Sustainable agriculture practice using Trichoderma and nonpathogenic Fusarium to control Vanilla Shoot Rot disease

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Abstract. Vanilla shoots rot (VShR) disease in Indonesia is generally detrimental to the nursery, but due to the extreme climate change, the VShR disease attacks the vanilla plants grown in the field increases. This paper describes the development of research on the effectiveness of Trichoderma sp. and nonpathogenic Fusarium (FusNP) in controlling VShR, starting from isolation and identification of antagonistic fungi, in vitro testing to research results in the field, compared with the use of synthetic fungicides. The nonpathogenic fungi isolated from the rhizosphere soil of healthy vanilla plants were dominated by Trichoderma spp and nonpathogenic fusarium because they used selective media. The study showed that Trichoderma sp. and FusNP were quite effective in controlling the disease VShR in the field, similar to the effectiveness of synthetic fungicides, whereas the incidence of control was 32%. The use of synthetic fungicides intensively causes environmental pollution in the form of pesticide residues on leaves and fruit vanilla.

Keywords: Phytophthora capsici. plant pathogen, vanilla shoot rot disease

1. Introduction
Vanilla (Vanilla planifolia Andrew.) is one of the plantation crops that is widely grown as a smallholder plantation. Vanilla is a source of income for farmers and also a source of foreign exchange because 99% of vanilla production is exported to Europe and America. Vanilla plant products are in the form of pods and extracts, are generally used as a flavor enhancer for various foods and beverages such as ice cream, bread, and milk mixtures. Vanilla is also used for perfume and aromatherapy. One of the obstacles in the development of vanilla in Indonesia is the disease. The vanilla cultivation system that requires climbing trees and shade makes vanilla plants prone to plant diseases [1]. The main disease in vanilla is vanilla stem rot (VSR) caused by Fusarium oxysporum f.sp vanillae [2, 3]. In Polynesia, vanilla shoots rot (VShR) is a serious disease because it can reduce production and cause the death of young vanilla plants [4].

VShR is caused by Phytophthora capsici [5]. Infected shoots initially show symptoms of yellowish-brown necrosis, then develop into dark brown and rot. VShR pathogen attack on mature vanilla plants can inhibit plant growth, while in nurseries and young plants can cause plant death. In addition, seedlings infected by VShR pathogens will be a source of inoculum to spread VShR in the garden [6]. VShR disease control by farmers is generally carried out using synthetic fungicides. Synthetic fungicides are expensive, ineffective, and high risk because they leave residues in vanilla products that are harmful to consumers and can contaminate the environment. Therefore, it is necessary to use alternative control methods that are effective and environmentally friendly. One of them is using biological agents.
The use of biological agents to control various plant diseases has been widely used, for example, Trichoderma spp. used to control *Rhizoctonia solani* and *Sclerotium rolfsii* [7;8]. The use of nonpathogenic *Fusarium oxysporum* (FoNP) to induce resistance of sweet potato plants to Fusarium rot disease [9]. The resistance of tomato plants to Fusarium wilt disease can also be increased by applying FoNP to tomato seedlings [10:11]. Soaking the pepper cuttings in conidia suspension FoNP and application of FoNP wettable powder formulation can reduce *P. capsici* attack on pepper nurseries by more than 50% [12].

This paper discusses the effectiveness of *Trichoderma* sp. and nonpathogenic Fusarium (FusNP) in controlling VShR, starting from isolation, selection, and laboratory testing, until its application in the field.

### 2. Isolation and evaluation of biological agents

Isolation, selection, and evaluation of candidate isolates of biological agents is a crucial step for the success of biological control. Isolation of biological agents was carried out from the rhizosphere and vanilla plant tissue which had the best growth in vanilla plantation areas endemic to VShR disease. The results of research by [13] have explained this process, the results of which are presented in Table 1.

