The shallot seeds used were Keta Monca varieties purchased from seed breeders. Shallot seeds
that are good to use are healthy and quality seeds with a shelf life of 2 months and there are visible
growing points on the roots. The day before planting the seeds were cut off about parts [11].
Figure 2. Legundi plant (scale 1:8 cm) and Legundi leaf extract for biofungicide.

2.2. Experimental design
The study was conducted at the Senteluk shallot planting center, Batu Layar, West Lombok using a randomized block design with a factorial experiment. As the main effect, namely the application method of fermented Legundi leaf extract biofungicide which consists of two levels, namely: seed treatment, and spraying treatment for plants aged 7 days after planting. Meanwhile, the simple effect is the application dose of Legundi leaf extract biofungicide consisting of 5 levels, namely: 0.00 mL, 2.50 mL; 5.00 mL; 7.50 mL and 10.00 mL. Methods and doses of biofungicides of fermented Legundi leaf extract were combined, each of which was repeated three times, so that there were 30 treatments.

2.3. Trial Execution
The size of the experimental plots was 2 m x 4 m and each experimental plot was covered with plastic mulch with planting holes made with a distance of 20 cm x 20 cm. Phonska fertilizer as much as 100.0 kg/ha was used as basic fertilizer. Legundi leaf extract biofungicide was given according to the treatment, namely soaking the seeds before planting and spraying after the plants were 7 days old. Weeds that grow around the plant are removed. Follow-up fertilization in the form of Urea as much as 165.0 kg/ha and KCl 50.0 kg/ha was carried out when the plants were 35 days after planting. Shallots were harvested when the plants were 62 days after planting.

Variable Observation
Observation of disease intensity was carried out until the plant was 35 dap with the following formula:

\[ I = \frac{n}{N} \times 100\% \]

- \( I \) = Disease intensity
- \( n \) = Number of diseased plants
- \( N \) = Total number of plants observed
Observation of plant height was carried out by measuring plant height starting from the base of the stem to the growing point of the plant or to the tip of the new young leaves growing. Observation of the number of tubers per plant was carried out after the plants were harvested. Observation of harvested dry tuber weight was carried out by weighing all parts of the plant/plot at the time of harvest, then converted to tons/ha. Observation of the weight of stored dried shallot bulbs was carried out by weighing all parts of the plant/plot that had been stored and air-dried for 30 days, then converted to tons/ha.

2.4. Data analysis
Observational data were analyzed using Diversity Analysis at the 5% significance level and further tested using the Honestly Significance Different (HSD) test at the same level.

3. Results and Discussion

3.1. Effect of Method and Dosage of Biofungicide Extract of Fermented Legundi Leaves of T. harzianum on Disease Intensity

Based on the analysis of diversity, it turned out that the interaction between methods and doses of biofungicide Legundi leaf extract was significantly different on disease intensity at 35 dap and the results of a follow-up test using HSD 0.05 are listed in Table 1.

Table 1. Average disease intensity at 35 dap as a result of the use of fermented Legundi leaf extract biofungicides.

| Biofungicide Dosage Treatment of Fermented Legundi Leaf Extract T. harzianum | Withering Disease Intensity (%) |
|---|---|
| | Seed Soak | Plant Spraying |
| Without Biofungicide | 50.67 a | 59.33 a |
| Biofungicide 2.5 mL | 0.00 a | 15.60 b |
| Biofungicide 5.0 mL | 0.00 a | 10.60 b |
| Biofungicide 7.5 mL | 0.00 a | 10.30 b |
| Biofungicide 10.0 mL | 0.00 a | 10.20 b |

The results of the 0.05 HSD test in Table 1 show that the control of wilt disease intensity was highest compared to all doses of biofungicide treatment, both applied by soaking the seeds before planting or by spraying the plants after 7 days after planting. However, from these two application methods, it turned out that the treatment by soaking the shallot seeds with doses ranging from 2.5 mL to 10.0 mL was more effective than the spraying treatment of shallot plants at the age of 7 days after planting, even until the plants aged 35 days after planting were not infected. Fusarium wilt disease or shallot plants become resistant and their induced resistance to Fusarium wilt increases. It is suspected that from the beginning the shallot seeds planted had defense against pathogenic fungal attacks because they had been treated with a biofungicide from the fermented Legundi leaf extract of T. harzianum. This means that simultaneously the effect of secondary metabolites contained in Legundi leaf extract and the antagonism effect of the fungus T. harzianum is able to control Fusarium wilt disease. According to Nugroho and Alam [2], Legundi leaf extract has pharmacological effects on pest organisms including antibacterial, antifungal, insecticide, anticancer, analgesic, tracheospasmytic, antiallergic and antipyretic. Similarly, Sudantha and Suwardji [12] reported that the use of fermented biofungicide
Trichoderma spp. in liquid, powdery and solid formulations treated by soaking the seeds and early planting can increase the resistance of shallots induced to Fusarium wilt disease.

