

Compatibility of Mycorrhizae and Pineapple-Based Liquid Fertilizer on Local Variety of Shallot for Suppression of Fusarium Wilt Disease

Kecocokan Antara Mikoriza dan Pupuk Organik Cair Berbasis Nanas pada Bawang Merah Varietas Lokal untuk Menekan Penyakit Layu Fusarium

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ABSTRACT

A comprehensive approach is needed to control shallots twisted disease (*penyakit moler*) caused by infection of *Fusarium oxysporum*. The use of mycorrhiza is known to be quite promising for controlling *Fusarium* wilt. Many farmers combine biological control agents with plant waste—pineapple peel waste—, but the synergistic effects are not yet clearly known. This research aims to determine the compatibility of liquid fertilizer and mycorrhiza as an effort to develop new techniques in managing *Fusarium* twisted disease. The research was arranged using a completely randomized design with 8 treatments and 3 replications. The tests conducted included measuring the percentage of mycorrhizal infectivity and disease severity, bulb production, and biomass of shallot plants. The analysis of the content of pineapple peel-based liquid organic fertilizer (PLOF) showed that it contains 11.14% organic carbon source, 0.14% nitrogen, as well as other macro and micronutrients. The combination of mycorrhiza and PLOF can form a symbiosis with plant roots with a colonization rate of 66.67%. Meanwhile, the combination of the two treatments could not suppress the severity of twisted disease, nor could it increase bulb production and biomass of shallot plants.

Key words: bulb production, disease severity, moler, PLOF

ABSTRAK

Pendekatan yang komprehensif diperlukan untuk mengendalikan penyakit moler pada bawang merah akibat infeksi *Fusarium oxysporum*. Penggunaan mikoriza diketahui cukup menjanjikan untuk mengendalikan penyakit layu *fusarium*. Banyak petani mengombinasikan agens hayati dengan limbah tanaman seperti limbah kulit buah nanas, tetapi efek sinergis belum diketahui secara jelas. Penelitian ini bertujuan untuk menentukan kompatibilitas pupuk cair dan mikoriza sebagai upaya mengembangkan teknik baru dalam pengelolaan penyakit layu *Fusarium*. Penelitian disusun menggunakan rancangan acak

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lengkap dengan 8 perlakuan dan 3 ulangan. Pengujian yang dilakukan meliputi pengukuran persentase infektivitas mikoriza, persentase keparahan penyakit, produksi umbi, dan biomasa tanaman bawang merah. Hasil analisis kandungan pupuk organik cair berbahan dasar kulit nanas (PLOF) mengandung sumber karbon organik sebesar 11.14%, nitrogen 0.14%, serta makro dan mikro nutrient lainnya. Kombinasi mikoriza dan PLOF dapat bersimbiosis dengan akar tanaman dengan tingkat kolonisasi 66.67%. Sementara itu, kombinasi keduanya tidak dapat menekan keparahan penyakit moler, serta tidak dapat meningkatkan produksi umbi dan biomasa tanaman bawang merah.

Kata kunci: keparahan penyakit, moler, PLOF, produksi umbi

INTRODUCTION

Shallot is one of the high-risk plant that have been cultivated over century (Hidayah *et al.* 2023; Lasmini *et al.* 2022). The twisted disease (local name: moler disease) is one devastating disease on shallot and has the potential to reduce the bulb production more than 50% (Supyani *et al.* 2021). As a high-risk crop, high inputs are required to support shallot production. The chemical-bio fertilizer and the chemical-bio pesticide are the two highest inputs for shallot production (Suminartika *et al.* 2022). Moreover, the prices of those inputs are increasing significantly over the time (Ridho *et al.* 2021).

In response to this problem, Indonesian farmers often take a creative and innovative approach. One such approach is combining two inputs, such as biological agents and chemical pesticides, biological control agents with liquid organic fertilizer, and many more. However, this is often done without any scientific testing or professional consultation, which may lead to unpredictable results in disease suppression. One case study involved the use of a pineapple peel-based liquid organic fertilizer (PLOF) combined with mycorrhizae by a farmer in the Probolinggo shallot field. Therefore, this research was initiated to determine the effectiveness of mycorrhizae and PLOF treatment in twisted disease suppression. The results of this experiment were inconclusive, prompting the need for further scientific investigation into the compatibility and efficacy of these two agents in disease suppression. However, this result is important for developing a recommendation

to help shallot farmers improve disease management.

