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Reduction of Whitefly Infestation and Yellowing Disease Symptoms in Field-Grown Kopay Chili Pepper by Local Multispore Arbuscular Mycorrhizal Fungi

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Abstract

Chili pepper is frequently affected by yellowing disease symptoms associated with whitefly infestation, reducing plant performance under field conditions. Environmentally friendly strategies are needed to suppress vector infestation and disease symptom development. This study evaluated the effectiveness of local multispore arbuscular mycorrhizal fungi (AMF) in reducing whitefly infestation and yellowing disease symptoms in field-grown Kopay chili pepper. The experiment was conducted at the experimental field and Biology Laboratory of Payakumbuh State Agricultural Polytechnic, West Sumatra, Indonesia. A randomized complete block design was used with four AMF doses: 0, 50, 70, and 100 g per plant. Observed variables included leaf-sucking insect (LSI) infestation, yellowing disease symptom (YDS) incidence, and AMF root colonization. Disease assessment was based on visual field symptoms without molecular confirmation. The results showed that local multispore AMF reduced LSI infestation and YDS incidence. The 100 g AMF treatment produced the lowest LSI infestation from 32% in the control to 7%. This treatment also reduced yellowing disease symptom incidence from 40% in the control to 5%, while AMF-treated plants showed high root colonization ranging from 56.7% to 61.7%. These findings indicate that local multispore AMF, particularly at 100 g per plant, has potential as a biological input to support environmentally friendly management of yellowing disease symptoms in chili pepper. However, because disease diagnosis was based on visual symptoms only, further studies using molecular confirmation of the causal pathogen are needed.

Keywords: *Bemisia tabaci,* biological control, *Capsicum annuum,* induced resistance, root colonization

Introduction

Chili pepper (*Capsicum annuum* Linnaeus) is one of the most important horticultural commodities in Indonesia. It has high economic value and contributes substantially to household consumption and the food industry (Jeksen and Sari, 2022). Chili production is often unstable because of pest and disease attacks, particularly yellowing disease symptoms, which are commonly associated with begomovirus infection transmitted by the whitefly *Bemisia tabaci* (Djaya, 2023). In Indonesia, yellow leaf curl disease in chili has also been reported as a complex viral disease. Trisno et al. (2021) reported that dual infection of Pepper Yellow Leaf Curl Virus and Chilli Veinal Mottle Virus can contribute to yellow leaf curl disease development in chili. In the field, affected chili plants commonly show yellowing of young leaves, vein thickening, leaf curling or cupping, reduced leaf size, and stunted growth, which may ultimately reduce fruit production and quality (Fakhira et al., 2025). In Indonesia, yellowing disease is considered one of the most economically damaging diseases of chili, with yield losses potentially ranging from 20 to 100% under conditions of high vector pressure (Arfiani and Nasruddin, 2023; Djaya, 2023).

The whitefly *B. tabaci* is recognized as the primary vector of begomovirus-associated yellowing disease in chili pepper; therefore, reducing whitefly infestation is an important component of disease management. Current management practices generally rely on pesticide application to suppress vector populations (Malado et al., 2024). However, excessive use of synthetic pesticides can cause adverse effects, including pest resistance, environmental contamination, and negative impacts on non-target organisms (Jamin et al., 2024). Therefore, sustainable strategies that can reduce vector infestation while improving plant tolerance to disease are urgently needed, especially under open-field cultivation systems. Because yellowing disease development in the field is closely related to the presence and activity of *B. tabaci*, management strategies that reduce whitefly infestation may indirectly contribute to lower disease symptom development.

One potential approach is the use of arbuscular mycorrhizal fungi (AMF). AMF form mutualistic associations with plant roots and contribute to improved nutrient uptake, especially phosphorus, better root development, enhanced plant vigor, and increased tolerance to biotic and abiotic stresses. Bendezu et al. (2021) stated that AMF are soil fungi belonging to the phylum Glomeromycota that form mutualistic symbioses with plant roots. In addition to improving plant nutrition, AMF can contribute to plant protection through mycorrhiza-induced resistance, which may involve defense priming, improved physiological status, and reduced susceptibility to pests and pathogens (Pozo and Azcón-Aguilar, 2007; Jung et al., 2012; Kaur and Suseela, 2020). Recent studies also indicate that AMF may influence plant interactions with aboveground herbivores by altering plant nutrition, defense-related metabolism, and physiological resistance, although the response can vary depending on plant species, AMF identity, and insect feeding guild (Frew et al., 2022). Jamarun and Yunisman (2017) showed that several antagonistic fungi were able to colonize chili roots and contribute to the suppression of anthracnose disease. Similarly, Yanty et al. (2024) reported that *Beauveria bassiana* could suppress *Colletotrichum truncatum* and improve chili plant growth.

