Characterization and virulence of *Fusarium oxysporum* f. sp. *cubense* cause wilt disease in banana plants and its biological control using endophytic fungi *Trichoderma* spp. at West Nusa Tenggara, Indonesia

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**Abstract.** One of the obstacles in developing banana plants is the presence of *Fusarium* wilt disease caused by the fungus *Fusarium oxysporum* f. sp *cubense*. This fungus is difficult to control because it has a survival structure in the form of chlamydospores that can survive as a saprophyte. One way to control it is biologically using the antagonist endophytic fungi *Trichoderma* spp. This study aimed to determine the characteristics and virulence of the fungus *F. oxysporum* f. sp *cubense* and its control using the endophytic fungi *Trichoderma* spp. The study used an exploratory method carried out in an endemic area of *Fusarium* wilt disease to isolate the fungus *F. oxysporum* f. sp *cubense* and the endophytic fungi *Trichoderma* spp. in the banana plant tissue. The research was continued in the laboratory, in the greenhouse, and in the field to test the effectiveness of the fungi *Trichoderma* spp. The results showed that seven isolates of the fungus *F. oxysporum* f. sp *cubense* with different characteristics. Six isolates of the endophytic fungi *Trichoderma* spp. which is effective in suppressing the growth of the fungus *F. oxysporum* f. sp *cubense*, namely *T. harzianum*, *T. koningii*, *T. aureoviride*, *T. hamatum*, *T. viride*, and *T. piluliferum*.

1. Introduction
One of the obstacles in the development of banana (*Musa* sp *L.* ) in West Nusa Tenggara to improve quality and production is the presence of *Fusarium* wilt disease caused by the fungus *Fusarium oxysporum* f. sp *cubense*. Symptoms caused by *Fusarium* wilt disease on banana plants are yellow leaves then turn brown and dry up, leaf stalks are broken around the false stems. Typical symptoms when the base of the false stem is split longitudinally, brown or black lines are seen in the vascular tissue. Based on the results of a preliminary survey in banana plant centers in West Nusa Tenggara, it is known that the intensity of *Fusarium* wilt disease ranges from 50 - 100% [1].

Until now, *Fusarium* wilt is one of the diseases on banana plants that is difficult to control because the fungus *F. oxysporum* f. sp *cubense* has a survival structure in the form of chlamydospores that can survive...
in the soil as a saprophyte for a relatively long time of about three to four years even without a host plant. In addition, the difficulty of controlling this disease is due to its transmission through infected banana seedlings, so that its spread becomes fast and widespread [2].

Thus, it is necessary to find an alternative to control Fusarium wilt that is effective and environmentally friendly. One control technique that has good prospects in the future is biological technology using the endophytic fungi *Trichoderma* spp., which can later be used to increase the resistance induced by banana seedlings to *Fusarium* wilt disease.

Endophytic fungi are fungi that live in healthy plant tissue without causing disease symptoms or damage to the host plant. This symbiosis is diverse and can be mutualistic, neutralistic, or antagonistic. Colonization of plant tissue by endophytic fungi occurs in the same way as plant pathogens or mycorrhizae. Colonization consists of several stages in a series, including host recognition by fungi, spore germination, penetration of the epidermis, and tissue colonization [3]. Induction resistance is the resistance of plants to pathogen infection because plants have been infected by other microorganisms before, both of the same type or other types [4].

Induced resistance in various plants due to the presence of the endophytic fungi *Trichoderma* spp. has been widely reported. Sulistyowati, Deci, and Gendall reported that the endophytic fungus *Trichoderma asperellum* isolated from citrus stem tissue acted as an antagonist against the fungus *Phytophthora* spp. and *Diplodia* spp [5]. Budi, Mariana, and Rachmadi said that the endophytic fungi *Trichoderma* spp. found in the stem and root tissue of tidal swamp rice can reduce the incidence of diseases caused by the fungus *Rhizoctonia solani* up to 80% [6]. The results of isolation from healthy vanilla plant tissue found six species of endophytic fungi *Trichoderma* spp. effective for controlling *Fusarium* wilt disease [7]. Sudantha found six species of endophytic fungi on soybean plants, one of which is effective for controlling *Fusarium* wilt disease is *T. koningii* [8]. Furthermore, the results of research by Sudantha and Suwardji on corn plants using the endophytic fungus *T. koningii* were effective for downy mildew [9]. Sudantha et al. the use of endophytic fungi *T. koningii* and *T. harzianum* was effective for controlling *Fusarium* wilt disease on shallots [10]. Mulaw et al. isolated from the endofit fungus *Trichoderma* on the roots of coffee plants in East Africa, the species *T. flagellatum* was used to control *Fusarium* wilt disease. In addition, it is effective for controlling plant diseases caused by the fungi *Alternaria alternata*, *Botryotinia fuckeliana*, and *Sclerotinia sclerotiorum* [11]. Furthermore, Larran et al. said that the fungus *T. hamatum* isolated from wheat plants in Buenos Aires was effective for the control of brown spot disease caused by the fungus *Pyrenophora tritici-repentis* [12].

