Biodiversity of *Trichoderma* antagonist saprophytic fungi and its use for biocontrol of Fusarium wilt disease on shallots at Lombok Island, West Nusa Tenggara, Indonesia

I M Sudantha¹ and S Suwardji²

¹ Agroecotechnology Study Program, Faculty of Agriculture, University of Mataram, Indonesia
² Soil Science Study Program, Faculty of Agriculture University of Mataram, Indonesia

Email: sudantha@unram.ac.id

Abstract. One of the obstacles in the development of shallots in West Nusa Tenggara (NTB) is the presence of Fusarium wilt disease. The use of antagonistic saprophytic fungi *Trichoderma* spp. is a biological control technique. The aim of this research is to explore the biodiversity of *Trichoderma* spp. and its use for controlling Fusarium wilt disease. Research using exploratory methods conducted at the shallot planting center on Lombok Island included isolation of antagonistic saprophytic fungi, purification and identification of isolates. Furthermore, research was carried out in the laboratory using experimental methods including in-vitro antagonism tests by direct opposition and culture steam. The research was continued in Greenhouses in planta and in field conditions in the Highlands of Sembalun Village, Medium Plains of Santong Village and Senteluk Village Lowlands. The results concluded that: there were 6 isolates of saprophytic fungi that were antagonistic, namely *T. viride*, *T. longibrachiatum*, *T. koningii*, *T. piluliferum*, *T. harzianum*, and *T. hamatum*. The mechanism of antagonism is physically through space competition, mycoparasites and antibiosis. Three isolates, namely *T. viride*, *T. harzianum* and *T. hamatum* were effective in suppressing Fusarium wilt disease and even causing immunity.

1. Introduction

Fusarium wilt disease caused by the fungus *Fusarium oxysporum* f.sp. *cepae* is one of the main diseases of shallot. This fungus causes shallots to wilt quickly, leaves turn yellow, leaves twist and stem rots. Fusarium wilt disease has caused damage and reduced tuber yield by up to 50% [1]. Fusarium wilt disease develops in shallot planting centers in NTB, including Senteluk West Lombok, Sembalun Bumbung, East Lombok, Rada Bima Village which causes damage and reduces the yield of tubers by more than 45% [2].

Until now, the fungus *Fusarium oxysporum* f. sp. *cepae* is difficult to control, this is due to its ability to adapt to extreme climates both in the highlands and lowlands. This fungus can dormancy in the soil as a saprophyte for a long time between three to four years even though there are no shallot plants, this is because it has a dormant structure in the form of chlamydospores, besides that in extreme climates this fungus can also survive [3]. In addition, Fusarium wilt spreads quickly through infected shallot seeds used by farmers due to the use of uncertified seeds [4].
Thus, it is necessary to find an alternative to control Fusarium wilt that is effective and environmentally friendly. Environmentally friendly control methods are biological technology using *Trichoderma* spp. which can later be used to increase the induced resistance. Biodiversity of saprophytic fungi *Trichoderma* spp. very useful in developing shallot that have induced resistance to Fusarium wilt disease.

Saprophytic fungi are fungi that take food from the rest of organic matter or dead material. Basically saprophytic fungi are divided into two groups, namely obligate saprophytic fungi and facultative parasitic fungi. Obligate saprophytic fungi are fungi that pass through their entire life cycle as a saprophyte without being a parasite. For example, *Trichoderma* sp. usually live and complete their life cycle in soils containing organic matter. This fungus is widely used as a controlling agent for various soil-borne pathogens. Another example is decomposer fungi or decomposers which are able to remodel organic materials from plant residues. This fungus is commonly found in trash cans and above ground in gardens or forests. While facultative parasitic fungi are saprophytic fungi that sometimes act as parasites when conditions are favorable for themselves, for example the fungus *Rhizoctonia solani* [5].

Many studies have been carried out on antagonistic saprophytic fungi to control soil-borne pathogens that attack various plants in Indonesia. Sudantha [6] reported that there were 7 types of *Trichoderma* spp. effectively control vanilla stem rot disease caused by the fungus *F. oxysporum f. sp. vanillae*. Sudantha *et al.* [7] reported that there are 6 types of saprophytic fungi *Trichoderma* spp. namely *T. harzianum*, *T. koningii*, *T. aeroviride*, *T. hamatum* and *T. viride* which were effective in controlling the *Fusarium* fungus that causes wilt disease in banana plants.

Antagonistic saprophytic fungi can suppress pathogenic fungi in three ways, namely being able to live as mycoparasites that can penetrate pathogenic hyphae and chlamydospores so that the hyphae are destroyed, produce secondary metabolites in the form of gliotoxin and viridine which can inhibit the growth of pathogenic fungi, and are able to compete for nutrients and space [8]. Sudantha [9] confirmed that the mechanism of antagonism between the fungi *Trichoderma* spp. with soil-borne pathogenic fungi, namely by competition, mycoparasites, and antibiosis.

This study aims to reveal the biodiversity of saprophytic fungus *Trichoderma* spp. and its use for controlling Fusarium wilt disease on shallots.

2. Method
Research using exploratory methods conducted at the shallot planting center on Lombok Island West Nusa Tenggara included isolation of antagonistic saprophytic fungi, purification and identification of isolates. Furthermore, research in the laboratory using experimental methods includes in-vitro antagonism testing by direct opposition and culture steam. In addition, in-plant tests were carried out in the Greenhouse and in the Field which were carried out at Highlands of Sembalun Village, on the Medium Plains of Santong Village and in the Lowlands of Senteluk Village.

