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Arbuscular Vascular Mycorrhizes (MVA) to Control Wilt Disease in Tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Background: Pests and diseases can cause suboptimal tomato production. One alternative that can be used to control wilt disease in tomato plants is to use biological agents. Biological agents that have the potential to control wilt disease in tomato plants are mycorrhizae. This study aimed to determine whether mycorrhizal treatment was effective in the growth of tomato plants and the control of wilt.

Methods: The research design was a randomized block design (RBD) arranged factorially. This study used two factors and the first was the dose of mycorrhizal application (M) with three treatments consisting of M1 = mycorrhizal 25 spores/plant, M2 = mycorrhizal 50 spores/plant and M3 = mycorrhizal 75 spores/plant. The second factor was Mycorrhiza (T) application time using two treatments consisting of T1 = when the tomato seeds were sown and T2 = when transplanting. All treatment combinations were repeated 4 times. The varieties used are varieties that farmers usually plant, namely the F1-resistant variety, which was an introduced hybrid plant. The type of plant was a determinate plant.

Result: The results showed that the mycorrhizal treatment with 75 spores significantly increased tomato plant growth on parameters such as plant height, number of leaves, fruit weight and reduced disease incidence.

Key words: Fusarium, Mycorrhizae, Ralstonia, Tomato, Wild disease.

INTRODUCTION

Tomatoes continue to develop into an essential commodity in international trade. Suboptimal tomato production can be caused by pest and disease attacks (Ma *et al.*, 2023). (Gatahi, 2020) said, it was conveyed that tomato cultivation cannot be separated from various obstacles affecting its production. The pathogens that cause wilt disease in tomato plants are *Fusarium oxysporum* f. sp. *lycopersici* (Fol) and *Ralstonia solanacearum* (RS). FoL attacks can reach 60 to 70% (Srinivas *et al.*, 2019) and even up to 85% (Sukorini *et al.*, 2023). In comparison, RS attacks on tomato plants are 77 to 90% (Wamani *et al.*, 2023). The genus Fusarium fungi includes several species of plant pathogens, among the most damaging phytopathogens worldwide, causing diseases in many crops, such as cereals and other crops (Todorović *et al.*, 2023). It can harm over 100 essential cash crops such as banana, tomato, cucumber, *M. charantia*, watermelon, muskmelon, cotton and beans (Yan *et al.*, 2023). In addition to causing diseases, these fungi produce a wide range of mycotoxins, which may be present in feed and food products. While *Ralstonia solanacearum* can infect a wide range of hosts, including banana, tobacco, potato, tomato, ginger, eggplant and chili (Ahmed *et al.*, 2022). This bacterium can attack more than 200 plant species from 50 different families and cause losses of up to 100% (Garćia *et al.*, 2019).

One alternative that can be used to control wilt disease in tomato plants is to use biological agents. The use of biological agents that live around the roots is greatly influenced by environmental conditions, especially soil humidity and temperature, so it is less effective. Germination of *Fusarium proliferatum* spores, a seed-borne pathogen of plants (Soni *et al.*, 2021), showed that *in vitro* treatment with chemical pesticides was better than biological treatment using *Pseudomonas fluorescens* and *Trichoderma harzianum*. However, research (Murali Sankar *et al.*, 2019) shows that *Pseudomonas fluorescens* has a high population of 8.2×10^5 cfu/g soil. *Pseudomonas chlororaphis* has better growth-promoting and disease-controlling properties in chickpeas with superior root colonization capabilities. Biological agents that have the potential to control wilt disease in tomato plants are mycorrhiza (Meddad-Hamza *et al.*, 2023). On the other hand, the impact of mycorrhizal fungal colonization on legume plants can reduce wilt disease, showing changes in soil biological fertility, especially in terms of phosphate and nitrogen content, so that it can be used as a biofertilizer (Benelhadj Djelloul *et al.*, 2024). Applying mycorrhizal fungi to tomato plants can significantly affect the intensity of FoL

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attacks, reducing the intensity of attacks by 28.26%, starting from the initial disease severity of 69.85% (Meddad-Hamza *et al.*, 2023). While controlling *R. solanacearum*, *G. Mosseae* could reduce disease severity from 55% at 20 days to 0%, but could not significantly reduce disease severity in other types. Research on the time of application and the number of spores given to reduce FoL or RS attacks has never been conducted. Related research on the existing application time is on oil palm plants (Hendarjanti and Sukorini, 2022). This research aimed to determine the interaction between the number of spores and the application time of Arbuscular Vascular Mycorrhiza (MVA) on the growth and control of wilting in tomato plants (*Solanum lycopersici* L.).