The mechanism of antagonism of endophytic fungi in suppressing the development of pathogens so that plants become resistant because as mycoparasites that can penetrate the mycelium and chlamydospores of pathogenic fungi resulting in lysis and crystallization, produce antibiotics (gliotoxin and viridine) which can inhibit the growth of pathogenic fungi, and have the ability to grow well. Faster so that there is competition for space and nutrients with other fungi [13]. Sudantha confirmed that the mechanism of antagonism between the fungi *Trichoderma* spp. with soil-borne pathogenic fungi, namely by competition, mycoparasites, and antibiosis [14].

Based on the description that has been stated, a study has been carried out that aims to determine the characteristics and virulence of the fungus *F. oxysporum f. sp cubense* causing wilt disease and biological control using the endophytic fungus *Trichoderma* spp. in West Nusa Tenggara.
2. Method
The study used two methods, namely exploratory and experimental. The exploratory method includes the isolation of endophytic fungi, purification, and identification of isolates, while the experimental method is carried out through a series of experiments in the laboratory, greenhouse, and field.

2.1. Exploratory method

2.1.1. Harvesting banana plants infected with Fusarium wilt disease and healthy plants. A sampling of healthy banana plants and those infected with Fusarium wilt disease was carried out on banana plantations spread over 16 locations in West Nusa Tenggara covering Lombok Island, each in two sub-districts for Mataram City (Ampenan and Cakranegara Districts), West Lombok District (Lobar) (Gerung District), and Sheets), Central Lombok District (Loteng) (Jonggat and Praya Districts) and East Lombok District (Lotim) (Aikmel and Masbagik Districts). Meanwhile, Sumbawa Island is in two sub-districts for West Sumbawa Regency (Taliwang and Jerewe Subdistricts), Sumbawa Regency (Empang and Plampang Subdistricts), Dompu Regency (Monta Baru and Woha Subdistricts), and Bima Regency (Madapangga and Manggalewa Subdistricts).

2.2. Experimental methods in the laboratory

2.2.1. Isolation, purification, identification of fungus F. oxysporum f. sp cubens and virulence test. Banana plants suspected of containing endophytic fungi were taken from various varieties of healthy banana plants among diseased plants, while the fungus F. oxysporum f. sp cubense was isolated from banana plants that showed Fusarium wilt symptoms. Five plants were selected in each banana variety and location, then the roots, pseudostem base, and leaves of each plant were put into plastic bags, then taken to the laboratory.

The roots or the base of the banana plant stem between the diseased and healthy parts were cut to a size of 0.5 cm, dipped in 1% sodium hypochlorite in 10% ethanol, then washed with sterile water. The diseased root fragments were then grown on a PDA medium. To avoid contamination by bacteria into the medium, added antibiotics such as Streptomycin. Then the cultures were incubated at room temperature. The fungus that grows is then purified and cultured to be used as a source of inoculum. Fungus F. oxysporum f. sp cubense obtained from each soil sampling location was then purified.

2.2.2. Isolation, purification, and identification of endophytic fungi Trichoderma spp. Endophytic fungi were isolated from samples of banana roots, stems, and leaves that were healthy or not infected with wilt disease. Roots, stems, and banana leaves were cut into strips of 1.0 - 1.5 cm long, sterilized the surface using 10% Chlorox (sodium hypochlorite) for two minutes and 96% absolute alcohol (for 30 seconds) three times, and sterile distilled water three times. Each material that has been surface-sterilized is placed on PDA (Potato Dextrose Agar) medium in a Petri dish and removed again, which aims to determine whether the surface of the material has been sterilized was truly sterile or as a control (material to check). Furthermore, it was cut along 0.5 cm and split, and then grown on a PDA medium that had been given lactic acid or antibiotics to avoid contamination by bacteria.

The endophytic fungi that grew were then isolated, grown on a PDA medium in another Petri dish. To ensure that the growth is endophytic fungi, then the control medium (material to check) must not be found growing fungi. The growing endophytic fungi were transferred to a Petri dish containing PDA medium by
single conidium transfer technique or hyphae 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 phialides, and phialides density (if any). Furthermore, it was identified using the identification key book by Barnett [15], Alexopoulos and Mims [16], and Rifai [17].