2.1. Exploratory method, including: collection of shallots plants infected with Fusarium wilt disease and soil samples
Sampling of soil and shallot plants infected with Fusarium wilt disease was carried out at shallots planting centers on the island of Lombok, namely: Sembalun Bumbung Village, Sembalun District, East Lombok Regency, Santong Village, Kayangan District, North Lombok Regency, and Senteluk Village, Batu Layar District, West Lombok Regency. At each location, five diseased shallot plants and five soil samples weighing 1 kg each were selected, and then brought to the laboratory.
2.2. Experimental methods in the laboratory, including:

2.2.1. Isolation, purification and identification of *Fusarium*. Isolation of the fungus *Fusarium* is done by isolating from shallot plant tissue that shows wilting symptoms. The roots, leaves and tubers of diseased plants were cut, dipped in 70% alcohol and then put into sterile distilled water. The diseased roots, leaves and tubers were grown on PDA medium. The grown fungus were then purified and identified for use as a source of inoculum [6].

2.2.2. Isolation, purification and identification of saprophytic fungi *Trichoderma* spp. Isolation of saprophytic fungi *Trichoderma* spp. was carried out by isolating it from the rhizosphere or the soil around the roots of the shallot plant. The isolation method that will be used is the dilution cup method with a dilution level of up to $10^{-4}$. The growing saprophytic fungi were transferred to a Petri dish containing PDA medium by single conidium transfer technique or hyphal tip transfer technique, then tagged. Observations were made macroscopically including colony color, colony growth direction, colony thickness, colony diameter and colony growth speed; and microscopically include hyphae color, conidia shape, conidia color, presence or absence of phialid and phialid density. Then identified using the identification key book Barnett and Hunter [10], Rifai [11], Alexopoulos and Mims [12], Domsch, Gams and Anderson [13].

2.2.3. Test of antagonism of saprophytic fungus *Trichoderma* spp. with *Fusarium*. The antagonism test was carried out by means of an inoculum of *F. oxysporum* isolate and each saprophytic fungal isolate was placed at a distance of 4 cm in the middle of the PDA medium in a Petri dish with a diameter of 9 cm. *Fusarium* fungus inoculum was in the form of culture pieces with a diameter of 4 mm on PDA medium, then the cultures were incubated at room temperature. Observations were made on the growth of *Fusarium* fungus colonies and the presence of inhibition zones between two opposing saprophytic colonies. The inhibition of *Fusarium* fungus mycelium growth by saprophytic fungi was calculated based on the Sudantha formula [6], namely:

$$I = \frac{(r_1 - r_2)}{(r_1)} \times 100\%$$

$I$ = percentage of inhibition, $r_1$ = radius of *F. oxysporum* colonies growing in the opposite direction to the saprophytic fungus, and $r_2$ = radius of *Fusarium* fungus colonies growing towards the saprophytic fungi. For the antagonism between *Fusarium* and saprophytic fungi that secrete antibiotic compounds, apart from measuring the radius of the colony, the distance of the inhibition zone (d) was measured, namely the zone of the tip of the saprophyte colony and the tip of the colony of *F. oxysporum*. The calculation of the percentage of inhibition was carried out on the data from the measurement of the radius of the fungal colony of *F. oxysporum* on the third day after inoculation of the saprophytic fungi.

2.2.4. Vapor test of saprophytic fungi *Trichoderma* spp. with fungus *F. oxysporum* f. sp. *ceae*. Cultures of *Fusarium* fungus with a diameter of 4 mm were grown on PDA medium in a Petri dish (15 ml). Saprophytic fungi were also cultured on PDA medium in 90 mm diameter Petri dishes. The trick is to plant a piece of saprophytic fungus culture with a diameter of 4 mm from a three-day-old culture in PDA medium in the middle of a Petri dish which already contains 15 ml of PDA medium. On the bottom of the Petri dish containing the culture of this saprophytic fungus, the culture of *F. oxysporum* was then cupped. Observation of the growth of the fungus *F. oxysporum* was carried out by measuring the diameter of the culture colonies every 24 hours until the culture was five days old.
2.3. **Experimental methods in the greenhouse**
The experimental design used in this research was a Completely Randomized Design (CRD) consisting of two factors. The first factor is the saprophytic fungi *Trichoderma* spp. (T) which consists of four levels, namely:
- T0 = Without fungus *Trichoderma* sp.
- T1 = With saprophytic fungus *T. harzianum*
- T2 = With saprophytic fungus *T. viride*
- T3 = With saprophytic fungus *T. hamatum*

The second factor is the shallots variety (V), namely:
- V1 = Bali Karet Varieties
- V2 = Ampenan Varieties
- V3 = Keta Monca Varieties

The experimental treatment was a combination of *Trichoderma* spp. and shallot varieties, each of which was repeated 3 times so that there were 36 experimental units.

2.3.1. **Observation of wilt disease (%)**. Observations were made by counting the number of plants affected by wilt disease until the plants were 35 days after planting (dap). The calculation of the incidence of disease is carried out using the absolute formula, namely:

\[
I = \frac{a}{a+b} \times 100\%
\]  

(2)

a = number of plants infected with wilt disease
b = number of healthy plants

2.3.2. **Data analysis.** The diversity analysis at the 5% significance level was used to determine the effect of the treatment and to determine the difference between treatments, the HSD test was carried out at the 5% significance level.