#### Table 1. Total fungal population on two kinds of selective media.

| Sample         | Height of place (m from sea level) | Vegetation                | Population on Media |
|----------------|-----------------------------------|---------------------------|---------------------|
| Sukabumi-Jabar | 250                               | Gamal (homogen)           | SFA: $3 \times 10^5$  | Martin Agar: $87 \times 10^4$ |
| Ciomas-Banten  | 450                               | Gamal, dadap duri, banana | SFA: $31 \times 10^5$  | Martin Agar: $77 \times 10^4$ |
| Batu-Jatim     | 650                               | Kaliandra (homogen)       | SFA: $27 \times 10^5$  | Martin Agar: $68 \times 10^4$ |

Source: [13]

The population and variety of species of fungi are influenced by the variety of vegetation in a garden area. In areas with homogeneous plants (monoculture), there is generally less variety of microbial species than in garden areas where more than one type of plant is planted (polyculture). However, the results of the isolation of rhizosphere soil from vanilla plantations in Sukabumi with relatively uniform Gamal vegetation showed that the total fungi obtained were more than those from Banten, where the vegetation was more diverse. This is probably caused by the population of pathogenic VShR fungi that are endemic in Sukabumi [13].

### 3. Pathogenicity test of fungi isolates

After obtaining pure isolates, it was continued with the pathogenicity test of the isolates isolated from vanilla plants. The results of the [13] pathogenicity test showed that 63 isolates were pathogenic to vanilla and 51 isolates were not pathogenic to vanilla. The pathogenic isolates were generally from the Fusarium genus, while the nonpathogenic from the Trichoderma and Fusarium genera. Generally, the type of Trichoderma obtained was *T. harzianum*. So much inoculum in the garden. Vanilla stem rot disease is the major disease of vanilla plants which is the most detrimental because it attacks all plant stages from seedling to mature plants.

The isolation results from the surface of the vanilla plant were relatively small. Because the surface structure of the hairless vanilla plant was less supportive for fungal colonization. Smooth, hairless plant surfaces are less able to hold water which is needed by fungi, on hairy plant surfaces such as plants from the Solanaceae family, quite a lot of fungi can be isolated. The isolation of several types of fungi such as fusarium, myroctecium, which are antagonistic to potato leaf blight pathogens, had been succeeded [14].
Table 2. Pathogenicity test results of fungal isolates against vanilla cuttings.

| Source isolate      | Pathogenic | Nonpathogenic | Total Isolate |
|---------------------|------------|---------------|---------------|
| Rizosfer            | 59 (61%)   | 38 (39%)      | 97            |
| Part of plant       |            |               |               |
| -surface leaf       | 4 (57%)    | 3 (43%)       | 7             |
| -stem endophyte     | -          | 4 (100%)      | 4             |
| Root endophyte      | -          | 6 (100%)      | 6             |

Source: [13]

4. In vitro biological agent antagonist test

Dozens of isolated Trichoderma isolates were selected based on their color and colony shape on PDA. So that fifteen different Trichoderma isolates were obtained. The isolates were tested for their inhibition against VShR pathogens in vitro. Tests carried out [13] on V8 juice agar media are presented in Figure 1. The inhibitory capacity of biological agents on the growth of *P. capsici* ranged from 44.5 to 73.5%, isolates with the highest inhibition were used for field testing. Inhibition mechanism in the form of space competition, nutrition, and mycoparasites. Meanwhile, Trichoderma isolates that showed antibiosis activity were not found, this was because the media used was V8 juice agar, which had a different composition from potato dextrose agar (PDA). In PDA, *Trichoderma* spp. can overgrow and dominate the available space and nutrients because its composition is suitable for expressing its antagonistic potential. Many studies have reported that *Trichoderma* spp. has the ability to produce antibiotics, for example some isolates of *T. viride*. This species has a high antibiosis ability, but on V8 juice media, the mechanism is not visible [13].

Figure 1. The ability of *Trichoderma* spp to inhibit the development of VShR pathogens.

5. Testing the effectiveness of *Trichoderma* sp. and FusNP in the field

After obtaining the best isolates of biological agents from in vitro testing, research was carried out in the field. The study was conducted [15] on vanilla clone one which had just been transferred to the field for one month. The planting area is classified as endemic for vanilla stem rot (VSR) and Vanilla shoot rot. Before planting, trim the shade trees in such a way that only 20% of the light enters. All test plants were given 1 kg cow manure/plant as essential fertilizer.