Several studies have shown that AMF colonization may improve plant resistance to pathogens, including viral diseases, by enhancing nutrient acquisition and activating systemic defense responses. Most previous evaluations have focused on single-species or commercial AMF inocula, often under controlled or greenhouse conditions (Kaur and Suseela, 2020). However, field-based evidence on the ability of locally derived multispore AMF inocula to simultaneously reduce whitefly infestation, other leaf-sucking insect infestation, and yellowing disease symptoms in chili pepper remains limited. In particular, the effectiveness of local multispore AMF isolate PU10 from West Sumatra, containing *Glomus* sp., *Acaulospora* sp., and *Sclerocystis* sp., has not been evaluated in field-grown Kopay chili pepper. Moreover, the appropriate inoculum dose for achieving high root colonization while reducing whitefly infestation and yellowing disease symptoms under West Sumatran field conditions has not been established.

The novelty of this study lies in the simultaneous field evaluation of local multispore

AMF isolate PU10 for root colonization, whitefly infestation, other leaf-sucking insect infestation, and yellowing disease symptom incidence in Kopay chili pepper. Because disease diagnosis in this study was based on visual field symptoms without molecular or serological confirmation, the disease is referred to as yellowing disease symptoms rather than a confirmed begomovirus infection. Therefore, this study aimed to determine the effective dose of local multispore AMF inoculum for reducing whitefly infestation and yellowing disease symptoms in field-grown Kopay chili pepper in West Sumatra.

Methods

This research was conducted at the experimental field of Payakumbuh State Agricultural Polytechnic and the Biology Laboratory of Payakumbuh State Agricultural Polytechnic, West Sumatra, Indonesia.

Research Design

The experiment was arranged in a randomized complete block design with four AMF inoculum doses and six replications. The treatments were control (without AMF inoculum), 50 g AMF inoculum, 70 g AMF inoculum, and D = 100 g AMF inoculum. Each dose was applied per plant by placing the inoculum directly into the planting hole at transplanting.

Each plot contained approximately 20 chili plants arranged in four rows of five plants, with a planting distance of 50 cm × 60 cm. Ten plants from the center of each plot were used as observation plants, while border plants were excluded from observation. The plot size was 6 m x 6 m. The distance between plots was 50 cm, and the distance between blocks was 100 cm. Blocks were established based on the location of the research plots.

AMF Inoculum

The AMF inoculum used in this study was local multispore isolate PU10, containing *Glomus* sp., *Acaulospora* sp., and *Sclerocystis* sp., with a spore density of 100 spores per 50 g of inoculum. The inoculum was obtained from the Biology Laboratory of Payakumbuh State Agricultural Polytechnic, Lima Puluh Kota Regency, West Sumatra. The inoculum contained spores, hyphae, and colonized root fragments.

Crop Management

The field was prepared manually by clearing weeds and tilling the soil. Beds were prepared one week after tillage. Manure was applied at 0.5 kg per plant approximately one week before transplanting. SP-36 and KCl were applied at 4.5 g per plant at transplanting, while urea was applied at 6 g per plant in two equal doses at 2 and 4 WAT. During the growing season, weeding and irrigation were applied uniformly to all plots. No synthetic insecticides, fungicides, or acaricides were applied during the experiment to avoid interference with insect infestation and yellowing disease symptom development.

AMF Application and Seedling Transplantation

AMF inoculum was applied at transplanting. Planting holes were prepared, and the inoculum was placed at the bottom of each planting hole according to the assigned treatment dose: 0, 50, 70, or 100 g per plant. The inoculum was placed in direct contact with the seedling root zone before covering with soil. One-month-old Kopay chili seedlings were then transplanted into the prepared holes. Only uniform and apparently healthy seedlings were selected for transplanting.