2.4. **Experimental methods in the field**
The experimental uses a Randomized Block Design (RBD) which consists of two factors. Main effect is the saprophytic *Trichoderma* spp. (T) there are four levels, namely:
- T0 = Without fungus *Trichoderma* sp.
- T1 = With saprophytic fungus *T. harzianum*
- T2 = With saprophytic fungus *T. viride*
- T3 = With saprophytic fungus *T. hamatum*

Simple effect is the location of planting shallots (L) at three altitudes, namely:
- L1 = The Highlands Sembalun Bumbung Village, East Lombok
- L2 = The Medium Plains of Santong Village, North Lombok
- L3 = The Lowlands of Senteluk Village, West Lombok

The treatment was a combination of factors of the saprophytic fungi *Trichoderma* spp. and the height of the planting site which was repeated 3 times so that 36 experimental units were obtained.

2.4.1. **Observation of disease incidence (%)**. Observations were made by counting the number of plants affected by Fusarium wilt until the plants were 35 dap. The calculation of the incidence of disease is carried out using the absolute formula, namely:
\[ I = \frac{a}{a+b} \times 100\% \]  

3. Results and discussion

3.1. Identification results of saprophytic fungi *Trichoderma* spp.

The results of the identification of the types of saprophytic fungi found in the shallot rhizosphere to the species level were mainly carried out microscopically based on the color of the hyphae, the shape of the conidia or phialospore, the shape of the conidiophores and the shape of the phialide. Thickness, growth pattern and colony diameter, because each type of fungus has its own characteristics in terms of its macroscopic appearance, as presented in Figures 1-6 and the following description.

The results of the identification of the saprophytic fungi *Trichoderma* spp. found in the rhizosphere or the area around the roots of shallot plants to the species level, mainly carried out microscopically based on the color of the hyphae, conidia or *phialospore* shape, conidiophores and *phialide* forms, while to ascertain the differences in the types of fungi belonging to the same genus, macroscopic observations were also carried out including color, thickness, growth pattern and colony diameter, because each type of fungus has its own characteristics in terms of its macroscopic appearance, as presented in Figures 1-6 and the following description.

3.1.1. *Trichoderma harzianum* Rifai aggr. The macroscopic characterization of the fungus *T. harzianum* was that the colonies spread evenly and grew rapidly, three days after inoculation covering the surface of the Petri dish (90.00 mm). Once the conidia are formed, the colonies turn greenish white and bright green. Microscopic characterization of hyphae is septate, branched, thin-walled and colorless. The branching system is like a cone/pyramid. *Phialide* grows at each end of the branching numbering 1-5, short conical shape. *Phialospores* are produced at each end of the *phialide*, round to oval in shape, pale green in color, measuring 2.5 – 3.3 x 2.5 – 2.8 (Figure 1).

3.1.2. *Trichoderma viride* Pers. Ex S. F. Gray aggr. The macroscopic characterization of the fungus *T. viride* was that the fungal colonies grew rapidly and evenly, three days after inoculation covering the Petri dish (90.00 mm). Aerial hyphae appear on the surface. Fungal colonies are white, after conidia are formed, they turn dark green to bluish green. Colonies grow thick and dense. Microscopic characteristics were *hyaline* mycelia, smooth-walled, insulated, branched, spores (*phialospores*) were round, green in color and 3-5 in diameter. Many branched conidiophores. *Phialide* formed more than 2 – 3 at the end of the branching conidiophores, and at each end of the *phialide* formed *phialospore* (Figure 2).

3.1.3. *Trichoderma hamatum* (Bon.) Bain aggr. The macroscopic characterization of the fungus *T. hamatum* was that the colonies of this fungus grew naturally, four days after inoculation covering the surface of the Petri dish (90.00 mm). Initially growing thin on the surface of the dish, the mycelia are translucent or white in color with little or no air hyphae. Old colonies are whitish green to grayish green. Microscopic characterization of hyphae is *hyaline*, branched, thin-walled and septate. Many branched conidiophores, short fertile branches consisting of 2-4 cells where *phialide* grows, while sterile branches...
grow lengthwise without the presence of phialide. Fertile branches have 2 – 5 phialides, each phialide produces an elliptical phialospore measuring $3.5 - 6 - 2.5 - 2.8$, pale green in color (Figure 3).

3.1.4. Trichoderma longibrachiatum Rifai aggr. The macroscopic characterization of the fungus T. longibrachiatum was that the fungal colonies grew rapidly, after three days of inoculation they covered the surface of a Petri dish (90.00 mm). It is white at first, with the formation of conidia turning greenish white and then dark green. Microscopic characterization of mycelia insulated, multi-branched, hyaline, and smooth-walled. Phialospore elliptical shape measuring $3 - 6 \times 2 - 3$ pale green. The main branch of the conidiophores grows elongated with few side branches. At each side branch a bottle-like phialide is formed and at the end a phialospore is formed (Figure 4).

3.1.5. Trichoderma koningii Oud. aggr. The macroscopic characterization of the fungus T. koningii was growing evenly and quickly, after three days of inoculation covered Petri dishes (90.00 mm). At first it is white and after the formation of the phialospore it turns greenish white. Colonies grow thick and dense. Microscopic characteristics were hyaline mycelia, many branched, smooth-walled, and insulated, round to elliptical phialospores measuring $3 - 5 \times 2 - 3$, light green in color. Many branched conidiophores, on the main branch there are 2-3 groups of branches. The branching system is like a pyramid. The phialide is conical in shape, at the end there is a phialospore (Figure 5).