MATERIALS AND METHODS

Place and Time

This research was conducted from August to November 2023 (rainy season) at the Agronomy Laboratory, the University of Muhammadiyah Malang and the Ngadaprejo Farmers' Land, Junrejo District, Batu City, East Java. The altitude was 739 meters above sea level and the soil type was Andosol. The average temperature was 18- 23°C and the Relative humidity was 76-97%. The land for the research location was a former tomato plantation owned by a farmer, 87% of which was infected with wilt disease.

Research design

The research design was a randomized block design (RBD) arranged factorially. This study used two factors and the first was the dose of mycorrhizal administration (M) with three treatments consisting of M1 = mycorrhizal 25 spores/plant, M2 = mycorrhizal 50 spores/plant and M3 = mycorrhizal 75 spores/plant. The second factor was Mycorrhiza (T) application time, using two treatments consisting of T1 = when the tomato seeds were sown and T2 = when transplanting. So, there are six treatment combinations. Each treatment contained five plant samples. All treatment combinations were repeated 4 times. The varieties used are varieties that farmers usually plant, namely the F1-resistant variety, which was an introduced hybrid plant. The type of plant was a determinate plant.

Application of vesicular arbuscular mycorrhiza in tomato plants

The soil used for the planting medium in this study originated from the research area previously used for tomato cultivation, so it has the potential for pathogens to settle in the soil. The soil is then put in a polybag, sprayed with water and left for one week before planting. Then, Mycorrhiza of 25, 55 and 75 spores/ plants can be applied to the soil for each sample. The mycorrhiza used was a personal collection of mycorrhiza propagated in corn plants. Furthermore, the spores in the soil were counted using the method (Sasvári *et al.*, 2012) to determine the spore content in 100 grams of soil. Based on the number of spores contained in the soil, the application of 25/50/75 spores was determined.

Data analysis

Data were analyzed using ANOVA. If the treatment has a natural effect, it is tested further with the Duncan multiple range test at a 5% level.

RESULTS AND DISCUSSION

Plant height (cm)

The treatment of giving the amount of mycorrhiza had a very significant effect on all plant ages. The results of further tests with DMRT at the 5% level are presented in Table 1.

Table 1 shows that treating 75 mycorrhizal spores at the sowing time achieved the longest plant height at all observation ages.

The number of leaves (Leaf blade)

The number of leaves shows a very significant interaction effect. The highest number of leaves at all observation ages in the treatment of mycorrhizal administration time was 75 spores during seedlings. This was not significantly different from the treatment of 75 spores during transplanting. The results of further tests with the DMRT test at the 5% level are presented in (Table 2).

Fruit weight (grams)

The fruit weight per plant was observed at tomato harvest 63 Days After Planting (DAP). Mycorrhizal and application

Table 1: Analysis of the interaction of vesicular arbuscular mycorrhiza (VAM) and the time of application of tomato plant height.

Treatment	Average plant height (cm) during Observation week after planting (WAP)						
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP
Mycorrhiza 25 Spores + Seedlings	26.34a	31.34a	37.40a	71.39a	83.05b	93.50a	112.73c
Mycorrhiza 50 Spores + Seedlings	34.24d	38.34d	38.49b	77.54b	94.52e	96.62b	119.44d
Mycorrhiza 75 Spores + Seedlings	33.89d	38.88d	46.76c	85.95d	98.25f	106.73d	128.52e
Mycorrhiza 25 Spores + Transplanting	25.56a	30.61a	37.59a	71.78a	80.61a	92.87a	101.66a
Mycorrhiza 50 Spores + Transplanting	28.72b	33.27b	38.49a	77.54c	89.38c	94.98ab	104.92b
Mycorrhiza 75 Spores + Transplanting	31.38c	35.38c	43.63b	82.73c	93.01d	99.34c	120.20d
Coefficient of variation (CV%).	3.41	3.19	3.53	5.27	6.22	4.72	9.24

Description: - wap (Week after planting) - Numbers followed by different letters in the same column indicate significant differences based on the 5% DMRT test.

time interacted with the fruit weight variable. The results of further tests with the DMRT test at the 5% level are presented in (Table 4).

Table 3 shows that 75 mycorrhizal spores at the time of transplanting produced the highest tomato fruit weight and were not significantly different from the treatment of giving 50 mycorrhizal spores at the time of sowing.

Root infection by mycorrhizae and disease incidence(%)

There was a significant interaction between the number of mycorrhizae applied and the time of mycorrhizae application, combined with root infection and disease incidence. The results of further testing with the DMRT test at the 5% level are presented in Table 4. Table 4 showed that the observation of the percentage of root infection of the treatment of giving 75 spores of mycorrhiza at the time of seeding resulted in the highest mycorrhiza root infection and was not significantly different from the treatment of applying 75 spores of mycorrhiza at the time of transplanting. At the age of 6 MST and 7 MST, the highest attack was in the treatment of 25 spores, which was given at the time of transplanting.