Observed Variables

Yellowing disease incidence

Yellowing disease symptom incidence was recorded weekly from 2 to 12 WAT by observing the ten sampled plants in each plot. A plant was classified as symptomatic when it showed one or more typical field symptoms, including irregular leaf yellowing, yellowing and thickening of leaf veins, leaf curling or cupping, reduced leaf size, and stunted growth. No molecular or serological confirmation was performed; therefore, disease assessment was based only on visual field symptoms. The final observation at 12 WAT was used for statistical analysis. Yellowing disease symptom incidence was calculated using the following formula:

$D\ (\%) = \frac{a}{A}$ ………………………………………………… (1)

Where D = Disease incidence (%), a = number of symptomatic plants, A = total number of observed plants.

Leaf-sucking insect infestation

The percentage of leaf-sucking insect infestation was recorded weekly from 2 to 12 WAT. Infestation was assessed by visual inspection of the ten sampled plants in each plot. Each plant was observed across the upper, middle, and lower canopy sections for the presence of whitefly (*Bemisia tabaci*), thrips (*Thrips* sp.), and aphid (*Aphis gossypii*). Among these insects, *B. tabaci* was considered the main vector associated with yellowing disease symptoms, whereas thrips and aphids were recorded as additional leaf-sucking insects. A plant was classified as infested when at least one individual of the respective insect species was found. Infestation was calculated separately for each insect species using the following formula:

$I\ (\%) = \frac{n}{N} x\ 100$ ……………………………………… (2)

Where I = infestation, n = number of infested plants, N = total number of observed plants

AMF root colonization

AMF root colonization was observed at 8 WAT. Root samples were collected from 3 plants per plot and washed thoroughly under running water. The roots were placed in test tubes and soaked in 10% KOH for 24 h, rinsed with water, soaked in 10% HCl for 24 h, and rinsed again with water. The roots were stained with lactophenol trypan blue for 24 h and subsequently cleared in lactophenol for 15 min. Ten 1-cm root segments were arranged on microscope slides, covered with cover glasses, and observed under a microscope at 100X magnification. Root colonization was classified according to the Institute of Mycorrhizal Research and Development, USDA, as cited by Setiadi (1992). AMF root colonization was calculated using the following formula:

$RC\ (\%) = \frac{b}{B} x\ 100$ ……………………………………… (3)

Where RC = Root colonization (%), b = number of colonized root segments, B = total number of observed root segments

Table 1. Classification of AMF root colonization percentage in chili plants

Root colonization (%)	Class	Category
0-5	1	Very low
6-25	2	Low
26-50	3	Moderate
51-75	4	High
>75	5	Very high

Data Analysis

All data were expressed as percentages and presented as mean ± standard deviation. Percentage data were checked for normality and homogeneity of variance before analysis. Treatment effects were analyzed using analysis of variance (ANOVA) based on a randomized complete block design. When significant treatment effects were detected, data were separated using Duncan's New Multiple Range Test (DMRT) at the 5% significance level.

Results

Yellowing Disease Incidence

The observations showed that symptomatic chili plants exhibited irregular yellowing on the leaves, yellowing and thickening of leaf veins, leaf curling or cupping, reduced leaf size, and stunted shoot growth. These symptoms were more frequently observed in the control treatment than in AMF-treated plants (Figure 1).

Figure 1. Chili plant leaves; (A) treated with AMF and showing no visible yellowing disease symptoms; (B) shoot with yellowing disease symptoms.

AMF application reduced yellowing disease symptom incidence in chili plants at 12 WAT. Application of 50 g AMF reduced symptom incidence to 15%, while 70 g AMF reduced it to 10%. The lowest symptom incidence was observed in the 100 g AMF treatment, with only 5% of plants showing yellowing disease symptoms. The reduction in symptom incidence followed a clear dose-related trend. Compared with the control, yellowing disease symptom incidence was reduced by 62.5% in the 50 g AMF treatment, 75.0% in the 70 g AMF treatment, and 87.5% in the 100 g AMF treatment. This trend indicates that higher AMF doses were associated with lower yellowing disease symptom incidence under field conditions (Table 2).