2.2.3. Test of antagonism of endophytic fungi Trichoderma spp. with fungus F. oxysporum f. sp cubense. The antagonism test was carried out using inoculum isolates of the fungus F. oxysporum f. sp cubens, and each endophytic fungal isolate was grown at a distance of 4 cm in the center of the PDA medium in a Petri dish with a diameter of 9 cm. Fungal inoculum F. oxysporum f. sp cubens 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 the fungal colonies of F. oxysporum f. sp cubens and the presence of a zone of inhibition between two opposing endophytic fungal colonies. Inhibition of mycelium growth of the fungus F. oxysporum f. sp cubens by endophytic fungi is calculated based on the formula, namely:

\[
I = \left(1 - \frac{r_2}{r_1}\right) \times 100\%
\]

where:
- \(I\) = percentage of inhibition,
- \(r_1\) = radius of fungal colonies of F. oxysporum f. sp cubens growing in the opposite direction to the endophytic fungal site,
- \(r_2\) = radius of the fungal colony F. oxysporum f. sp cubens growing towards endophytic fungi.

2.2.4. Endophytic fungus Trichoderma spp. culture vapor test with F. oxysporum f. sp cubens. This test aims to determine the presence of endophytic fungal culture vapors containing antibiotics. For this purpose, a fungal culture of F. oxysporum f. sp cubens by planting a piece of culture with a diameter of 4 mm 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 endophytic fungal culture with a diameter of 4 mm from a three-day-old culture in a 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 cultured endophytic fungi, the cultures of F. oxysporum f. sp cubens. Observation of the growth of the fungus F. oxysporum f. sp cubens was done 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 study was a Completely Randomized Design consisting of two factors.

The first factor is the endophytic fungi Trichoderma spp. (T) which consists of four levels, namely:
- T0 = No Endophytic Fungus Trichoderma sp
- T1 = With Endophytic Fungus T. harzianum
- T2 = With Endophytic Fungus T. viride
T3= With Endophytic Fungus *T. hamatum*

The second factor is the banana variety (V), namely:
- V1= Pisang Kepok Varieties
- V2= Pisang Ketip Varieties
- V3= Pisang Susu Varieties

The treatment was a combination of the endophytic fungal *Trichoderma* spp: factor and banana varieties which were repeated three times to obtain 36 experimental units.

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

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

(2)

- \(a\) = number of plants infected with *Fusarium* wilt disease
- \(b\) = number of healthy plants

2.3.2. *Data analysis*. The data were analyzed using the Analysis of Variance (ANOVA) at the 5% significance level and further tested using the BNJ at the 5% significance level.

2.4. *Experimental methods in the field*

The experimental design used in this research uses a Randomized Block Design, which consists of two factors.

The first factor is the application method of the endophytic fungus *T. koningii* (T), which consists of four levels, namely:
- T0 = Without Endophytic Fungus *T. koningii*
- T1= Soaking Seeds with Endophytic Fungus *T. koningii*
- T2= Injection Seeds with Endophytic Fungus *T. koningii*
- T3= Sprinkling Seeds with Endophytic Fungus *T. koningii*

The second factor is the banana varieties (V), namely:
- V1= Pisang Kepok Varieties
- V2= Pisang Ketip Varieties
- V3= Pisang Susu Varieties

The treatment was a combination of the endophytic fungal factor *Trichoderma* spp. and banana varieties which were repeated three times to obtain 36 experimental units.

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

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

(3)
a = number of plants infected with *Fusarium* wilt disease
b = number of healthy plants

2.4.2. Data analysis. The data were analyzed using the ANOVA at the 5% significance level and further tested using the BNJ at the 5% significance level.

