

# Distribution of arbuscular mycorrhizal fungi and *Trichoderma* spp. in the rhizosphere and roots of garlic (*Allium sativum* L.) cultivated in Adiyaman province, Türkiye

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**Received:** December 25, 2025 | **Accepted:** February 17, 2026 | **Published Online:** March 11, 2026 | **Final Version:** March 22, 2026

## Abstract

Limited knowledge of root diseases and microbial interactions in commonly grown crops such as garlic in intensive production systems when coupled with losses caused by the *Fusarium* species complex (FSC) and the low use of chemical control in traditional production areas makes it strategic to develop protection techniques and investigate local microbial diversity for sustainable plant protection strategies (SPPS). This study aims to microscopically examine *Trichoderma* spp. in rhizosphere soil and arbuscular mycorrhizal fungi (AMF) in both rhizosphere and root tissues of garlic cultivated in Adiyaman province, Türkiye, while assessing their potential role in biological control. Root and soil samples were collected from five garlic fields in Kaşlıca village, Tut district, Adiyaman province in 2024. After detecting the presence of pathogens, microscopic examinations were conducted to determine the AMF and *Trichoderma* spp. spore density in soil and AMF root colonization rate, and the obtained data were statistically analyzed. Analyses of soil and plant tissue samples collected from different sites revealed significant variation in microbiological and chemical parameters. The higher abundance of *Trichoderma* spp. in certain locations highlights their importance as biocontrol agents in garlic cultivation. These results suggest that soil health and plant-microbial interactions can vary depending on field conditions, and indicate that microbial composition and soil chemistry jointly influence garlic health and pathogen suppression. The findings provide information for the development of biological control strategies specific to agricultural products and the evaluation of regional microbial diversity in terms of agricultural sustainability.

**Keywords:** *Allium sativum*, FSC, native microbial populations, rhizosphere microbiota, sustainable agriculture

## INTRODUCTION

Garlic (*Allium sativum* L.) one of the medicinal plants that has been used extensively since ancient times, is one of the most important representatives of the *Allium* genus of the Alliaceae family (Londhe et al., 2011). Garlic is a globally important agricultural crop, valued not only for its culinary and medicinal properties but also for its economic importance in smallholder farming systems (Lanzotti, 2006). As a phytonutrient, it is widely used in the treatment and prevention of various diseases thanks to its biologically active components, antimicrobial and antioxidant properties. The strong antioxidant activity of garlic peel extract supports its potential for use in herbal treatment (Hernández-Montesinos et al., 2023). Allicin is considered one of the most well-known bioactive compounds found in garlic and contributes significantly to its antimicrobial and therapeutic properties (Kim et al., 2016).

In Türkiye, especially in the Southeastern Anatolia Region, garlic cultivation plays a vital role in rural livelihoods (TÜİK, 2024). However, the sustainability of garlic production is increasingly challenged by both biotic and abiotic stress factors, including soil-borne pathogens, nematodes and viruses, nutrient depletion and climate-induced variability, in addition to intensive agricultural activities (Ahmad et al., 2021; Eom and Hyun, 2023). The intense effect of changing biochemical

**Citation:** Demirel, O., Gunes, H., Can, C. (2026). Distribution of arbuscular mycorrhizal fungi and *Trichoderma* spp. in the rhizosphere and roots of Garlic (*Allium sativum* L.) cultivated in Adiyaman province, Türkiye. International Journal of Agriculture, Environment and Food Sciences, 10 (1): 65–76. <https://doi.org/10.31015/jaefs.2026.1.8>

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value parameters on plant resistance mechanisms also provides very important results on the plant-root relationship (Jerčić et al., 2023). Utilizing genome-assisted selection and innovative biotechnological applications to improve genetic diversity is one of the measures (Khar et al., 2020). In this context, the need for biologically based, innovative and sustainable plant protection approaches is increasing (Demirel et al., 2024b).