MATERIALS AND METHODS

Isolation of Mycorrhiza

The arbuscular mycorrhizae were collected from the soil of shallot fields in Probolinggo (7°45' 57.384" S 113°11'35,778" E), which was characterized as alluvial-regosol soil. The isolation methods used were based on those described by Malik *et al.* (2022) and Patil *et al.* (2022). Healthy shallots were collected from the surrounding soil at a depth of 30 cm and a width of ½ of the planting distance. The mycorrhizae were propagated using zeolite and living maize roots (Goh *et al.* 2022; Tenzin *et al.* 2022). Root organ cultures and characterized by KOH/HCl staining. Subsequently, the mycorrhizal spores were subjected to sieving using 25, 140, and 230 mesh-stratified sieves (707 µm, 105 µm, 63 µm). The 2×10^6 spore mL⁻¹ sample was stored and collated for the subsequent stage of the procedure.

Isolation and Propagation of *Fusarium oxysporum*

Fusarium oxysporum was isolated and inoculated using methods as described by Gibert *et al.* (2022) and Khoo *et al.* (2023). Briefly, the fungi was isolated from shallot bulbs with *Fusarium* wilt symptoms on potato dextrose agar (PDA) medium (Hi Media, India) and propagated on carnation leaf agar (CLA) (Fisher *et al.* 1982) to a density of 1×10^6 spores mL⁻¹. The propagation results were then made into a suspension to be used as a pathogen inoculum.

Treatments and Experimental Design

This research was conducted in a controlled environment using a completely randomized design with four replications. The shallot variety utilized in this study was var. Biru Lancor, a local variety that is widely cultivated in Probolinggo, the largest shallot producer city in East Java, Indonesia. The planting material was sterilized, and a combination of commercial burn husk and organic material was used. Three bulbs were planted in each polybag. Pineapple peel-based liquid organic fertilizer (PLOF) was collected from farmers and subjected to testing for contamination, nitrogen, phosphorus, potassium, and micro-mineral content at Soil Chemistry and Plant Nutrition Laboratory, Department of Soil Science and Land Resources, Padjadjaran University. Mycorrhizal infectivity tests were conducted to determine the ability of mycorrhizal colonization on shallot roots, and observations of disease severity were made to measure the level of suppression against *F. oxysporum* wilt disease. This test consists of the following treatments: (1) control treatment with sterile water; (2) single mycorrhiza treatment; (3) single pineapple peel-based liquid organic fertilizer (PLOF) treatment; (4) combination of mycorrhiza + PLOF. Inoculation of the pathogen *F. oxysporum* was used as a negative control and was also used in the disease control ability test. The application of mycorrhiza was done before applying base fertilizer and planting the plants on soil media. In the PLOF treatment, liquid fertilizer was given as much as 100 mL per polybag during the preparation of planting media.

Observation and Data Analysis

On day 28 after treatment, the following observations were made: mycorrhizal colonization, disease severity, plant biomass and shallot bulb production. Mycorrhizal colonization was measured according to the protocol established by Goh *et al.* (2022), while disease severity was assessed according to Sintayehu *et al.* (2011). The root samples observed were mycorrhiza-treated roots without pathogen inoculation and roots from

treatments with *F. oxysporum* inoculation. This is to observe the effect of mycorrhizal colonization on the ability of biocontrol agents and shallot plant growth. Observational data including the percentage of mycorrhizal colonization, disease severity, plant biomass, shallot bulb production, and other quantitative data were analyzed using one-way analysis of variance (ANOVA) using Microsoft Excel. Then, Duncan multiple range test at 95% confidence level was used to examine the differences in each treatment.

RESULTS

Colonization of Mycorrhizal on Shallot Roots

Mycorrhiza applied in each treatment was successfully established and colonized shallot roots. Observations of mycorrhizal colonization without the application of pathogen showed the highest percentage of mycorrhizal colonization in a single application (20.00%) followed by a combination application of mycorrhiza + PLOF (18.33%) (Table 1). Mycorrhizal colonization of shallot roots with pathogen application also showed a high percentage of infection, i.e. 66.67% and 33.67% in the combination treatment of mycorrhiza + PLOF + *F. oxysporum* and mycorrhiza + *F. oxysporum*, respectively (Table 1). These results indicate that the ability of mycorrhiza colonization in shallot roots inoculated with the pathogen *F. oxysporum* is higher than those without the pathogen.