3. Results and discussion

3.1. Results of characterization and virulence test of fungus *F. oxysporum f. sp cubense*
Based on the results of laboratory observations on samples of stems, leaves, and fruit that showed symptoms of illness from 16 locations of banana plantations, eight isolates of the fungus *F. oxysporum f. sp cubense* with the same characteristics. In PDA medium pH 6.0, which was incubated at 25°C, in general, this fungus formed white colonies with a diameter of 90 mm after being incubated for 2-9 days (Figure 1 A and 1 D). In the eighth PDA medium, isolates of the fungus *F. oxysporum f. sp cubense* forms macroconidia after incubation for 7-14 days with a large number of 3 to 5 septa, bent shape like a crescent moon and pointed at the end, measuring 24 - 44 long and 3-5 wide. Macroconidia formed after 3-7 days of incubation in large numbers, round to oblong in shape, 5-9 long, and 2-3 wide. The characteristics of the eight fungal isolates of *F. oxysporum f. sp cubense* is the same as that proposed by Booth (1971), namely short to medium-sized macroconidia, bent to almost straight (27 – 46 x 3 – 5 ), thin-walled, and usually three septate and at the ends tend to be tapered. Microconidia are not septate and are oval to elliptical, measuring 5 – 12 x 2.2 – 3.5 (Figs 1 B and 1 E). The longest incubation period for *Fusarium* wilt caused by the fungus Foc-01 Mataram is 35 days (Figure 1 C) and the fastest by the fungus Foc-6 Sumbawa, which is the fastest, which is 4 days (Figure 1 F).

Fungal colonies of *F. oxysporum f. sp cubense* isolate Foc-01 Mataram, 8 days old, filled a Petri dish

Morphology (1= macroconidia, 2 = microconidia, 3 = hyphae)

The incubation period for *Fusarium* wilt is 35 days after inoculation of the pathogen on the kepok banana variety
The incubation period for Fusarium wilt is 4 days after inoculation of the pathogen on the kepok banana variety. Colony morphology of the fungus *F. oxysporum f. sp cubense* isolate Foc-06 Sumbawa, two days old filled a Petri dish.

**Figure 1.** Colonies and morphology of several isolates of the fungus *F. oxysporum f. sp cubense* isolates Foc-01 Mataram and isolates Foc-06 Sumbawa and incubation period of *Fusarium* wilt disease on virulence test

3.1.1. **Fungal virulence test** *F. oxysporum f. sp cubense* (Foc). The results of the analysis of the diversity of the incubation period of *Fusarium* wilt disease on the virulence test of the fungus *F. oxysporum f. sp cubense* showed that there was a significantly different interaction between the isolates of Foc fungus and the banana varieties tested. The results of further test interactions between isolates of Foc fungi and banana varieties on the incubation period of *Fusarium* wilt disease in the virulence test are presented in Table 1.

**Table 1.** The average incubation period of *Fusarium* wilt caused by several isolates of the fungus *F. oxysporum f. sp cubense* on virulence test

| Origin of Fungus isolate *F. oxysporum f. sp cubense* (Foc) | The average incubation period of *Fusarium* wilt on banana seedlings (days) | Banana plant varieties |
|------------------------------------------------------------|-----------------------------------------------------------|------------------------|
|                                                            | Pisang kepok | Pisang susu | Pisang hijau | Pisang ketip |
| Foc-01 Mataram                                             | 35.00 a*)    | 44.30 b     | 45.50 b      | 49.00 c      |
|                                                            | E**)         | E           | E            | E            |
| Foc-02 Lobar                                               | 21.00 a      | 27.70 b     | 31.00 b      | 35.40 c      |
|                                                            | C            | C           | C            | C            |
| Foc-03 Lotim                                               | 29.00 a      | 37.50 b     | 38.60 b      | 41.20 c      |
|                                                            | D            | D           | D            | D            |
| Foc-04 Loteng                                              | 20.10 a      | 26.00 b     | 32.40 b      | 35.30 c      |
|                                                            | C            | C           | C            | C            |
| Foc-05 Sumbawa Barat                                       | 9.00 a       | 15.00 b     | 16.90 b      | 19.20 c      |
|                                                            | B            | B           | B            | B            |
Table 1 shows that the eight isolates of the fungus *F. oxysporum f. sp cubense* can cause *Fusarium* wilt disease in all banana varieties tested with different incubation periods. The banana variety that was most sensitive to *Fusarium* wilt disease was the Pisang kepok variety, followed by the Pisang susu variety, the Pisang hijau variety, and the Pisang ketip variety. The most virulent isolate in causing *Fusarium* wilt disease was Foc-06 from Plampang Sumbawa with the shortest incubation period for *Fusarium* wilt disease, which was 4.70 days on average for the Pisang kepok variety, followed by Foc-07 Dompu, Foc-08 Bima, Foc-05 West Sumbawa, Foc-02 Lobar and Foc-03 Lotim, while the less virulent was Foc-01 Mataram with an average incubation period of 35.00 days for the Pisang kepok variety. Thus, for further experiments in the laboratory and plastic house, isolates of the fungus Foc-06 Sumbawa were used. The emergence of symptoms of *Fusarium* wilt disease in banana plants that are more susceptible to banana varieties is thought to be due to the high virulence level of the fungus *F. oxysporum f. sp cubense* in causing disease and the absence of growth and development inhibitors of the fungus. According to Agrios [18], virulent pathogens can rapidly infect their hosts and subsequently produce more inoculum than less virulent pathogens.