The role of the fungus *T. harzianum* in controlling Fusarium wilt disease was also reported by several researchers, namely: The fungus *T. harzianum* can suppress soil-borne pathogenic fungi through three mechanisms, namely as mycoparasites that can penetrate mycelium and chlamydospores of pathogenic fungi resulting in lysis and crystallization, produce antibiotics (gliotoxin and viridin) that can inhibit the growth of pathogenic fungi, and have the ability to grow faster so that there is competition for space and nutrients with other fungi [13], [14], [15], [16] and 17. Furthermore, the role of *Trichoderma* in controlling pathogenic fungi physically by means of hyphae entanglement, cell lysis, enzymatic mycoparasite antibiosis, cross-protection and induced systemic resistance [18], [19], [20], [21] and [22].

Figure 3A describes healthy shallot plants due to treatment with the Legundi biofungicide, while Figure 3B describes plants infected with the *F. oxysporum* fungus which show symptoms of poor growth, namely some of the leaves are curved and twisted, the color of the leaves is pale green to yellow and over time it becomes dry. This is in accordance with the results of the study of Duriat *et al.* [23] i.e. the shallot plant wilts rapidly, the roots rot, the plant looks like it will collapse, and the leaves are yellowish with a slightly curved shape. Supriyadi *et al.* [24] in his research also reported that red bottom plants infected with Fusarium wilt disease showed yellowing of leaves from the tip to the base of the leaves, twisted plant leaves, and in advanced attacks caused the plants to fall and die.

The superiority of the fungus *T. harzianum* in controlling several plant diseases was also reported by several researchers, including: The fungus *T. harzianum* has the potential to be used to control onion bulb rot disease by the pathogen *F. proliferatum* [25]. Seed treatment with the fungus *T. harzianum* on soybeans can reduce the occurrence of charcoal rot disease caused by the fungus *M. phaseolina* [26]. The fungus *T. harzianum* can inhibit disease and is able to stimulate stem length, roots and production [27]. Secondary metabolites produced by microbes have the potential as biopesticides as a substitute for chemical pesticides that tend to damage the environment [28]. *Trichoderma* fungi have high adaptability to several ecological environments including drought [29].
3.2. Effect of Method and Dosage of Biofungicide Extract of Fermented Legundi Leaves of T. harzianum on Plant Height and Number of Shallots

Plant height and number of shallots analyzed by Diversity Analysis showed that there was a significant interaction between the method and dose of biofungicide of fermented Legundi leaf extract of T. harzianum on plants aged 35 days after planting, further tests using 5% HSD as presented in Tables 2 and 3.

Table 2. Effect of biofungicide method and dose of Legundi leaf extract fermented by T. harzianum on plant height at 35 dap.

| Biofungicide Dosage Treatment of Fermented Legundi Leaf Extract T. harzianum | Shallot Plant Height (cm) at 35 dap |
|-----------------------------------------------------------------------------|-----------------------------------|
|                                                                             | Seed Soak                         |
|                                                                             | Plant Spraying                     |
| Without Biofungicide                                                        | 12.25 a                           |
|                                                                             | A                                 |
| Biofungicide 2.5 mL                                                        | 28.77 b                           |
|                                                                             | B                                 |
| Biofungicide 5.0 mL                                                        | 28.67 b                           |
|                                                                             | B                                 |
| Biofungicide 7.5 mL                                                        | 28.70 b                           |
|                                                                             | B                                 |
| Biofungicide 10.0 mL                                                       | 28.90 b                           |
|                                                                             | B                                 |

Biofungicides applied at various doses showed better growth in plant height and number of leaves than those without biofungicides, either treated by soaking the seeds or by spraying plants. Of the two methods of biofungicide treatment, it turns out that soaking the seeds is better than spraying on plants (see Table 2 and Table 3). The difference in the growth of shallots was due to the biofungicide treatment by soaking the seeds with doses ranging from 2.5 mL to 10.0 mL which was more effective in suppressing disease compared to spraying treatment of plants aged 7 days after planting. Thus, with treatment with biofungicide, shallot plant growth was better than without biofungicide. In this case, the content of biofungicide in the form of the fungus T. harzianum plays a role in stimulating the growth of shallots. Several researchers reported that the fungus T. harzianum can increase seed germination and
plant growth [30]. The fungus *T. harzianum* has the same role as the growth regulator of Benzyl Amino Purine which can stimulate plant growth and increase the yield of shallot bulbs [11]. *T. harzianum* mushroom given in the form of tablet biocompost at a dose of 5 g/plant resulted in better shallot plant height growth [31]. *T. harzianum* given to soybean plants caused higher plant height growth and yields compared to controls [32]. *Trichoderma* spp. can stimulate plants to produce hormones that can spur plant growth to be optimum. The roots formed are longer and the stems are taller, and the tubers are more numerous [33].