Diseases caused by seed and soil-borne diseases are among the most important biotic stress limiting agricultural activity areas for many plants (Kocalar et al., 2020; Demirel Durak et al., 2022; Altinok et al., 2023; Demirel et al., 2024a; Demirel et al., 2025). Fusarium basal rot, primarily caused by *F. proliferatum* and *F. oxysporum*, is recognized as one of the most destructive fungal diseases of garlic, resulting in severe yield and quality losses both in the field and during storage (Gálvez and Palmero, 2022; Mondani et al., 2021). These pathogens establish latent infections in root and tuber tissues and are known to produce diverse secondary metabolites (mycotoxins such as fumonisin, moniliformin and beauvericin) that compromise product quality and food safety, but also provide many benefits such as anticarcinogenicity of purified-cyclic depsipeptide secondary metabolites (Jeong et al., 2024; Rämö et al., 2025). As traditional chemical control methods in agriculture are increasingly inadequate, the exploitation of beneficial microorganisms such as *Trichoderma* spp. and Arbuscular Mycorrhizal Fungi (AMF) emerges as a crucial and sustainable alternative. These biological agents not only offer significant potential for disease suppression, particularly against *Fusarium* spp., but also align with global strategies for environmentally friendly crop protection (Mondani et al., 2021; Güneş et al., 2022; Sharma et al., 2024).

Among abiotic stresses, drought stands out as a primary and globally constraint on crop production, with profound implications for food security and sustainable agriculture. Its impact becomes even more detrimental when accompanied by phosphorus (P) deficiency, severely reducing plant performance in arid and semiarid regions (Rani et al., 2020; Khar et al., 2022). Although P is the most critical macronutrient for plant growth after nitrogen (N), its bioavailability in soil decreases significantly due to moisture loss, which disrupts vital processes such as root development, water use efficiency and nutrient uptake. Moreover, the occurrence of much more phenotypic variation in different agronomic traits in different climates is associated with the multifaceted effects of drought (such as morphological, physiological, growth and yield-quality) in garlic (Kovačević et al., 2023). In this context, AMF not only support P-supply in onions, members of the *Allium* genus, but also increase the capacity to resist drought stress by stimulating antioxidant systems and regulating osmotic balance; thus, microbial-based applications come to the fore in sustainable plant protection strategies (Arunachalam et al., 2024). Practices that optimize root colonization and P-uptake efficiency of AMF modulate plant stress responses and support Reactive Oxygen Species (ROS) homeostasis through root tissue, especially under low P concentration conditions (Boutasknit et al., 2021). In this context, the combined evaluation of mycorrhizal symbiosis and P-fertilization constitutes an important biological framework that contributes to the development of rational agroecological strategies for drought management.

On the other hand, one of today's promising approaches is to take advantage of the active roles of beneficial soil microorganisms such as *Trichoderma* spp. in plant resistance. These microorganisms are widely recognized for enhancing plant growth, nutrient uptake, and resistance to pathogens from biotic stress factors (Smith and Read, 2009; Yao et al., 2023). However, their interactions (AMF + *Trichoderma* spp.) remain relatively unexplored in *Allium* spp.

According to their mechanisms, while AMF improve P-acquisition and drought tolerance that represent effective and critical traits for sustaining crop yields under climate change, i.e. water use efficiency, by establishing mutualistic relationships with plant roots, *Trichoderma* species are active against a wide range of phytopathogens and abiotic stress through multifaceted defense system mechanisms such as mycoparasitism, antagonism, antibiosis (secondary metabolite and antibiotic production), competition (nutrient and spatial) and induced systemic resistance (Han et al., 2022; Shahriar et al., 2022). Moreover, especially in plants with limited root structure such as *Allium* species (garlic, onion, leek, etc.), plant growth is supported by effectively expanding the root surface area thanks to AMF colonization. In short, the role of AMF in disease resistance is multifaceted. As a physical barrier, AMF colonization thickens root cell walls, making pathogen penetration more difficult. With induced systemic resistance (ISR), AMF activates defense genes in the plant, creating a prepared defense state against pathogens. By providing intensive production of phenolic compounds, AMF shows antifungal effects on infected plants. By creating competition for nutrients, AMF consume nutrients in the root zone and limit the development of pathogens.

The discovery of local AMF and *Trichoderma* populations and their combined use, understanding their functional potential, together with knowledge of the characteristics of plant growing areas, are seen as an important basis for the development of plant-specific bioformulations, biostimulants and sustainable crop management practices (Szczałba et al., 2019). Combination uses of AMF bring about significant improvements in many aspects (Boutasknit et al., 2021). Recent studies indicate that co-inoculation of AMF and *Trichoderma* species, replacing single applications within the limited knowledge in the genus *Allium*, produces synergistic effects on plant health and productivity, especially under stress conditions (Yağmur et al., 2024). Considering the study conducted in Costa Rica, it shows that *Trichoderma* spp. isolated from garlic and onion rhizospheres effectively suppress *Sclerotium cepivorum*, the causative agent of white rot, and promote plant growth under greenhouse and field conditions (Alvarado-Marchena and Rivera-Méndez, 2016). Similarly, AMF and its different combination applications have been associated with significant effects on garlic and onion and, within limited information, a higher bulb-yield in garlic (Golubkina et al., 2020). All this information reveals the importance of integrating regional microbial resources into agricultural production systems.