Mycorrhiza takes time to infect the roots of plants. Where mycorrhizal hyphae colonize the roots of tomato plants by forming external hyphae to expand the absorption area of tomato plant roots in obtaining water, nutrients and other nutrients, the infection process begins with germination in the soil and begins to form hyphae; the growing hyphae will penetrate the roots, which will later develop in the cortex. In roots that have been infected by mycorrhiza, arbuscules will form and from these arbuscules, mycorrhiza can help absorb nutrients needed by plants (Mohammadi *et al.*, 2020). The mycorrhiza that is given has infected the roots of tomato plants and is actively carrying out mutualistic symbiosis with tomato plants; this can support the growth of tomato plants, especially plant height, so that there is a difference in tomato plant height between treatments. Other studies also explain that giving AMF Glomus sp. as much as 20 grams can increase the growth of castor oil plant seedlings, especially plant height. AMF with phosphate enzyme content can free P and N elements that were initially unavailable and then become available in the soil (Zhang *et al.*, 2014; Ettlili *et al.*, 2022). The nutrient N is a stimulant for plant growth, especially plant height. The analysis of variance between treatments significantly affected all observation variables, meaning that each treatment of the amount of mycorrhiza given could affect the growth and yield of tomato plants. One of the functions of leaves in tomato plants is to carry out the process of photosynthesis so that growth and development continue to increase (Zai *et al.*, 2021). Photosynthesis produces energy in the form of ATP and NADPH compounds, where ATP is a source of energy for the metabolism of the plant's body. The availability of nutrients such as P affects the formation of ATP. This is related to mycorrhiza in plants, which can increase the absorption of nutrients, especially phosphorus. Increasing the P content in plant tissue can accelerate cell division, especially in the meristem tissue in plants, which affects the growth and development of the plant leaves.

Judging from the results, the provision of mycorrhiza affects the weight of the fruit between treatments. The dose of 75 spores is optimal for increasing the growth and development of tomato plants, significantly increasing fruit weight. Mycorrhiza can increase nutrient absorption and maintain soil water levels that are good for tomato plant growth. This is also in line with (Mohammadi *et al.*, 2020) opinion that mycorrhiza can increase nutrient absorption. Plants given mycorrhiza are more resistant to drought because the roots of mycorrhizal plants recover quickly after experiencing a lack of water. Mycorrhizal hyphae can still absorb the soil's pores when the plant roots can no longer absorb water. In addition (Franczuk *et al.*, 2023) also explained that mycorrhiza can replace approximately 50% of phosphate needs, 25% of potassium needs and 40% of nitrogen needs because mycorrhiza can increase the absorption of N, P, K, Ca and Mg elements compared to organic fertilizers. From this incident, it can be explained that the absorption of nutrients assisted by mycorrhizal hyphae affects the weight of tomato fruit. The more mycorrhizal spores given, the better it is for the growth of tomato plants.

Table 2: Analysis of the interaction between the provision of vesicular arbuscular mycorrhiza (VAM) and the time of the application of the number of tomato leaves.

Treatments	Average number of leaves (strands) during Observation week after planting (WAP)						
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP
Mycorrhiza 25 Spores + Seedlings	20.55b	43.43b	72.42b	84.25b	99.56a	118.15b	126.78a
Mycorrhiza 50 Spores + Seedlings	29.33c	52.11c	83.62c	88.83c	122.35c	133.85d	142.36c
Mycorrhiza 75 Spores + Seedlings	27.11c	59.33d	87.16c	97.56d	122.92c	140.34e	153.80d
Mycorrhiza 25 Spores + Transplanting	15.77a	40.55a	67.27a	81.51a	98.44a	114.47a	124.49a
Mycorrhiza 50 Spores + Transplanting	21.22b	44.64b	66.01a	81.60a	114.38b	122.53c	133.66b
Mycorrhiza 75 Spores + Transplanting	28.22c	58.33d	86.15c	96.81d	121.78c	138.84e	152.33d
Coefficient of Variation (CV%)	4.88	7.31	8.83	6.64	10.47	10.10	11.53

Description: - wap (Week after planting) - Numbers followed by different letters in the same column indicate significant differences based on the 5% DMRT test.

The observation results of tomato plant root infection with the treatment of different amounts of mycorrhizal spores at different times of application show that all tomato plant roots inoculated with mycorrhizal spores are infected, but have different infection values. The correlation test results between the Mean Percentage of Root Infections (%) and the average percentage of disease incidence (%) in the 7 WAP showed that R^2 = 89.02%. This indicated that the higher the average percentage of root infections, the lower the disease incidence.