### 3.2. Characterization and antagonism test for endophytic fungi *Trichoderma* spp.

#### 3.2.1. Results of characterization of endophytic fungi *Trichoderma* spp.

The results of the identification of the endophytic fungus *Trichoderma* spp. found in healthy banana plant tissue to the species level was 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, while to ascertain the differences in the types of fungi belonging to the same genus, macroscopic observations were also carried out including color, thickness, pattern, growth and colony diameter, because each type of fungus has its characteristics in terms of its macroscopic appearance, as shown in Figure 2-7 and the following description.

**3.2.1.1. Trichoderma koningii Oud. aggr.** The macroscopic characterization of the fungus *T. koningii* was that the fungal colonies grew rapidly three days after inoculation covering the Petri dishes (90 mm). At first, it is white and after forming the phialospore turns greenish white to dark green. Colonies grow thick and dense. Microscopic characterization of hyaline mycelia, insulated, branched, Phialospore spherical green, 3 – 5 in diameter. Many branched conidiophores. Phialide formed more than 2 – 3 at the end of the branching conidiophores; each end of the phialide formed phialospore (Figure 2).
3.2.1.2. *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 3).

3.2.1.3. *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 4).

3.2.1.4. *Trichoderma polysporum* (Link ex Pers.) Rifai aggr. The macroscopic characterization of the fungus *T. polysporum* was that the fungal colonies of this fungus on the PDA medium grew rapidly, covering the entire surface of the dish (90.00 mm) after three days of inoculation with white mycelia and remained white in old colonies. Hyphae branched, hyaline, septate. Infertile conidiophores there are many branches and at the ends there are phialides, sterile conidiophores grow elongated with branches between 2 – 3. The phialide shape is like a fruit per fruit, measuring 4 – 6.5 x 3 – 3.5. Colorless phialospore elliptical in size 2.7 – 3.7 x 1.5 – 2.2 (Figure 5).

3.2.1.5. *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 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 (Figure 6).

3.2.1.6. *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 7).
3.3. In-vitro antagonism test results with direct opposition method and vapor culture test

The results of the diversity analysis showed that all isolates of the endophytic fungi *Trichoderma* spp. significantly different in inhibiting the growth of the fungus *F. oxysporum f. sp cubens* both by the direct opposition method and the steam method of culture of *Trichoderma* spp. The results of further tests between isolates of the endophytic fungi *Trichoderma* spp. which are significantly different from each other are presented in Table 2 and Table 3.
Table 2. The average percentage of fungal growth inhibition *F. oxysporum f. sp cubens* in opposition to some endophytic fungi *Trichoderma* spp.

| Treatment of fungus *F. oxysporum f. sp cubens* in opposition to the endophytic fungi *Trichoderma* spp. | Average inhibition (%) |
|---------------------------------------------------------------|-------------------------|
| *T. koningii*                                                 | 45.70 c*                |
| *T. harzianum*                                                | 43.60 c                 |
| *T. viride*                                                   | 43.50 c                 |
| *T. polysporum*                                               | 40.30 b                 |
| *T. hamatum*                                                  | 40.20 b                 |
| *T. piluliferum*                                              | 40.10 b                 |
| Tanpa jamur endofit                                           | 0.00 a                  |

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

Table 3. The average percentage of fungal growth inhibition *F. oxysporum f. sp cepae* on PDA medium in a Petri dish cupped over cultures of several saprophytic fungi *Trichoderma* spp. after being incubated for eight days

| Treatment of fungi *F. oxysporum f. sp cepae* cupped on a culture of the saprophytic fungus *Trichoderma* spp. | Average inhibition (%) |
|----------------------------------------------------------------------------------------------------------------|-------------------------|
| *T. koningii*                                                                                                   | 76.60 c*                |
| *T. harzianum*                                                                                                | 75.20 c                 |
| *T. viride*                                                                                                    | 75.10 c                 |
| *T. polysporum*                                                                                                | 65.60 b                 |
| *T. hamatum*                                                                                                   | 64.40 b                 |
| *T. piluliferum*                                                                                               | 63.40 b                 |
| Without endofit fungi                                                                                          | 0.00 a                  |