3.1.6. Trichoderma piluliferum Rifai Webster & Rifai aggr. The macroscopic characterization of the fungus T. piluliferum was that the fungal colonies grew rather slowly, after five days of inoculation covering the Petri dish (90.00 mm). Mycelia are white and remain white even though conidia have formed. Colonies grow thin. Microscopic characterization of conidiophores growing irregularly, forming many side branches and phialide at each end of the branching. The shape of the phialide is like a small bottle to a pyramidal shape, and at each end of the phialide produces a phialospore that is round to oval measuring $2.5 - 3.5$ (Figure 6).
3.2. In-vitro antagonism test with direct opposition method and steam culture of Trichoderma spp.

From the analysis of diversity, it is known that all isolates of the saprophytic fungus *Trichoderma* spp. significantly different in inhibiting the growth of *Fusarium* both with the direct opposition method and the steam culture method of *Trichoderma* spp. The results of further tests between isolates of the saprophytic fungus *Trichoderma* spp. which are significantly different from each other are presented in the table below.

| No. | Treatment of fungi *F. oxysporum* in opposition to the saprophytic fungi *Trichoderma* spp. | Average inhibition (%) |
|-----|--------------------------------------------------------------------------------------------|------------------------|
| 1   | *T. harzianum*                                                                             | 46.60 c *)              |
| 2   | *T. viride*                                                                                | 43.30 c                 |
| 3   | *T. hamatum*                                                                              | 43.20 c                 |
| 4   | *T. longibrachiatum*                                                                      | 40.60 b                 |
| 5   | *T. koningii*                                                                             | 40.40 b                 |
| 6   | *T. piluliferum*                                                                          | 40.40 b                 |
| 7   | Without the saprophytic fungus *Trichoderma* sp.                                         | 0.00 a                  |

*) Numbers followed by the same letter were not significantly different p ≤ 0.05

| No. | Treatment of fungus *F. oxysporum* cupped on a culture of the saprophytic fungi *Trichoderma* spp. | Average inhibition (%) |
|-----|-----------------------------------------------------------------------------------------------|------------------------|
| 1   | *T. harzianum*                                                                              | 77.20 c *)              |
| 2   | *T. viride*                                                                                 | 76.60 c                 |
| 3   | *T. hamatum*                                                                               | 75.80 c                 |
| 4   | *T. longibrachiatum*                                                                       | 66.20 b                 |
| 5   | *T. koningii*                                                                              | 65.50 b                 |
| 6   | *T. piluliferum*                                                                           | 64.80 b                 |
| 7   | Without the saprophytic fungus *Trichoderma* sp.                                           | 0.00 a                  |

*) Numbers with the same letter were not significantly different p ≤ 0.05

From Table 1 and Table 2 it can be said that 6 isolates of the saprophytic fungus *Trichoderma* spp. can inhibit the growth of *Fusarium* fungus in both the direct opposition method and the steam method of *Trichoderma* spp. fungus culture, but the level of inhibition is different. The saprophytic fungus
Trichoderma spp. the best in inhibiting the growth of the fungus *F. oxysporum* f. sp. *cepae* were *T. harzianum*, *T. viride* and *T. hamatum* (Figures 7 and 8) with inhibition percentages of more than 43% for the direct opposition method or mycoparasite competition mechanism and more than 75% for the steam method of fungal culture *Trichoderma* spp. or are on an antibiosis mechanism.

In Figure 7 shows the saprophytic fungus *Trichoderma* spp. in the direct opposition test did not show the inhibition zone, but it could grow continuously past the fungal colonies of *F. oxysporum* f. sp. *cepae* causing inhibited growth of *Fusarium* fungus, this is suspected to be the saprophytic fungus *Trichoderma*. 

**Figure 7.** Antagonism test with direct opposition method in which *T. harzianum* (A) and *T. viride* (B) inhibited the growth of *Fusarium* (F). 

**Figure 8.** Growth of the fungus *F. oxysporum* (F) on PDA medium cupped on a culture of *T. harzianum* (T) and control (C) or without culture of after *T. harzianum* seven days of incubation.
spp. has the ability to grow quickly and can entangle the hyphae of the *Fusarium* fungus. In Figure 8 it can be seen that the saprophytic fungus *Trichoderma* spp. in the cupping test, it can reduce the diameter of the *Fusarium* fungus, it is suspected that the saprophytic fungus secretes antibiotic compounds or volatile alkaloids that can inhibit the growth of the *Fusarium* fungus. Several researchers have reported this incident, such as Abadi [14] who reported that the fungus *T. harzianum* caused hyphae of *Ganoderma boninense* to lyse when hyphae contact occurred between the two fungi with an antagonist effectiveness score of four. According to Cook and Baker [15], the general mechanism of antagonism of the fungus *Trichoderma* spp. in suppressing pathogens as mycoparasites and aggressive competitors. At first the mycelia growth of the fungus *Trichoderma* spp. elongated, then convoluted and penetrated the hyphae of the host fungus, so that the hyphae of the host underwent vacuolation, lysis and finally disintegration, then the antagonist grew inside the hyphae of the pathogen. The fungi *T. harzianum* and *T. hamatum* act as mycoparasites against *R. solani* and *S. rolfsii*, producing the enzymes -(1,3) glucanase and chitinase that cause exolysis of the host hyphae. The fungus *T. hamatum* also produces cellulase enzymes, thus increasing its ability as mycoparasites in *Phytium* spp. The enzyme -(1,3) glucanase produced by the *Trichoderma*.