Table 1 Mycorrhizal infectivity in colonizing shallot roots

Treatment	Mycorrhiza infectivity (%)
Control (without mycorrhiza)	0.00 ± 0.00 a
Mycorrhiza	20.00 ± 10.00 b
Mycorrhiza + PLOF	18.33 ± 1.53 b
Mycorrhiza + <i>F. oxysporum</i>	33.67 ± 4.04 c
Mycorrhiza + PLOF + <i>F. oxysporum</i>	66.67 ± 5.77 d

Note: Numbers in the same column followed by the same letter are not significantly different based on Duncan's multiple range test at 95% confidence level.

Root staining also showed a symbiosis between mycorrhiza and shallot roots. In control treatment (the roots that were not colonized by pathogens and mycorrhizae), no hyphal structures or spores were seen (Figure 1a), while plants treated with single mycorrhizae or their combinations showed the presence of hyphal structures or spores. Intracellularly, spores were present in the root cortex of shallots treated with single mycorrhiza (Figure 1b and 1c) and the combination of mycorrhiza + PLOF + *F. oxysporum* (Figure 1f and 1g); while the combination treatments of mycorrhiza + PLOF and mycorrhiza + *F. oxysporum* showed hyphal structures (Figure 1d and 1e respectively). The mycorrhizae were identified as endomycorrhizae, where arbuscules or hyphae colonized the internal root tissue.

The Effect of Mycorrhiza and its Combination with PLOF on Twisted Disease Severity

A single application of mycorrhiza or PLOF showed lower twisted disease severity compared to the negative control (Table 2). The single treatment of mycorrhiza was able to reduce disease severity to 11.11%, showing

a significant difference against the negative control which was an application of the *F. oxysporum* without biocontrol agents and liquid organic fertilizer. On the other hand, although the single application of PLOF could reduce disease severity by 22.22%, this result was not significantly different when compared to the negative control. In contrast, the application of shallot plants with a combination of mycorrhiza and PLOF increased the severity of twisted disease to 76.85%, higher than the control and other treatments.

Symptoms of twisted disease on shallots can be easily observed. The symptoms appear on 14-21 days after inoculation. Symptoms then develop more severely when infection moves to

Table 2 Severity of twisted disease by *F. oxysporum*

Treatment	Disease severity (%)
Water	0.00 ± 0.00 a
Negative control	38.89 ± 0.00 b
PLOF	22.22 ± 19.24 ab
Mycorrhiza	11.11 ± 19.24 a
Mycorrhiza + PLOF	76.85 ± 11.22 c

Note: Numbers in the same column followed by the same letter are not significantly different based on Duncan's multiple range test at 95% confidence level.