Table 2. Yellowing disease symptom incidence in chili plants treated with different AMF doses at 12 weeks after transplanting.

Treatment	Yellowing disease incidence (%) (% ±SD)
Control	40 ± 0.0 a
50 g AMF	15 ± 5.0 b
70 g AMF	10 ± 3.3 c
100 g AMF	5 ± 1.6 d

Note: Values in the same column followed by the same letter are not significantly different according to DMRT at the 5% significance level

Leaf-sucking Insect Infestation

AMF application affected the infestation of leaf-sucking insects recorded on chili plants at 12 WAT. The AMF-treated plants showed lower infestation, with mean values of 12%, 9%, and 7% in the 50 g, 70 g, and 100 g AMF treatments, respectively.

Among the observed insects, whitefly (*Bemisia tabaci*) infestation was 30% in the control treatment and decreased to 10% in all AMF treatments. This indicates that AMF application reduced whitefly infestation compared with the control, although the three AMF doses produced the same whitefly infestation value. A clearer dose-related reduction was observed in aphid infestation, which decreased from 40% in the control to 15%, 10%, and 5% in the 50 g, 70 g, and 100 g AMF treatments, respectively. Thrips infestation also decreased from 25% in the control to 10% in the 50 g AMF treatment and 5% in both the 70 g and 100 g AMF treatments.

Overall, the 100 g AMF treatment produced the lowest mean leaf-sucking insect infestation. Compared with the control, this treatment reduced mean infestation from 32% to 7%, representing a 78.1% reduction (Table 3).

Table 3. Percentage of leaf-sucking insect infestation in chili plants treated with different AMF doses at 12 weeks after transplanting

Treatment	*Bemisia tabaci* (mean ± SD)	*Thrips* sp. (mean ± SD)	*Aphis gossypii* (mean ± SD)	Overall (mean ± SD)
Control	30 ± 10.00	25 ± 8.33	40 ± 13.33	32 ± 10.66 a
50 g AMF	10 ± 3.33	10 ± 3.33	15 ± 5.00	12 ± 4.00 b
70 g AMF	10 ± 3.33	5 ± 1.66	10 ± 3.33	9 ± 3.00 c
100 g AMF	10 ± 3.33	5 ± 1.66	5 ± 1.66	7 ± 2.33 d

Note: Values in the same column followed by the same letter are not significantly different according to DMRT at the 5% significance level

AMF Root Colonization in Chili Plants

AMF application increased root colonization in chili plants at 8 WAT. All AMF-treated plants showed high root colonization. The 50 g AMF treatment resulted in 56.7% root colonization, while the 70 g and 100 g AMF treatments resulted in 60.0% and 61.7% root colonization, respectively.

Root colonization increased sharply after AMF application, but the difference among the 50 g, 70 g, and 100 g AMF treatments was relatively small. This indicates that AMF colonization may have reached a high level even at 50 g AMF, while higher doses were more clearly associated with lower mean leaf-sucking insect infestation and lower yellowing disease symptom incidence (Figure 2, Table 4).

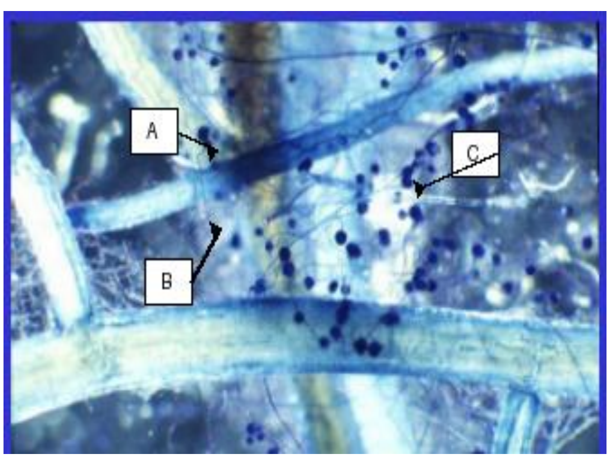

Figure 2. AMF colonization in chili roots: (A) internal hyphae; (B) external hyphae; (C) mycorrhizal spores.