The success of using *Trichoderma* fungus as a control agent was reported by several researchers as follows: In vitro *Trichoderma* fungus can inhibit *Fusarium* fungus on vanilla plants by physically entangling *Fusarium* hyphae and penetration into cells, competition for nutrients and space and antibiosis [7,16–18]. With this mechanism, the plant will increase its induction resistance to *Fusarium* wilt [3,19]. Thus, there are five ways *Trichoderma* can suppress the growth of pathogenic fungi, namely: (1) Competition in space and nutrition, (ii) parasitizing other fungi, (iii) producing metabolites, (iv) cross-protection, and (v) resistance. Systemically induced [20].

### 3.3. Experimental results in the greenhouse

The analysis of variance of *Fusarium* wilt data showed that *Trichoderma* spp. showed significant differences in inhibiting *Fusarium* wilt in three shallot varieties carried out in the greenhouse. The results of further tests between isolates of the saprophytic fungus *Trichoderma* spp. which are significantly different from each other are listed in Table 3.

| No. | Treatment of saprophytic fungi *Trichoderma* spp. | Average Occurrence of Fusarium Wilt Disease (%) |
|-----|---------------------------------|-----------------------------------------------|
| 1   | *T. harzianum*                  | 0.00a*)                                        |
|     |                                 | Bali Karet Varieties                           |
|     |                                 | 0.00a*)                                        |
|     |                                 | Ampenan Varieties                              |
|     |                                 | 0.00a*)                                        |
|     |                                 | Keta Monca Varieties                           |
|     |                                 | A**)                                          |
| 2   | *T. viride*                     | 5.70a                                          |
|     |                                 | Bali Karet Varieties                           |
|     |                                 | 5.90a                                          |
|     |                                 | Ampenan Varieties                              |
|     |                                 | A**                                           |
| 3   | *T. hamatum*                    | 5.70b                                          |
|     |                                 | Bali Karet Varieties                           |
|     |                                 | 5.80b                                          |
|     |                                 | Ampenan Varieties                              |
|     |                                 | B                                             |
| 4   | Without the saprophytic fungus *Trichoderma* sp. | 60.70a                                        |
|     |                                 | Bali Karet Varieties                           |
|     |                                 | 60.90a                                        |
|     |                                 | Ampenan Varieties                              |
|     |                                 | C                                             |
|     |                                 | Keta Monca Varieties                           |
|     |                                 | C                                             |

Information: Numbers in column 1) and in row 2) followed by the same letter in the 0.05 HSD test means that there is no significant difference

From Table 3 it can be explained that the application of *Trichoderma* on shallots was able to reduce *Fusarium* wilt in both the Bali Karet, Ampenan and Keta Monca varieties. The type of fungus *Trichodema*
spp. as *T. harzianum* was the most capable of suppressing Fusarium wilt disease, and there was no Fusarium wilt disease or induced resistance to Fusarium wilt disease in the Bali Karet, Ampenan and Keta Monca varieties.

In the varieties of Bali Karet, Ampenan and Kenta Monca, the intensity of wilt disease reached more than 60% in the control, when treated with *Trichoderma* it decreased to less than 6%, even in the treatment of *T. harzianum* fungus it became 0% or resistance was induced or became immune. The decrease in the incidence of this disease is related to the results of in-situ experiments that the saprophytic fungus *T. harzianum* can suppress the development of the *Fusarium* pathogen that causes Fusarium wilt disease through competition, mycoparasite and antibiosis mechanisms (Tables 2 and 3 and Figures 2 and 3). According to Sudantha [9] that plants that are susceptible to disease can be manipulated for their resistance through environmental manipulation or often referred to as ecological resistance or false resistance. One form of ecological resistance to plant diseases is induced resistance. Petrini [21] said that plant-induced resistance to disease can be done by treating seedlings or plants with antagonist suspension. According to Guest [22] that induced resistance to disease can occur due to the role of antibiotics such as phytoalexins. Immediately after infection by pathogenic fungi, cell membranes release free radicals. The tissue turns brown due to the activity of the polyphenol oxidase enzyme which produces phenol oxidase which will turn into quinone which is toxic to pathogenic fungi. These free radicals are associated with lignin and reactive oxygen and hydroxyproline cell wall glycoproteins, causing the cell wall to be indestructible by pathogenic fungal enzymes.

As an illustration in Figure 9A, it can be seen that the application of *T. harzianum* by soaking the tubers caused the plants to become healthy, which was characterized by higher height and number of leaves compared to the control. Figure 9B shows the control plants or those that were not treated with *T. harzianum* caused the plants to wilt, the leaves curled and twisted, the leaf color turned pale green to yellow and eventually dried and died.

![Image](image_url)

**Figure 9.** Treatment with *T. harzianum* resulted in healthy plants (a). In plants without treatment *T. harzianum* caused shallots to become infected with Fusarium wilt (b)

### 3.4. Field experiment results

The disease incidence data analyzed using Analysis of Variance showed that all isolates of the saprophytic *Trichoderma* fungi were significantly different in inhibiting the intensity of Fusarium wilt until the plants were 5 weeks old after planting at three locations where the altitude was planted. The
results of further tests between isolates of the saprophytic fungus *Trichoderma* spp. which are significantly different from each other are presented in Table 4.