Garlic production in Türkiye's Southeastern Anatolia Region faces increasing challenges from soil-borne pathogens and declining soil fertility, making the exploration of beneficial rhizosphere microorganisms a scientifically grounded research

problem. The rhizosphere microbiome, which plays an important role in determining plant resistance and yield quality in general, has a high potential to help obtain valuable information in garlic cultivation. However, the limited information on phytopathogens in addition to the roots and soil-associated local microbial populations of garlic plants, especially in different ecological and underexplored areas of Türkiye (such as Adıyaman, Şanlıurfa and Hatay provinces) (Dedecan, 2024), highlights the importance of the study and the significance of the information to be obtained. Previous studies have separately highlighted the role of *Trichoderma* spp. and AMF in enhancing plant growth and suppressing pathogens, yet region-specific and combination group choosing data remain scarce. By focusing on naturally occurring microbial communities in Kaşlıca village, Tut district, Adıyaman Province, this study not only establishes an aim of microscopic characterization and evaluation of biological control potential, but also contributes novel insights into the variability of soil health under field conditions. Moreover, this work provides region-specific evidence that strengthens global understanding of plant-microbe interactions under field conditions. The findings are expected to enrich the literature on sustainable crop protection and microbial ecology, while offering practical implications for garlic cultivation and broader agroecological management strategies.

## MATERIAL AND METHOD

### Survey and Sampling

This study was conducted in garlic production areas in the Tut district of Adıyaman province, Southeastern Anatolia Region of Türkiye. Plant and soil sampling was conducted from 5 fields (numbered 1, 2, 3, 4 and 5) with different microclimatic characteristics during the 2024 production season (April–June). The samples were microscopically analyzed for the presence of pathogens by 10-fold serial diluted soil solutions with sterile distilled water (sdH<sub>2</sub>O) and tissues sterilized with 1.5% NaOCl incubated on potato dextrose agar (PDA) (22±2 °C). Fields that were healthy after incubation, i.e., petri dishes where no fungal growth was observed, were excluded from the study. The collected roots of each plant were considered one sample and included in the study. The plant materials used in the study were roots of imported Chinese garlic seeds and rhizosphere soil from the cultivated area. A total of 50 duplicated plant roots, five from each area (in falcon tubes at 4±2 °C), and their rhizosphere soil were collected separately and transported to the laboratory under appropriate conditions (in polyethylene bags at 22±2 °C). Rhizosphere soil samples were taken from the root area at a depth of ~20 cm (Lorenz et al., 1994). The entire study was conducted in the Population Genetic Laboratory of the Biology Department, Faculty of Arts and Sciences, Gaziantep University.

### Root Preparation

Root samples collected from healthy and infected surveys were first washed in running tap water and dried. After the roots were fixed (fixation process), they were stained with lactophenol and prepared for microscopic observation. First, the roots were fixed and placed in long-term storage (22±2 °C) for ease of use. For this process, the entire root of each plant was placed in a sufficient amount of AFA Fixation Solution (90 mL of 70% Ethyl Alcohol, 5 mL of Formaldehyde, and 5 mL of Acetic Acid). The roots were then stored in this preservative solution in sterile containers (20 mL per sample) until staining (Phillips and Hayman, 1970). For the study, 1.6 g pieces were taken from the roots in the preservative solution (including ~5% error and repetitions), and ~0.5 g of the pieces for each replicate were used for microscopic observation in three replicates.

In the second stage, the roots placed in the AFA solution to be used in the study were stained with lactophenol blue. For this purpose, the roots were soaked in 20 mL of 10% KOH for half a day, then rinsed with sdH<sub>2</sub>O and then soaked in 20 mL of 10% HCl for half an hour. Then, the roots were transferred to a lactophenol dye solution containing lactophenol blue (0.05%), glycerol (80 mL), and sdH<sub>2</sub>O (30 mL) (20 mL per sample) and soaked in this solution for two hours. The Grid-Line Intersect Method was used to determine the percentage of AMF colonization in the stained roots (Giovannetti and Mosse, 1980). Finally, the stained roots were heated in hot water at 50 °C for 5 minutes and after being washed in sdH<sub>2</sub>O, the heated roots were kept in lactic acid (20 mL per sample) for 1 hour to make them ready for microscopic analysis.