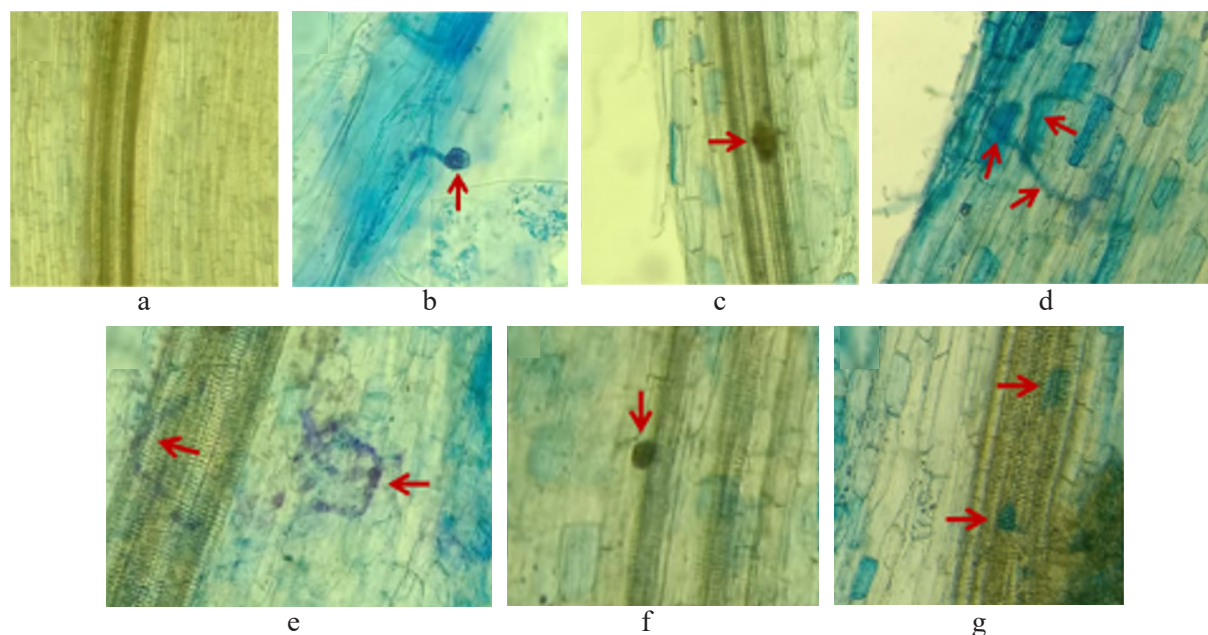


Figure 1 Mycorrhiza colonization in shallot root tissue. a, Control treatment without mycorrhiza; b and c, Single mycorrhiza treatment; d, Mycorrhiza + PLOF treatment; e, Mycorrhiza + *F. oxysporum* treatment; f-g, Mycorrhiza + PLOF + *F. oxysporum*.

the layer of bulbs, causing rot of the bulbs and roots so that they are easily uprooted (Figure 2).

leaves, leaf length, number of roots, and root length of shallot plants.

The Effect of Mycorrhiza and PLOF Combination on Bulb Production and Biomass of Shallot Plants

The treatment combination of mycorrhiza and PLOF was not significant in increasing bulb production and biomass of shallot plants (Table 3). The number of bulbs produced per clump of shallots with a treatment of single mycorrhiza, single PLOF, or a combination of both ranged from 6.33 and 6.67. These results were not significantly different from the control which produced 6.00 bulbs per clump. Each treatment did not cause a significant difference in the weight and diameter of shallot bulbs, except for the single PLOF treatment which produced a bulb weight of 7.35g. Similar results are also found for the biomass of shallot plants where the provision of each treatment did not cause a significant difference in the fresh weight, number of

DISCUSSION

Infection of *F. oxysporum* on shallot plants causes rotten leaves from the tip to the base, as well as twisted and pale yellow leaves, as described by Wahyuni *et al.* (2023). Moreover, this disease also causes shallot bulbs to rot and die. Infection of this disease in the field can cause pre- and post-emergence damping off, and decay on the roots of older plants. The pathogenic fungi *F. oxysporum* is highly transmissible through soil, water, and air (Calderón *et al.* 2023; Henry *et al.* 2023). In addition, the finding that this pathogen is also infects shallot plantlets makes it difficult to handle *Fusarium* in the laboratory (Widono *et al.* 2022). This is thought to be the cause of the appearance of disease symptoms in shallot treatments that were not inoculated with *Fusarium*.