Table 4. Intensity of Fusarium wilt to plants 5 weeks after planting at three shallots planting locations

| No. | Treatment of saprophytic fungi *Trichoderma* spp. | Highlands of Sembalun Bumbung Village, East Lombok | Medium Plains of Santong Village, North Lombok | Lowlands of Senteluk Village, West Lombok |
|-----|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 1   | *T. harzianum*                               | 0.00 a*)                                      | 0.00 a*)                                      | 0.00 a*)                                      |
|     |                                              | A**)                                          | A**)                                          | A**)                                          |
| 2   | *T. viride*                                  | 11.10 a                                       | 11.20 a                                       | 11.40 a                                       |
|     |                                              | B                                             | B                                             | B                                             |
| 3   | *T. hamatum*                                 | 11.70 b                                       | 11.81 b                                       | 11.80 b                                       |
|     |                                              | B                                             | B                                             | B                                             |
| 4   | Without the saprophytic fungus *Trichoderma* sp. | 70.70 a                                       | 70.80 a                                       | 70.90 a                                       |
|     |                                              | C                                             | C                                             | C                                             |

Notes: The numbers in column 1) and in row 2) followed by the same letter are not significantly different in the 0.05 HSD Test.

From Table 4 it can be explained that the saprophytic fungus *Trichoderma* spp. effectively inhibited the occurrence of Fusarium wilt both at the location of shallots in the highlands, medium plains and lowlands. The type of fungus *Trichodema* spp. The most capable of suppressing Fusarium wilt disease is the fungus *T. harzianum*, even Fusarium wilt does not occur, in other words there is induced resistance to Fusarium wilt at onion planting locations in the highlands, medium plains and lowlands. This means that the fungus *T. harzianum* has a high adaptability to the environment. In control or without *Trichoderma* shallots, both in the highlands, medium plains and lowlands, the wilting intensity was high, reaching more than 70%. The incidence of disease after treatment with *T. viride* and *T. hamatum* fungi was seen in shallots grown in the highlands, medium and lowlands decreased to less than 11%, even in the treatment with *T. harzianum* there was no Fusarium wilt. immune. The decrease in the incidence of this disease is related to the results of in-situ experiments that the saprophytic fungus *T. harzianum* can suppress the development of the fungus *F. oxysporum* f. sp. *cepae* causes Fusarium wilt disease through competition, mycoparasite.

As an illustration in Figure 10A, shallot plants grow healthy with higher plant height and number of leaves because they are treated with the saprophytic fungus *T. harzianum*, while Figure 10B shows shallot plants infected with Fusarium wilt disease which show symptoms of poor growth, namely some of the leaves wither and twisted, the leaves change color to pale green to yellow and then become dry. The same symptoms were also shown in the study of Sudantha et al. [4] conducted in Sembalun, East Lombok Regency.
The effectiveness of the fungus *T. harzianum* in suppressing pathogenic fungi in several plants and adaptation to the environment is found in several papers, namely: The fungus *F. proliferatum* that causes onion bulb rot is inhibited by *T. harzianum* [23]. Coating soybean seeds with *T. harzianum* can reduce the occurrence of charcoal rot disease caused by *Macrophomina phaseolina* [24]. *T. harzianum* can act as a biofungicide because of its ability to suppress disease and stimulate root and stem elongation and plant yields [25]. *T. harzianum* has good prospects as a biological fungicide and growth promoter because it can eliminate Fusarium wilt and can stimulate the growth of shallot plants [26]. Even according to Sudantha *et al.* [27] and [28] that the fungus *T. harzianum* and Benzyl Amino Purine Growth Regulators have the same role in promoting the vegetative and generative growth of shallots. Furthermore, *Trichoderma* microbes that produce secondary metabolites can be formulated into biopesticides that can replace chemical pesticides that are not environmentally friendly [29]. *Trichoderma* can adapt to a dry environment [30]. Furthermore, *T. harzianum* formulated as tablet biocompost was able to increase plant height and increase tuber weight in the face of adaptation to extreme climate change [31].

The saprophytic fungus *T. harzianum* has good prospects as a disease control component and as a decomposing agent of organic matter in various plants as reported by several researchers as follows: 0 tons/ha [32]. *T. harzianum* bioactivator applied to shallots can increase the yield of shallots up to 15.0 tons/ha [33]. *T. harzianum* in the form of a bioactivator accompanied by Arbuscular Mycorrhizal Fungi can increase the growth and bulbs of shallots [34]. *T. harzianum* as a bioactivator can increase the induced resistance of soybean plants to Fusarium wilt disease and increase plant growth and yield [35]. *T. harzianum* administered to soybeans caused resistance to Fusarium wilt and increased pod growth and weight [36]. *T. harzianum* can increase the weight of shallot bulbs [37]. *T. harzianum* which was formulated in a stimulator biocompost formulation that was applied to maize caused the plant height and the number and weight of corn cobs to increase [38].

4. Conclusions
The conclusions that can be drawn from this study are: there are 6 species of antagonistic saprophytic fungi, namely: *T. viride, T. longibrachiatum, T. koningii, T. piluliferum, T. harzianum*, and *T. hamatum*. The mechanism of antagonism is physically through space competition, mycoparasites and antibiosis.