The wet sieving method developed by Gerdemann and Nicholson (1963) was used to prepare rhizosphere soil samples collected from healthy and infected surveys for microscopic observation and to determine AMF spore density. Firstly, in order to remove coarse particles, all soils brought from the field were subjected to a 2 mm pre-sifting process and the amount to be used in the study (10 g) was weighed into 250 mL sterile beakers and the remaining was re-bagged and stored under appropriate conditions (22±2 °C) (Lorenz et al., 1994). Using the method adapted from Güneş (2022), sdH<sub>2</sub>O (100 mL) was added to the sifted soils transferred into the beaker and mixed with a glass rod to obtain a homogeneous solution. The mixing process was carried out in three replicates at 5-minute intervals and allowed to stand for 15 minutes (22±2 °C) to allow plant parts and/or insoluble soil particles to settle. The supernatant was carefully filtered from the separated beakers, first through an 80-µm sieve and then through a 45-µm sieve, and collected in new sterile 100 mL beakers. The AMF-containing liquid was then transferred to centrifuge tubes, 5% glucose was added, and after shaking, the liquid was centrifuged at 2000 rpm for 3 minutes. The supernatant was transferred to a petri dish.

### Soil pH Preparation and Measurements

pH measurement, an indicator of soil hydrogen activity, was performed by adapting the method of Lorenz et al. (1994). During the preparation phase, soil samples (10 g) from each survey area were weighed and transferred into 250 mL beakers, and sdH<sub>2</sub>O (90 mL) was added (Jackson, 1958). For observations using homogeneous soil solutions in the study, which was carried out in three replicates, each beaker containing stirrer magnet was carefully placed on a magnetic stirrer and stirred at medium speed (~200 rpm) for 15 min.

### Microscopic Observation of *Trichoderma* spp. and AMF Density in Soil

In order to determine the spore density of *Trichoderma* spp. and AMF in the soil, spore density per gram of soil was determined within the adapted method by observing the spores under a stereo microscope and recording their numerical data using homogeneous solutions prepared from the soil in the rhizosphere region of the plants (Güneş, 2022).

### Microscopic Analysis of AMF Colonization in Root

Roots (healthy and infected), which were finished with dyeing and ready for analysis, were removed from lactic acid and analyzed under a microscope using the method adapted from Güneş (2022). In the analyses carried out with 3 replications, roots (0.5 g) were cut into 1-1.5 cm long pieces and distributed homogeneously in a petri dish divided into 1 cm<sup>2</sup> areas. They were then examined under a stereo microscope and during the counting, for each root segment that intersected the grids in the petri dish perpendicularly, - (dash) was noted. If AMF propagules (hyphae, vesicles, chlamydo spores) were present in that vertical root segment piece, they were marked with an X (cross) and the AMF root colonization ratio (%) was calculated by the formula *i* after the counting (using the Grid-Line method). The percentage of colonization was calculated according to the presence of arbuscules, vesicles and hyphae in 30 randomly selected segments from each root sample (Giovannetti and Mosse, 1980).

$$\text{AMF Colonization ratio (\%)} = \frac{\text{The number of roots colonized with AMF}}{\text{The total number of roots}} \times 100(i)$$

### Statistical Analysis

The obtained data were analyzed using SPSS 22.0 software (IBM, 2013). While the variations between different fields were tested with One-way ANOVA (Tukey HSD), AMF root colonization ratio (%) and AMF and *Trichoderma* spp. spore density in the soil between healthy and infected sampling were tested with independent t-test, and the significance level was accepted as  $p < 0.05$ . The relationship between soil pH measurement and *Trichoderma* spp. soil spore density was determined by a correlation test ( $p < 0.01$ ).

## RESULTS

Statistical evaluation of microscopic analyses of garlic root and soil samples collected from four cultivation areas in Kaşlıca village, Tut district, Adiyaman province, revealed significant differences in microbial content and soil chemistry between healthy and infected areas. This study is the first to identify microorganisms in garlic under *in vitro* conditions.