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

In Table 2 and Table 3, it can be seen that all isolates of the endophytic fungi *Trichoderma* spp. can inhibit the growth of the fungus *F. oxysporum f. sp cubens* in both the direct opposition method and the steam method of *Trichoderma* spp. mushroom culture, but the level of inhibition was different. Endophytic fungi *Trichoderma* spp. the most capable of inhibiting the growth of the fungus *F. oxysporum f. sp cubens* were *T. harzianum*, *T. viride*, and *T. hamatum* (Figures 8 and 9) with an inhibition percentage 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 an antibiotic mechanism occurs.
The fungus \textit{T. koningii} (T) with \textit{F. oxysporum f. sp cubens} (F)

\textbf{Figure 8}. Growth of the fungus \textit{F. oxysporum f. sp cubens} (F) was inhibited by the endophytic fungus \textit{T. koningii} (A) and \textit{T. harzianum} (B) in the antagonism test using the direct opposition method.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig8.png}
\caption{Growth of the fungus \textit{F. oxysporum f. sp cubens} (F) was inhibited by the endophytic fungus \textit{T. koningii} (A) and \textit{T. harzianum} (B) in the antagonism test using the direct opposition method.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig9.png}
\caption{Growth of the fungus \textit{F. oxysporum f. sp cubens} (F) on PDA medium cupped on a culture of \textit{T. koningii} (T) and control (C) or without a culture of \textit{T. koningii} after seven days of incubation}
\end{figure}

In Figure 8 it can be seen that in the direct opposition test of the endophytic fungi \textit{Trichoderma} spp. can grow continuously through the fungal colonies \textit{F. oxysporum f. sp cubens} causing the growth of the fungus \textit{F. oxysporum f. sp cubens} to become blocked but does not show the inhibition zone. It is suspected that the endophytic fungi \textit{Trichoderma} spp. can grow quickly or space competition occurs and can
entangle the hyphae of the *Fusarium* fungus or a mycoparasite mechanism occurs. In Figure 9 in the cupping test, it can be seen that the endophytic fungi *Trichoderma* spp. can inhibit the growth of the fungus *F. oxysporum f. sp cubens*, so that the diameter is smaller, while the control fungus *F. oxysporum f. sp cubens* grow normally that fills the surface of the Petri dish. It is suspected that endophytic fungi secrete volatile antibiotic or alkaloid compounds so that they can inhibit the development of *Fusarium* fungi or an antibiosis mechanism occurs. Several researchers have reported this incident, such as Abadi, who reported that the fungus *T. harzianum* grown together with the fungus *Ganoderma boninense* caused the hyphae of the fungus *G. boninense* to lyse [19]. According to Sudantha and Abadi [20] that the mechanism of antagonism between endophytic fungi *T. koningii*, *T. harzianum*, *T. viride*, and *F. oxysporum f. sp vanillae* through the mechanism of competition for space and nutrients and mycoparasites. According to Sudantha et al. that the mechanism of competition antagonism, mycoparasites, and antibiosis can be used to increase the induced resistance of banana plants to *Fusarium* wilt disease [21]. Further, Ghorbanpoura et al. said that the mechanism of antagonism of the antagonist fungus *Trichoderma* sp with pathogenic fungi through five ways, namely: competition with pathogenic fungi for space and nutrients, mycoparasitism, antibiotics, mediated by mycovirus cross-protection, and induced systemic resistance [22].

### 3.4. Experimental results in the greenhouse

The results of the analysis of variance showed that all isolates of the endophytic fungi *Trichoderma* spp. significantly different in inhibiting the incidence of *Fusarium* wilt disease in three varieties of banana plants at the age of 35 days after planting in the Greenhouse. The results of further tests between isolates of the endophytic fungi *Trichoderma* spp., which are significantly different from each other, are presented in Table 4.

| Treatment of endophytic fungi *Trichoderma* spp. | Average occurrence of *Fusarium* wilt disease (%) |
|---------------------------------------------------|-----------------------------------------------|
|                                                   | Pisang kepok varieties | Pisang ketip varieties | Pisang susu varieties |
| Endophytic Fungus *T. koningii*                   | 0.00 a*)               | 0.00 a*)               | 0.00 a*)             |
|                                                   | A**)                   | A**)                   | A**)                 |
| Endophytic Fungus *T. harzianum*                  | 5.70 a                 | 5.80 a                 | 5.90 a               |
|                                                   | B                      | B                      | B                    |
| Endophytic Fungus *T. viride*                     | 5.70 b                 | 5.80 b                 | 5.80 b               |
|                                                   | B                      | B                      | B                    |
| Without Endophytic Fungus *Trichoderma* spp.     | 60.70 a                | 60.80 a                | 60.90 a              |
|                                                   | C                      | C                      | C                    |