The saprophytic fungi *T. harzianum, T. viride* and *T. hamatum* were the most effective in inhibiting the growth of *F. oxysporum* f. sp. *cubens* by more than 43% using the direct opposition method or the
mechanism of space and nutrition competition and mycoparasites, and more than 75% for the steam method of culture of *Trichoderma* spp. or an antibiotic mechanism occurs.

The incidence of disease after treatment of the saprophytic fungus *Trichoderma* spp. seen in the varieties of Bali Karet, Ampenan and Kenta Monca decreased to less than 6%, even in the treatment of the fungus *T. harzianum* there was no Fusarium wilt infection or induced resistance.

Application of saprophytic fungus *T. harzianum* on shallots was very effective in suppressing the incidence of Fusarium wilt disease or induced resistance in the highlands, medium plains and lowlands. *T. harzianum* fungus has high adaptability to the environment.

**Acknowledgments**

This paper was compiled using some data from the 2021 Leading Higher Education Development Research (PP-UPT), therefore we would like to thank the Director of Resources of the Directorate General of Higher Education, Research, and Technology; Unram Chancellor and Head of LPPM Unram.

**References**

[1] Sudantha I M and Suwardji S 2021 *Trichoderma* biofungicides formulations on shallot growth, yield and Fusarium wilt disease resistance 6th Int. Conf. Clim. Chang. 2021 824

[2] Sudantha I M 2015 Pemanfaatan Bioaktivator dan Biokompos untuk Meningkatkan Kesehatan, Kuantitas dan Kualitas Hasil Bawang Merah (Laporan Penelitian Program Magister Pengelolaan Sumberdaya Lahan Kering Program Pascasarjana Unram)

[3] Sudantha I M 2013 *Patogen Tumbuhan Tular Tanah dan Pengendaliannya* (Agra Puji Press)

[4] Sudantha I M, Suwardji, Aryana I G P M, Pramadya I M A and Jayadi I 2018 Peningkatan Mutu Benih G0/Bibit Bawang Merah Dengan Teknologi Hayati Untuk Menunjang Sumberdaya Lahan Kering Program Pascasarjana Unram)

[5] Abadi A L 2003 Ilmu Penyakit Tumbuhan I Edisi Pertama (Malang: Bayumedia Publishing)

[6] Sudantha I M 2007 Karakterisasi dan Potensi Jamur Endofit dan Saprofit Antagonistik Sebagai Agens Pengendali Hayati Jamur Fusarium oxysporum f. sp. vanillae Pada Tanaman Vanili di Nusa Tenggara Barat (Universitas Brawijaya)

[7] Sudantha I M, Kusnarta I G M, Rahayu M and Sudana I N 2008 Karakterisasi dan Potensi Jamur Saprofit dan Endofit Antagonistik Untuk Meningkatkan Ketahanan Induksi Tanaman Pisang terhadap Penyakit Layu *Fusarium* di Nusa Tenggara Barat Kerja Sama Kemitraan Penelit. Pertan. dengan Perguru. Tinggi Badan Litbang Pertan. 106

[8] Cook R J and Baker K F 1983 *The Nature and Practice of Biological Control of Plant Pathogens* (St. Paul MN: APS Press, The American Phytopathological Society)

[9] Sudantha I M 2018 *Buku Teknologi Tepat Guna: Penerapan Biofungisida dan Biokompos pada Pertanian Organik* (Mataram: LPPM Unram Press)

[10] Barnett H L and Hunter B B 1998 *Illustrated Genera of Imperfect Fungi* (St. Paul, Minnesota: APS Press, The American Phytopathological Society)

[11] Rifai M A 1969 A revision of the marga *Trichoderma* Commonw. Mycol. Institute, Mycol 1–56

[12] Alexopoulos C J and Mims C W 1979 *Introductory Mycology* (New York, Chichester, Brisbane, Toronto, Singapore: Jhon Wiley & Sons)

[13] Domisch K H, Gams W and Anderson T 1980 *Compendium of Soil Fungi* (New York: Academic Press.)

[14] Abadi A L 1987 *Biologi Ganoderma boninense Pat. Pada Kelapa Sawit (Elaes guineensis Jacq) dan Pengaruh Beberapa Mikroba Tanah Antagonistik Terhadap Pertumbuhannya* (IPB)

[15] Cook R and Baker K 1983 *The Nature and Practice of Biological Control of Plant Pathogens* (St. Paul MN)
[16] Sudantha I and Abadi A 2006 *Biodiversitas Jamur endofit Pada Vanili (Vanilla planifolia Andrews) dan Potensinya Untuk Meningkatkan Ketahanan Vanili Terhadap Penyakit Busuk Batang* (Mataram)

[17] Sudantha I and Abadi A 2007 Identifikasi jamur endofit dan mekanisme antagonismenya terhadap jamur *Fusarium oxysporum* f. sp. vanillae pada tanaman vanili *J. Agroteksos* 17:23–8

[18] Sudantha I, Kusnarta I and Sudana I 2011 Uji Antagonisme Beberapa Jenis Jamur Sapropfit Terhadap Jamur *Fusarium oxysporum* f. sp. cubense Penyebab Penyakit Layu pada Tanaman Pisang Serta Potensinya Sebagai Agens Hayati *J. Agroteksos* 21:106–19