The fields numbered as 1, 2, 3, 4, and 5. The studies revealed that field number 3 was only healthy-excluded from study-, while other fields showed *Fusarium* infection through both plant tissues and soil sample analysis (S1).

### Microbial Diversity and Distribution

The independent sample t-test demonstrated a statistically significant difference in the abundance of *Trichoderma* species, which were detected at markedly higher levels in uninfected rhizosphere soils ( $p < 0.05$ ) (Table 1).

**Table 1.** Spore density of *Trichoderma* spp. in rhizosphere soil ( $p < 0.05$ )

	Case	N	Mean±Std. Deviation	Mean±Std. Error	F	t	df	Sig. (p)
Mean <i>Trichoderma</i> spp.	Healthy	60	6.67±0.24	6.67±0.03	34.90	2.29	118.00	<b>0.02</b>
	Infected	60	6.50±0.50	6.50±0.07			84.29	

According to the One-way ANOVA and Tukey HSD, significant differences at the 95% confidence level were observed between healthy and infected samples based on the fields (1, 2, 4 and 5), with each group further divided into two distinct subgroups (a and b) (Figure 1).

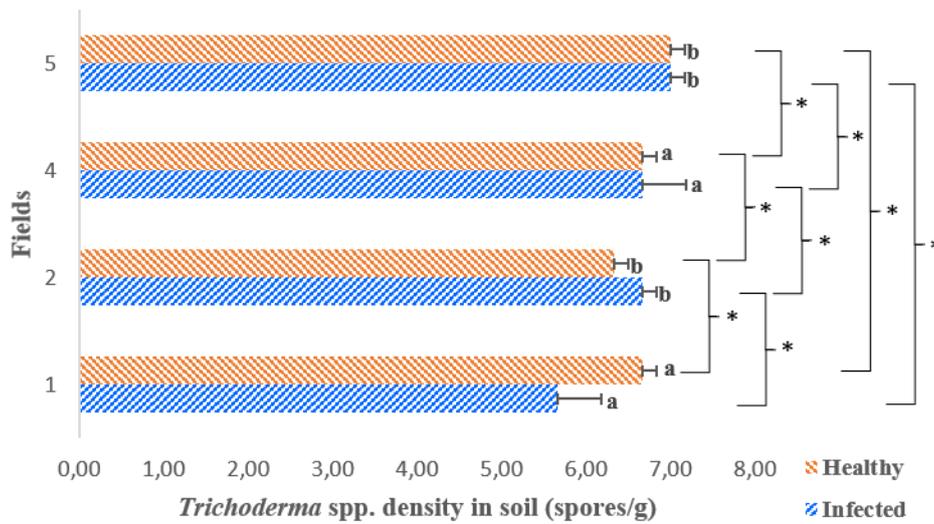


Figure 1. The soil *Trichoderma* spore density graph according to fields and sample cases (\*: $p < 0.05$ ).

AMF spore density were significantly lower in healthy rhizosphere soils and root tissues compared to their infected counterparts (S2), and the difference was statistically significant at the 95% confidence level by independent sample t-test ( $t_{soil} (114) = -9.38$  and  $t_{root} (118) = -15.00$ ,  $p < 0.05$ ). In the analysis of AMF spore density in soil according to the fields using One-way ANOVA test and Tukey assumption, healthy samples were collected under a single group, while infected samples were collected under different groups according to the fields ( $p < 0.05$ ) (Figure 2a). In contrast, in the analysis of AMF spore density in the root tissues according to the fields with One-way ANOVA test and Tukey assumption, healthy and infected samples were collected under a single group ( $p < 0.05$ ) (Figure 2b).