According to (Smith, 2009), MVA can cooperate with 80-90% of plant species. It also revealed that one type of plant that can cooperate or colonize with MVA is from the Solanaceae family. Tomato plant roots infected by mycorrhizae are characterized by the presence of external and internal hyphae, as well as vesicles. Mycorrhizal infection with 75 spores resulted in a negligible effect on the level of tomato wilt disease attack, where more spores were given and colonized the roots of tomato plants. The higher the percentage of root colonization by AMF, the smaller the percentage of disease attack intensity (Campo *et al.*, 2020). Mycorrhiza uses carbohydrates and plant root exudates, thus creating an unsuitable environment for pathogens around the plant. In addition, mycorrhizal fungi can secrete antibiotics that can be toxic to pathogens.

Table 3: Analysis of the interaction between vesicular arbuscular mycorrhiza (VAM) provision and application time on tomato fruit weight.

Treatments	Average weight of tomato fruit (g) observed 63 days after planting (DAP)
Mycorrhiza 25 Spores + Seedlings	153. 02b
Mycorrhiza 50 Spores + Seedlings	198.66de
Mycorrhiza 75 Spores + Seedlings	183.4cd
Mycorrhiza 25 Spores + Transplanting	117. 74a
Mycorrhiza 50 Spores + Transplanting	163. 44bc
Mycorrhiza 75 Spores + Transplanting	219.07e
Coefficient of Variation (CV%)	32.73

Description: Numbers followed by different letters in the same column indicate significant differences based on the 5% DMRT test.

The roots of tomato plants infected by mycorrhiza will experience morphological changes, namely the formation of lignin in the endodermis of the plant roots, so that it can be a barrier against pathogen attacks and tomato plants will increase their resistance to *Fusarium oxysporium* wilt disease (Pu *et al.*, 2022). Other studies also explain that plants that are symbiotic with mycorrhiza will experience increased flavonoid content because stimulation occurs when plants are infected with MVA. Colonization will form in the roots, making the plants more resistant. In addition (Vierheilig and Bago, 2005) also explains that plants infected with MVA will experience lignification in the roots and parenchyma, where lignification is part of the cell wall defence against pathogen infection.

Ralstonia solanacearum is a bacterium that causes wilt disease in tomato plants; it also attacks other plants, such as chili plants, tobacco and potatoes. Bacterial attacks on tomato plants found in experimental fields are suspected of *Psedomonas solanacearum* bacteria, currently known as *R. solanacearum*. This soil-borne pathogen can survive in plant debris for a long time and spread quickly to injured plant parts. In addition, this bacterium infects its host through the roots during transplanting. (Boutaj *et al.*, 2022) Also explained that attacks by *R. solanacearum* bacteria are also influenced by environmental factors such as high rainfall and high humidity, so pathogen growth and development cannot be suppressed optimally. The influence of the environment affects microbes such as mycorrhiza, which are less than optimal in suppressing attacks of bacterial wilt disease caused by *R. solanacearum* . This study showed that plants infected with mycorrhiza were still attacked by the *R. solanacearum* bacteria, as indicated by the brownish inner stem of the tomato.

Table 4: Mean root infection is the interaction between the provision of vesicular arbuscular mycorrhiza (VAM) and application time by mycorrhiza attacking tomato plants.

Treatments	Mean percentage of root infections (%)	Average percentage of disease incidence (%) in the Week of observation after planting (WAP)		
		5	6	7
Mycorrhiza 25 Spores + Seedlings	32.44a	0.00a	33.33c	57.89d
Mycorrhiza 50 Spores + Seedlings	41.78bc	0.00a	16.33b	22.35b
Mycorrhiza 75 Spores + Seedlings	53.78d	0.00a	12.56a	17.33a
Mycorrhiza 25 Spores + Transplanting	43.26bc	4.44b	14.33ab	25.67b
Mycorrhiza 50 Spores + Transplanting	38.74ab	1.11a	9.33a	43.53c
Mycorrhiza 75 Spores + Transplanting	47.89cd	0.00a	13.67a	21.43b
Coefficient of Variation (CV%)	6.71	1.59	7.79	14.33

Description: Numbers followed by different letters in the same column indicate significant differences based on the 5% DMRT test.

CONCLUSION

Based on the results of the study, it is known that there is an interaction between the Treatment of the number of mycorrhizae and the application time. The best combination of growth treatments is M3T1 (75 Spores + seedling time). This can be seen in several parameters, such as plant height and number of leaves. A dose of 75 mycorrhizae spores is known to suppress bacterial wilt attacks caused by *Ralstonia solanacearum* by 21.38%. Root infection of tomato plants treated with M3T1 (75 spores + seedling time) has a high mycorrhizae infection rate of 53.78%.

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Disclaimers

The views and conclusions expressed in this article are solely those of the author and do not necessarily represent the views of their affiliated institutions. The author is responsible for the accuracy and completeness of the information provided, but does not accept any liability for any direct or indirect losses resulting from using this content.

Informed consent

We did not use animals for this research.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the study's design, data collection, analysis, publication decision, or manuscript preparation.

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