The treatments tested were:
(a) Substrate of Trichoderma sp 1 kg/tree (label T),
(b) Flushing 50 ml of FusNP conidia suspension (population density 10^7 cfu/ml) onto vanilla roots (label F),
(c) Substrate of Trichoderma sp. 1 kg/tree and watering 50 ml of FusNP conidia suspension (population density 10^7 cfu/ml) to vanilla roots (TF label),
(d) Spraying Mancozeb 2 g/liter to all plants every week for six months (label M).
(e) control (plants only watered).

The research design was a randomized block design, and each treatment consisted of 20 vanilla plants in blocks measuring 3 x 10 m. Each treatment was repeated five times. The variables observed were the incidence and severity of VShR disease and the analysis of pesticide residues on plants and rhizosphere soil.

The results of the study [15] showed that all the treatments tested were able to inhibit the development of VShR disease, both the percentage of incidence and severity of the disease and the number of plants that died infected with VShR pathogens (Table 3). The incidence and severity of disease in vanilla plants treated with biological agents and synthetic fungicides were much lower than controls. Likewise, the percentage of vanilla plant mortality is measured. Vanilla plants that are attacked by VShR pathogens do not all die, depending on the age of the plant tissue and the length of the vanilla stem. If at the time of infection with the VShR pathogen, the stem length is more than 20 cm, then the plant tissue is quite old, likely the vanilla plant will still be able to survive, and new shoots will emerge from one of the existing buds. However, if the stem length is less than 20 cm at the time of the attack, the plant tissue is generally young, the plant will most likely die, especially if the environmental conditions are optimum for the development of VShR pathogens.

### Table 3. Mean incidence and severity of vanilla shoot rot disease 8 weeks after the application of biological agents and synthetic fungicides.

| Treatment       | Disease Incidence (%) | Scoring | Disease Severity (%) | Plant Death (%) |
|-----------------|-----------------------|---------|----------------------|-----------------|
| Trichoderma sp. (T) | 3.0                   | 5       | 3.0                  | 2.0             |
| Fus NP (F)       | 5.0                   | 5       | 5.0                  | 4.0             |
| T + F            | 2.0                   | 5       | 3.0                  | 2.0             |
| Mancozeb (M)     | 3.0                   | 5       | 3.0                  | 3.0             |
| Control          | 32.a                  | 5       | 32.0                 | 15.0            |

**Source:** [15]

The incidence of disease describes the number of plants infected with the pathogen (quantity), regardless of the effect of damage to plants (quality), so that the incidence of disease is greater than the severity of the disease, but some are the same in value. The high value of disease incidence does not represent a significant loss for vanilla plants because not all of them kill the plants, but they are still detrimental to vanilla plants because plant growth is severely hampered and becomes a source of VShR disease inoculum in vanilla plantations [15].

In almost all plants, the beginning of planting seedlings in the field is a critical time for pests and diseases because plants are generally still stressed and weak when they begin to adapt to a new environment. Plants that were treated with biological agents and synthetic fungicides were initially infected with the VShR pathogen, which is endemic in the studied area. However, the infection did not develop further so that it did not spread to surrounding plants, and the disease incidence remained low compared to controls.

The inoculum of VShR pathogens can be pieces of mycelia, sporangia, zoosporangia, and oosporangia [6, 16]. Zoosporangia that come out of the sporangium actively move in puddles of rainwater on the soil and water film layers on plants because they have a locomotion tool in the form of a flagellum.
In the rainy season, disease development due to Phytophthora attacks is speedy and can cause plant death in red chili and tomato cultivation.

There are several mechanisms of inhibition of the development of VShR pathogens by synthetic fungicides and biological agents used in this study, namely in the form of toxins and antibiotics that poison the pathogens and competition for nutrients and root colonization between root colonization biological agents and VShR pathogens. Mancozeb is one of the active ingredients of a synthetic fungicide that is toxic to VShR pathogens, but the biological agents of the genus *Trichoderma* sp. also produce various antibiotics and antimicrobial compounds that can inhibit the development of pathogens [17, 18].

The results of the study [19] reported that *Trichoderma* sp. is able to inhibit the development of *Phytophthora palmivora* pathogenic cocoa pod rot disease by various mechanisms, namely producing antibiosis substances, mycoparasites, and colonization of cocoa plant roots. As reported by [20], *Trichoderma* sp. endophytes were able to induce the resistance of chili plants so that they were able to avoid the attack of *Phytophthora capsici*.