In Table 4 it can be seen that all the endophytic fungi *Trichoderma* spp. can suppress the incidence of *Fusarium* wilt disease in both Pisang Kepok varieties, Pisang Ketip varieties, and Pisang Susu varieties. The type of fungus *Trichoderma* spp. specifically, *T. koningii* was the most capable of suppressing *Fusarium* wilt disease, and there was no occurrence of *Fusarium* wilt disease or induced resistance to *Fusarium* wilt disease in Pisang Kepok varieties, Pisang Ketip varieties, and Pisang Susu varieties.
Thus it can be said that the treatment with the endophytic fungi *Trichoderma* spp. can increase the resistance induced by banana seedlings to *Fusarium* wilt disease. This is in accordance with the opinion of Sudantha [23] that plant-induced resistance to disease can occur because the plant has been infected by antagonistic fungi, for example, resistance induced by vanilla plants due to *Fusarium* wilt disease that has been inoculated with the endophytic fungus *Trichoderma* spp. Guest [24] said that induced resistance occurs due to a combination of passive resistance with local responses due to cell death events and accumulation of antibiotics, which can be phytoalexins. Sudantha [25] reported that the endophytic fungus *T. koningii* was effective for controlling *Fusarium* wilt disease in various crops such as tobacco, corn, soybeans, and vanilla. Furthermore, Sudantha [26] said that the endophytic fungus *T. koningii* has the potential to be developed as a bio fungicide and bio activator. Talapatra et al. [27] reported that the endophytic fungus *T. viride* was effective in controlling plant diseases caused by the fungus *Alternaria sp.* and *Pythium sp.*

As an illustration of the effect of the endophytic fungi *Trichoderma* spp. In increasing the induced resistance of banana seedlings to *Fusarium* wilt disease, it can be seen in Figure 10 A, i.e. banana seedlings appeared healthy with normal growth, while Figure 10 B showed that banana seedlings infected with *Fusarium* wilt disease were in control or without treatment with the endophytic fungi *Trichoderma* spp., i.e. the leaves and petioles become brownish yellow and dry up.

![Figure 10](image)

**Figure 10.** Healthy banana with the treatment of endophytic fungi *Trichoderma* spp. (A). Banana infected with *Fusarium* wilt disease without endophytic fungi *Trichoderma* spp. treatment (B)

### 3.5. Field experiment results

The results of the analysis of variance showed that the application of the endophytic fungus *T.koningii* in three different ways showed significant differences in the incidence of *Fusarium* wilt disease. The results of further tests between the application methods of the endophytic fungus *T. koningii*, which differ significantly from each other, are presented in Table 5.
Table 5 shows that the application of the endophytic fungus *T. koningii* can suppress the incidence of *Fusarium* wilt disease in the Pisang Kepok, Pisang Ketip, and Pisang Susu varieties. All methods of application of the *T. koningii* fungus, either by immersing banana seeds, injecting banana seeds, and watering banana seedlings, were significantly different with or without the control of endophytic fungi, both on Pisang Kepok, Pisang Ketip, and Pisang Susu varieties. In fact, in all treatment methods for the endophytic fungus *T. koningii*, there was no *Fusarium* wilt disease or induced resistance to *Fusarium* wilt disease in Pisang Kepok, Pisang Ketip, and Pisang Susu varieties.

Table 5. The average incidence of *Fusarium* wilt disease with three treatments of the endophytic fungus *T. koningii* on the seeds of three varieties of banana plants aged 35 days after planting in the field

| Treatments the endophytic fungus *T. koningii* | The average incidence of *Fusarium* wilt disease (%) |
|-----------------------------------------------|----------------------------------------------------|
|                                              | Pisang Kepok Varieties | Pisang Ketip Varieties | Pisang Susu Varieties |
| Soaking Seeds with Endophytic Fungus *T. koningii* | 0.00 a*) | 0.00 a*) | 0.00 a*) |
| Injection of Seeds with Endophytic Fungus *T. koningii* | 0.00 a | 0.00 a | 0.00 a |
| Watering Seedlings with Endophytic Fungus *T. koningii* | 0.00 a | 0.00 a | 0.00 a |
| Without endophytic fungi | 70.70 a | 70.80 a | 70.90 a |

Information: *) The numbers in each column followed by the same letter are not significantly different.