Table 4. Percentage of AMF root colonization in chili plants treated with different AMF doses

Treatment	AMF root colonization (% ± SD)	Category
Control	5.0 ± 1.60 a	Very low
50 g AMF	56.7 ± 18.90 b	High
70 g AMF	60.0 ± 20.00 c	High
100 g AMF	61.7 ± 20.56 c	High

Discussion

The present study showed that local multispore AMF inoculation reduced yellowing disease symptom incidence, lowered leaf-sucking insect infestation, and successfully colonized the roots of field-grown Kopay chili pepper. This result indicates that AMF application may contribute to field-level suppression of yellowing disease symptoms through improved root colonization and reduced infestation by leaf-sucking insects. The strongest overall response was observed in the 100 g AMF treatment, which produced the lowest yellowing disease symptom incidence (Table 2) and the lowest mean leaf-sucking insect infestation (Table 3), while AMF-treated plants showed high root colonization (Table 4; Figure 2).

Those findings are consistent with previous reports that arbuscular mycorrhizal fungi can improve plant resilience to biotic stress by enhancing nutrient acquisition, modifying plant metabolic responses, and supporting disease resistance (Kaur and Suseela, 2020; Umer et al., 2025). AMF have also been reported to influence plant interactions with aboveground herbivores through changes in plant nutrition and defense-related responses, although the effect may vary depending on plant species, AMF identity, herbivore feeding guild, and environmental conditions (Frew et al., 2022). Therefore, local multispore AMF may contribute to field-level suppression of insect infestation and yellowing disease symptoms in chili pepper.

Yellowing disease symptom incidence was lower in all AMF-treated plants than in untreated plants, with the 100 g AMF treatment showing the lowest incidence (Table 2). The visual symptoms observed in this study included irregular leaf yellowing, vein thickening, leaf curling or cupping, reduced leaf size, and stunted shoot growth (Figure 1). These symptoms are consistent with common field symptoms of yellow leaf curl or yellowing disease in chili. Similar symptoms have been reported in chili plants affected by begomovirus-associated diseases, including yellowing, leaf curling, vein thickening, and growth reduction (Subiastuti et al., 2021; Arfiani and Nasruddin, 2023). In addition, Trisno et al. (2021) reported that yellow leaf curl disease in chili may involve complex viral infection, including dual infection of Pepper Yellow Leaf Curl Virus and Chilli Veinal Mottle Virus. Therefore, although the symptoms observed in this study were consistent with virus-associated yellowing disease, the causal pathogen cannot be definitively confirmed because molecular or serological diagnosis was not performed. Thus, the reduction observed in this study should be interpreted as suppression of yellowing disease symptoms rather than confirmed suppression of begomovirus infection.

The reduction in yellowing disease symptom incidence may be related to lower infestation by leaf-sucking insects, particularly whitefly. Among the leaf-sucking insects observed, whitefly (*Bemisia tabaci*) is the most important species in relation to yellowing disease symptoms because it is widely recognized as the primary vector of begomovirus-associated yellow leaf curl disease in chili pepper. In this study, whitefly infestation was lower in AMF-treated plants than in the control (Table 3). This finding is important because *B. tabaci* has been reported as the vector of Pepper Yellow Leaf Curl Virus in chili pepper, and field development of yellow leaf curl disease is strongly associated with the presence and activity of this vector (Selangga and Listihani, 2021; Taufik et al., 2023). However, since whitefly infestation was similar among AMF doses, the effect of AMF on whitefly should be interpreted as a general AMF treatment effect rather than a clear dose-dependent response.

Thrips and aphids were also lower in AMF-treated plants (Table 3). However, these insects should not be interpreted as confirmed primary vectors of the yellowing disease symptoms observed in this study. Their reduction more likely reflects a broader AMF-associated improvement in plant resistance or reduced plant suitability to leaf-sucking insects. AMF can influence aboveground herbivore responses through changes in plant nutrition, defense allocation, and induced resistance, although the direction and magnitude of the effect may depend on plant species, AMF identity, herbivore feeding guild, and field conditions (Frew et al., 2022). The reduction of thrips and aphids in this study supports the possibility that AMF improved general host resistance to leaf-sucking insects, rather than specifically suppressing confirmed disease vectors.