[19] Sudantha I, Kusnarta I, Rahayu M and Sudana I 2009 Karakterisasi dan Potensi Jamur Sapropfit dan Endofit Antagonistik Untuk Meningkatkan Ketahanan Induksi Tanaman Pisang terhadap Penyakit Layu Fusarium di Nusa Tenggara Barat. Laporan Penelitian Kerjasama Kemitraan Pertanian Perguruan Tinggi (KKP3T) (Mataram: Badan Litbang Deptan)

[20] Ghorbanpoura M, Omidvarib M, Abbaszadeh-Dahajic P, Omidvard R and Karimane K 2018 Mechanisms underlying the protective effects of beneficial fungi against plant diseases *Biol. Control* 117:147–57

[21] Petrini O 1991 Fungal endophytes of tree leaves *Microbial ecology of leaves* (Springer) pp 179–97

[22] Guest D 2005 Induced Disease Resistance in Plants *In Program and Abstract The 1st International Conference of Crop Security 2005* (Malang) p 264

[23] Ghanbarzadeh B, Safaie N, Mohammadi Goltapeh E, Rezaee Danesh Y and Khelghatibana F 2016 Biological control of *Fusarium* basal rot of onion using *Trichoderma harzianum* and *Glomus mossaeae* *J. Crop Prot.* 5:359–68

[24] Khaledi N and Taheri P 2016 Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by Macrohomina phaseolina *J. Plant Prot. Res* 56:21–31

[25] Khalili E, Javed M A, Huyp F, Rayatpanah S, Jamshidi S and Wahab R A 2016 Evaluation of *Trichoderma* isolates as potential biological control agent against soybean charcoal rot disease caused by Macrohomina phaseolina *Biotecnol. Biotechnol. Equip.* 30:479–88

[26] Sudantha I M and Suwardji S 2021 *Trichoderma* biofungicides formulations on shallot growth, yield and *Fusarium* wilt disease resistance *IOP Conference Series: Earth and Environmental Science* vol 824 (IOP Publishing) p 12032

[27] Sudantha I, Suwardji S, Aryana I G P, Pramadaya I and Jayadi I 2020 The Effect of Liquid Bio Fungicides Dosage *Trichoderma* spp. against *Fusarium* Wilt Diseases, Growth and Yield of Onion *Journal of Physics: Conference Series* 1594 (2020) 012013 (IOP Publishing)

[28] Sudantha I M, Aryana I G P M, Suwardji S, Jayadi I and Pramadaya I M A 2021 Growth and yield response of shallots applied with growth regulators benzyl amino purine (GR BAP) and liquid bioactivator of *Trichoderma harzianum* fungus *Proceeding International Conference on Science (ICST)* vol 2 pp 129–40

[29] Gupta V G, Schmoll M, Herrera-Estrella A, Upadhyay R S, Druzhinina I and Tuohy M 2014 *Biotechnology and biology of Trichoderma* (USA: Newnes)

[30] Thanapat S, Harada H, Jogloy S, Ekprasert J and Boonlue S 2020 Rhizosphere *Elsevier* 16

[31] Sudantha I M and Suwardji S 2021 The effect of biocompost *Trichoderma* spp. tablet in stimulating shallot growth and yield for climate change adaptation *IOP Conference Series: Earth and Environmental Science* vol 824 (IOP Publishing) p 12033

[32] Suwardji S, Sudantha I and Muliarta I 2018 Pemberdayaan kelompok tani dalam memproduksi biokompos dan aplikasinya untuk meningkatkan hasil umbi bawang merah dan rumah pangan lestari *Abdi Insa. Unram* 5:1–16

[33] Sudantha I M, Thei R S P and Jayadi I 2018 Produksi dan penerapan teknologi hayati (biokompos, bioaktivator dan bibit unggul bawang merah) pada budidaya tanaman bawang merah *Abdi Insa. Unram* 5
[34] Yusrinawati Y, Sudantha I and Astiko W 2017 The Effort of Increasing Growth And Harvest of Local Variety Red Onion With Applications of Some Dose of Indigenous Mycorrhizal And Bioactivator *Trichoderma* spp. in Dry Land *IOSR J. Agric. Vet. Sci.* **10** 42–9

[35] Sudantha I 2017 *Eksplorasi Sumberdaya Alam (Biokompos, Bioaktivator, Biochar dan FMA) Untuk Mengembangkan Tanaman Pangan Sistem Organik Di Lahan Kering* (Mataram: Universitas Mataram)

[36] Yudhiarti S, Sudantha M and Fauzi T 2020 Effect of Giving Arbuscular Mycorrhizal Fungi (AMF) and Bioactivator Dosage of *Trichoderma* spp. on the Growth and Products of soybeans (*Glycine max* L. Merr.) *Traektoriâ Nauk. Path Sci.* **6**

[37] Sudantha I and Suwardji S 2016 Growth an Yield of Onion (*Allium Cepa Var. Ascalonicum*) as CA Result of Addition of Biocompost and Boactivity Fermented with *Trichoderma* spp. *The 1st International Conference on Science and Technology (ICST)* (Mataram: Universitas Mataram)

[38] Apzani W, Sudantha I and Fauzi M 2015 Aplikasi biokompos stimulator *Trichoderma* spp. dan biochar tempurung kelapa untuk pertumbuhan dan hasil jagung (*zea mays* L.) di lahan kering *J. Agroteknologi* **9** 21–35