**) The numbers on each line followed by the same letter are not significantly different.

The fact that banana seedlings became immune after being treated with the endophytic fungus *T. koningii* was thought to be due to the mechanism of antagonism between the endophytic fungus *Trichoderma* and the fungus *F. oxysporum f. sp cubens* that occurred in vitro (Tables 1 and 2) also occurred in the field. The same thing happened to tomato plants, as reported by Sridanti and Sudantha [28] that the treatment of bio activators containing the fungus *Trichoderma* spp. in tomato plants caused induced resistance to *Fusarium* wilt disease caused by the fungus *F. oxysporum f. sp lycopersici*. Sudantha and Suwardji [29] also reported that the use of *Trichoderma* spp. in the form of bio fungicides can increase the resistance induced by shallots against *Fusarium* wilt disease. Bae et al. reported that the use of the endophytic fungus *Trichoderma* spp. for the control of the fungus Phytophthora capsici causing pepper plants to be very resistant and induced resistance to occur [30]. The same case also occurred as reported by Fontana et al. [31] that endophytic fungi have the potential to act as biological control agents, as elicitors in the process of resistance induction and in reducing abiotic stresses due to the ability of endophytic fungi to produce biologically active substances. Even Sudantha and Suwardji [32] said that the endophytic fungus *Trichoderma* given in the bio compost formulation could stimulate growth and increase the yield of shallots, and could increase the induced resistance to drought stress. Furthermore, Sudantha et al. [33] said that the fungus *Trichoderma* spp. has the same role as the growth regulator benzyl amino purine in promoting growth and increasing plant yields.
The successful use of *Trichoderma* spp. in increasing induced resistance, growth and yield have good prospects in the future to be developed in various plants, as reported by several researchers. Apzani et al. [34] reported that the use of the fungus *Trichoderma* spp. on corn could increase plant growth and yield. Sudantha [35] and Sudantha [36] said that the application of *Trichoderma* spp. and litter could increase the induced resistance of vanilla plants to *Fusarium* stem rot disease. Similarly, Sudantha and Abadi [37] explained that some vanilla clones could increase their resistance to stem rot disease after being treated with the endophytic fungus *Trichoderma* spp. Ningsih et al. [38] concluded that onion plants treated with a mixture of *Trichoderma* spp. and the element Boron could stimulate growth and increase yields. Furthermore, Yusrinawati et al. [39] explained that administration of *Trichoderma* spp. accompanied by mycorrhizal fungi can increase growth and yield. This success is supported by Thanapat et al. [40] said that the endophytic fungus *T. koningii* should be considered as a biofertilizer or biofungicide because of its ability to suppress pathogenic fungi and increase plant height, chlorophyll, and crop yields. Rajani et al. [41] said that endophytic fungi produce volatile organic compounds as pathogenic antifungal and antitoxic compounds.

As an illustration of the response of banana plants treated with the endophytic fungus *T. koningii* as shown in Figure 11 A, the banana seedlings grew healthy and normally, while Figure 11 B shows that the banana seedlings infected with *Fusarium* wilt disease were in control or without fungal treatment. endophytic *T. koningii*, i.e., leaves and petioles, turn brownish-yellow and dry out.

4. Conclusion
The results showed that seven isolates of the fungus *F. oxysporum f. sp cubense* with different characteristics. Six isolates of the endophytic fungi *Trichoderma* spp. which is effective in suppressing the growth of the fungus *F. oxysporum f. sp cubense*, namely *T. harzianum*, *T. koningii*, *T. aureoviride*, *T. hamatum*, *T. viride*, and *T. piluliferum*. Endophytic fungi *Trichoderma* spp. the most capable of inhibiting

![Figure 11](image-url). Healthy banana with the treatment of Endophytic *Trichoderma* spp. (A). Banana infected with *Fusarium* wilt disease without Endophytic *Trichoderma* spp. treatment (B)
the growth of the fungus *F. oxysporum* f. sp *cubens* were *T. koningii*, *T. harzianum*, and *T. viride* with an inhibition percentage of more than 43% for the direct opposition method or mycoparasite competition mechanism and more than 75% for the steam method of *Trichoderma* spp. or an antibiotic mechanism occurs. The application of the endophytic fungus *T. koningii* was able to suppress the incidence of *Fusarium* wilt disease in Pisang Kepok varieties, Pisang Ketip varieties, and Pisang Susu varieties. Methods of application of the fungus *T. koningii* by immersing seeds, injecting seeds, and watering banana seedlings effectively control *Fusarium* wilt disease and increase the immunity of banana seedlings against *Fusarium* wilt disease or induced resistance to *Fusarium* wilt disease in Pisang Kepok varieties, Pisang Ketip varieties and Pisang Susu varieties.

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