Successful AMF root colonization appears to be an important basis for the observed reduction in disease symptoms and insect infestation. AMF-treated plants showed high root colonization, whereas the control had very low colonization (Table 4; Figure 2). This indicates that local multispore AMF isolate PU10 was able to establish symbiosis with Kopay chili roots under field conditions. Root colonization may improve nutrient uptake, particularly phosphorus, and enhance plant vigor, which can increase plant tolerance to biotic stress. Recent studies have reported that AMF improve plant nutrient acquisition, root development, and resilience to biotic and abiotic stresses (Umer et al., 2025). Liu et al. (2024) also reported that mycorrhizal symbiosis can enhance phosphorus uptake and indole-3-acetic acid accumulation, thereby improving root morphology. Nevertheless, nutrient uptake, IAA, and plant growth variables were not measured in the present study, so these mechanisms should be considered plausible explanations.

In addition to improving plant nutrition, AMF may also contribute to plant protection through mycorrhiza-induced resistance. AMF colonization can prime plant defense responses, alter primary and secondary metabolism, and enhance plant readiness against biotic stress (Pozo and Azcón-Aguilar, 2007; Jung et al., 2012; Kaur and Suseela, 2020). Such mechanisms may help explain why AMF-treated chili plants showed lower leaf-sucking insect infestation and lower yellowing disease symptom incidence. However, defense-related parameters such as defense enzymes, secondary metabolites, salicylic acid, jasmonic acid, or pathogenesis-related proteins were not evaluated in this study. Therefore, AMF-induced resistance should be discussed as a possible mechanism, not as a confirmed mechanism.

Jamarun and Yunisman (2017) showed that antagonistic fungi were able to colonize chili roots and suppress anthracnose disease. Yanty et al. (2024) reported that *Beauveria bassiana* could suppress *Colletotrichum truncatum* and improve chili plant growth. Although these studies did not involve AMF or yellowing disease, they support the broader concept that beneficial fungi can contribute to chili disease management. The present study adds new information by showing that a local multispore AMF inoculum can colonize chili roots under field conditions and is associated with reduced whitefly infestation and yellowing disease symptoms.

The novelty of this study lies in the simultaneous field evaluation of local multispore AMF isolate PU10 on yellowing disease symptom incidence, leaf-sucking insect infestation, and root colonization in Kopay chili pepper. Unlike studies that mainly evaluate AMF under controlled conditions or focus only on plant growth, this study provides field-based evidence that local multispore AMF may support chili plant protection by reducing yellowing disease symptoms and leaf-sucking insect infestation while establishing high root colonization. However, the interpretation remains limited because pathogen identity, vector infectivity, plant nutrient status, growth response, and defense-related mechanisms were not directly assessed. Future studies should include molecular detection of the causal pathogen, more precise vector population monitoring, plant growth and yield measurements, and biochemical or molecular markers of plant defense.

From a practical perspective, the 100 g AMF treatment may be considered a promising preliminary dose for supporting environmentally friendly management of yellowing disease symptoms in field-grown Kopay chili pepper. This approach may be useful in chili cultivation systems where whitefly infestation and yellowing symptoms frequently occur. However, the recommendation should be applied cautiously because the causal pathogen, vector infectivity, plant nutrient status, growth response, and yield effects were not directly evaluated. Therefore, further field validation under different agroecological conditions is needed before broader application.

Conclusions

local multispore arbuscular mycorrhizal fungi (AMF) inoculation reduced leaf-sucking insect infestation and yellowing disease symptom incidence in field-grown Kopay chili pepper. The 100 g AMF treatment produced the lowest mean leaf-sucking insect infestation, reducing overall infestation from 32% in the control to 7%. This treatment also reduced yellowing disease symptom incidence from 40% in the control to 5%, while AMF-treated plants showed high root colonization ranging from 56.7% to 61.7%. These findings indicate that local multispore AMF, particularly at 100 g per plant, has potential as a biological input to support environmentally friendly management of yellowing disease symptoms in chili pepper. However, because disease diagnosis was based on visual symptoms only, further studies using molecular confirmation of the causal pathogen are needed.

Declaration

Author contributions

Yefriwati was the primary contributor to this study and served as the corresponding author. Darmansyah was a co-author. All authors read and approved the final version of the manuscript.

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Competing interest

The authors declare that they have no financial or personal relationships that could have influenced the work reported in this article.

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