3.3. Effect of Method and Dosage of Biofungicide Extract of Fermented Legundi Leaf Extract of *T. harzianum* on Shallot Yield

The method and dosage of fermented Legundi leaf extract biofungicides affect the yield component and the interaction effect of these two factors on the yield component is presented in Tables 4, 5 and 6.

### Table 4. Effect of biofungicide method and dose of fermented Legundi leaf extract of *T. harzianum* on the number of tillers of plants at 35 dap.

| Biofungicide Dosage Treatment of Fermented Legundi Leaf Extract *T. harzianum* | Number of Shallots per clump (cloves) |
|---|---|
| | Seed Soak | Plant Spraying |
| Without Biofungicide | 5.28 a A | 5.15 a A |
| Biofungicide 2.5 mL | 12.58 b B | 8.45 a B |
| Biofungicide 5.0 mL | 12.60 b B | 8.45 a B |
| Biofungicide 7.5 mL | 12.60 b B | 8.48 a B |
| Biofungisida 10.0 mL | 12.70 b B | 8.50 a B |

### Table 5. Effect of biofungicide dose of *Trichoderma* spp. on the weight of harvested dry tubers.

| Biofungicide Dosage Treatment of Fermented Legundi Leaf Extract *T. harzianum* | Onion Bulbs Weight (tons/ha) |
|---|---|
| | Seed Soak | Plant Spraying |
| Without Biofungicide | 6.25 a A | 6.18 a A |
| Biofungicide 2.5 mL | 16.60 b B | 12.00 a B |
| Biofungicide 5.0 mL | 16.75 b B | 12.10 a B |
| Biofungicide 7.5 mL | 16.80 b B | 12.40 a B |
| Biofungicide 10.0 mL | 16.90 b B | 12.50 a B |
Table 6. Effect of application and dosage of *Trichoderma* spp. on the weight of dry onion bulbs stored.

| Biofungicide Dosage Treatment of Fermented Legundi Leaf Extract *T. harzianum* | Dried Shallot Bulbs Weight Save (tons/ha) |
| --- | --- |
| | Seed Soak | Plant Spraying |
| Without Biofungicide | 5.30 a | 5.10 a |
| **Biofungicide 2.5 mL** | 15.10 b | 11.00 a |
| **Biofungicide 5.0 mL** | 15.20 b | 11.20 a |
| **Biofungicide 7.5 mL** | 15.45 b | 11.28 a |
| **Biofungicide 10.0 mL** | 15.60 b | 11.50 a |

In Tables 4, 5 and 6 it can be seen that the number of tubers/clumps, dry weight of harvested tubers and dry weight of storage per ha due to the use of biofungicide doses of fermented Legundi leaf extract of *T. harzianum* were significantly different from those without biofungicides either by soaking the seeds or spraying plants. The biofungicide treatment by soaking the seeds from 2.5 mL to 10.0 mL was better than spraying plants. The yield of shallots, especially the weight of harvested dried shallots obtained with the biofungicide treatment of fermented Legundi leaf extract of *T. harzianum* reached more than 16.0 tons/ha, this means that the productivity of shallots of the Keta Monca variety has reached.

The fact that the dose of *T. harzianum* fermented Legundi leaf extract biofungicide can increase the yield component of shallots is thought to be related to the role of the *T. harzianum* fungus contained in the Legundi extract biofungicide to control Fusarium wilt disease so that the vegetative and generative growth of plants is maximized. Tronsmo and Dennis [13] reported that spraying conidia of the fungi *T. viride* and *T. koningii* to protect strawberry plants from blight was found to promote early flowering. The same thing was reported by Sudantha [16] that soaking vanilla cuttings with the saprophytic fungus *T. harzianum* to control Fusarium wilt disease was able to stimulate flowering and fruiting. This is because the fungus *T. harzianum* present in the rhizosphere or plant root areas can release ethylene which is diffused into plant tissues through xylem which plays a role in stimulating plant generative growth. Salisbury and Ross [34] reported that hormones such as ethylene produced by *Trichoderma* spp. which can stimulate flowering in plants. Arianci [35] also mentioned that *Trichoderma* spp. can produce certain hormones to increase the weight and number of pods in soybean plants on peatlands.

The successful use of *T. harzianum* fungus in various formulations that can increase crop yields has been reported by several previous researchers, namely: The use of *T. harzianum* fungus in bioactivator formulations and Arbuscular Mychoriza Fungi can increase the yield of shallots in dry land [36]. The use of the fungus *T. harzianum* can increase the yield of maize and soybeans [37]. *T. harzianum* fungus in tablet and liquid bioactivator formulations can increase soybean yield [38].

4. Conclusion

The results showed that the Legundi extract biofungicide fermented with the fungus *T. harzianum* was effective in controlling Fusarium wilt disease, increasing the growth and yield of shallots by soaking the seeds before planting at doses starting at 2.5 mL. With the application of a biofungicide extract from the fermented Legundi leaf of *T. harzianum*, the plants became immune, and the dry shallots bulb weight increased to more than 16.0 tons/ha.
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