Various antimicrobial compounds that have been isolated and identified are Viridiofungin A (VFA) isolated from T. viride ([21], Peptaibols isolated from T. asperellum TR356 ([22] and Trichorzianol isolated from T. harzianum F031 [23]. These antimicrobial compounds generally have a broad spectrum, meaning they are able to inhibit the development of various types of pathogens.

The mechanism of inhibition of pathogens by FusNP is by competing for space and nutrient and inducing plant’s resistance to pathogens. For example, studies by ([24, 25, 26] showed that FoNP, even though in in vitro showed low inhibitory power, was able to induce resistance in banana plant against banana wilt disease pathogens when applied to the banana seedlings prior planting in the endemic area. Another study [27] also succeeded in isolating antagonistic fungi against ginger wilt disease pathogens from ginger plant rhizosphere. Root exudates secreted by FoNP that colonizes roots can inhibit the germination of pathogenic Fusarium conidia so that they do not develop and interfere with their host plants [28].

The reaction of vanilla plants to the application of biological agents in the form of *Trichoderma* sp. spp and FusNP varied, depending on the plant clones tested. This is consistent with the results of the study [29] that the response of vanilla clones or accessions varied when they were infected with root rot pathogens or colonized by FusNP.

### 6. Pesticide residue testing

The field research [15] tested pesticide residues in samples of plants and treated soil (M) intensively using the synthetic fungicide Mancozeb, compared to samples of plants and organic agricultural soil using the biological agent Trichoderma sp. and FusNP. Soil and plant samples were analyzed for the residual content of synthetic fungicides using gas chromatography and the residual values for synthetic fungicides were determined according to the standards of the National Standardization Agency (BSN) SNI No.7313-2008 concerning Maximum Residue Limit (BMR) of pesticides on agricultural products [30].

The results of the analysis of residues of synthetic fungicides reported [15] on vanilla leaves and fruits, as well as the rhizosphere around vanilla plants showed that the application of Mancozeb 2 g/L water once a week still left Mancozeb residues on the leaves and vanilla fruit, and vanilla rhizosphere soil (Table 4). However, fungicide application was stopped 15 and 30 days before plant and soil samples were analyzed. Meanwhile, the ability of synthetic fungicides to protect vanilla plants from VShR pathogen infection was not significantly different from that of biological agents (Table 3). This means that biological agents can be used instead of Mancozeb to protect vanilla plants from *P. capsici* infection. The utilization of biological agents has other advantages, namely lower costs and more environmentally friendly because it utilizes mulch from plant litter.
Table 4. Data of synthetic fungicide Mancozeb residue on the soil and vanilla leaves.

| Treatment      | Sample | 15 (daa)* | 30 (daa) |
|----------------|--------|-----------|----------|
| Trichoderma sp. (T) | leaf   | 0         | 0        |
|                 | Soil   | 0         | 0        |
| Fusarium (F)    | leaf   | 0         | 0        |
|                 | Soil   | 0         | 0        |
| T + F (TF)      | leaf   | 0         | 0        |
|                 | Soil   | 0         | 0        |
| Mancozeb (M)    | leaf   | 0.156     | 0.049    |
|                 | Soil   | 0.022     | 0.011    |

Note: *) day after application
Source: [15]

Mancozeb is a new generation of synthetic fungicide that is easily degraded by the environment. The residual value in the test 30 days after application (DAA) on the leaves was 0.049 mg/kg sample and only 0.011 mg/kg sample in the soil and was much lower than the results of the analysis at 15 dA, namely in the leaves of 0.156 mg/kg sample and in the soil. Soil was 0.022 mg/kg sample, so that the half-life of Mancozeb in the soil was 50%, while the half-life in the leaves was 58.97% [31].

7. Conclusion

Based on the explanation above, the nonpathogenic fungi isolated from the rhizosphere soil of healthy vanilla plants were dominated by Trichoderma spp and nonpathogenic fusarium because they used selective media. Trichoderma sp. and Fusarium NP were quite effective in controlling VShR in the field, equivalent to the effectiveness of synthetic fungicides with a disease incidence of around 3.0%, while the control reached 32.0%. The intensive use of synthetic fungicides causes environmental pollution in the form of pesticide residues on vanilla leaves and fruits and vanilla rhizosphere soil.

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