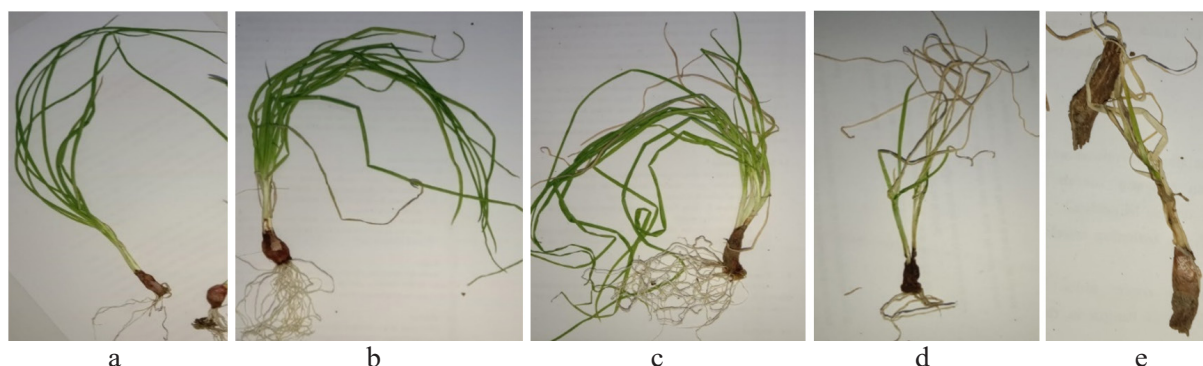


Figure 2 Symptoms of twisted disease on shallots. a, Healthy plants with no symptoms; b, Yellowing leaves and twisting roots; c, The number of yellow and twisted leaves increases, then the leaves dry out; d, Almost all leaves turn yellow and dry; and e, Plant twisted and turned yellow before it decayed.

Table 3 Shallot bulb production and plant biomass with mycorrhiza and PLOF treatment

Treatment	Bulb production			Plant biomass				
	Number of bulbs	Bulb weight (g)	Diameter (cm)	Fresh weight (g)	Number of leaves	Leaf length (cm)	Number of roots	Root length (cm)
Control	6.00 a	1.88 a	1.19 a	10.09 a	13.33 a	33.72 a	22.67 a	12.47 a
Mycorrhiza	6.33 a	2.37 a	1.46 a	12.31 a	18.00 a	33.67 a	45.80 a	11.10 a
PLOF	7.33 a	7.35 b	1.34 a	18.25 a	19.11 a	36.39 a	36.23 a	13.17 a
Mycorrhiza + PLOF	6.67 a	1.76 a	1.18 a	16.52 a	29.17 a	38.10 a	37.10 a	12.10 a

Note: Numbers in the same column followed by the same letter are not significantly different based on Duncan's multiple range test at 95% confidence level.

Fusarium infection is strongly influenced by environmental conditions and can occur at natural entry points or wounds (Degani *et al.* 2022; Labanska *et al.* 2022). Mycorrhiza can alter soil composition and host metabolism, and facilitate pathogen infection (Pu *et al.* 2022; Wang *et al.* 2022). In our study, the application of mycorrhiza in combination with PLOF did not increase shallot bulb production nor biomass of shallot plants. The combined treatment of mycorrhizal biocontrol agents and PLOF was not effective in reducing the severity of twisted disease caused by *F. oxysporum*. The combined application of *F. oxysporum* and PLOF caused higher disease severity on shallot plants. This is similar to the findings of Gadira *et al.* (2018) in their research which showed that the combination of fertilizer with mycorrhiza could not prevent *Fusarium* infection.

Mycorrhiza can form a symbiotic relationship with plant roots, which can increase plant resistance to pathogens. But in some cases, this interaction can trigger excessive or less effective activity of the resistance system against *Fusarium*. In addition, mycorrhiza requires elemental carbon (C) to grow and develop. The element of organic carbon plays an important role in the survival of all living things that interact with the soil, ranging from microorganisms such as fungi to plants (Widyati 2017). Based on the results of laboratory analysis of PLOF, this liquid fertilizer contains organic carbon of 11.14%, nitrogen of 0.14%, and other macro elements of around 0.3%. PLOF provides additional nutrients, especially high carbon (C), which can increase mycorrhizal activity, thus strengthening the symbiosis between mycorrhiza and shallot plants. However, these nutrients are also thought to increase plant susceptibility to *F. oxysporum* infection as this pathogen can also utilize the available nutrients for its development.

Mycorrhizae also alter soil composition and host plant metabolism, creating conditions more favorable to the growth and spread of *F. oxysporum* (Cheeke *et al.* 2017). The

carbon cycle in the soil altered by mycorrhiza affects the production of plant secondary metabolites, such as saponins, which can prevent bacterial growth but are not effective against pathogenic fungi (Widyati 2017). Mycorrhiza can facilitate pathogen infection by modifying the microenvironment around the roots, making it easier for *F. oxysporum* to infect host plants. Previous research by Gadira *et al.* (2018) showed that the combination of fertilizer with mycorrhiza could not prevent *Fusarium* infection, and could even worsen plant conditions. Our study also showed that the combination of PLOF and mycorrhiza was not effective in suppressing wilt disease in shallots. The treatment combinations may work synergistically and affect the increase in disease severity.

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