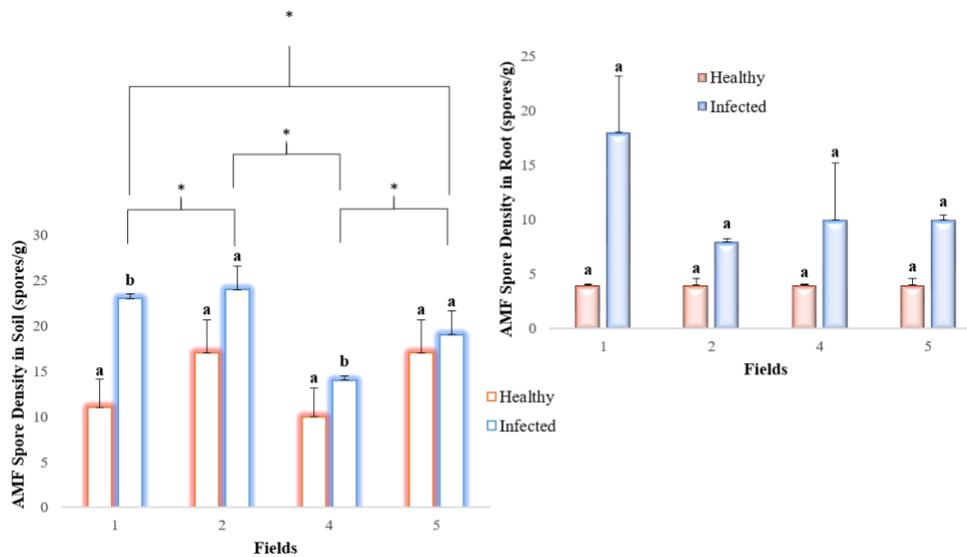
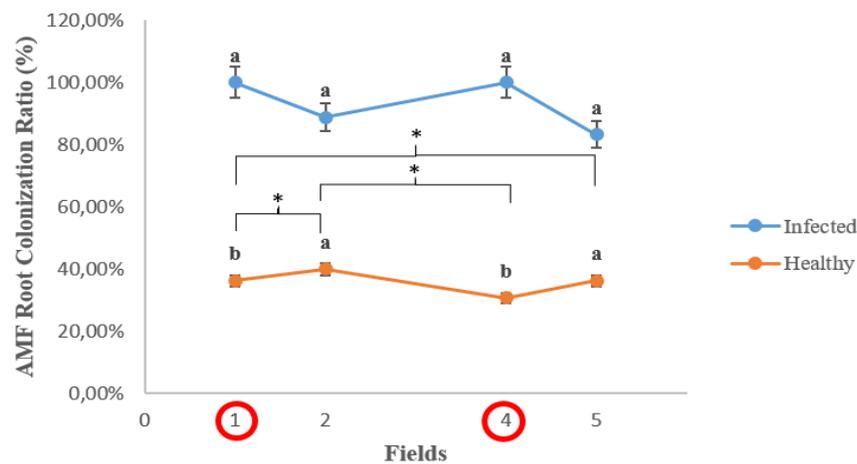


Figure 2. AMF spore density in soil (a) and root (b) according to fields and sample cases (\*: $p < 0.05$ ).

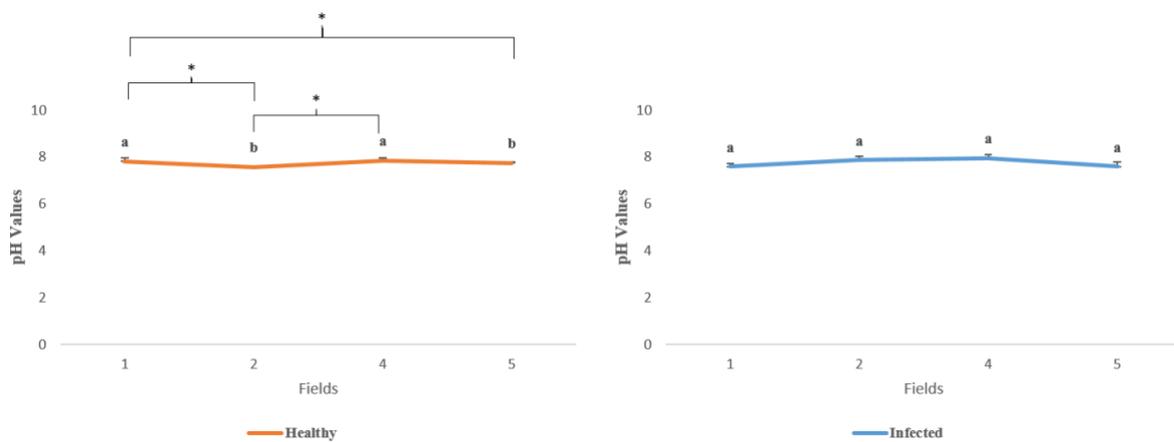
On the other hand, similar to the AMF spore analysis, the AMF root colonization ratio (%) (S3) value in healthy root samples was significantly lower than in infected samples, and the difference was indicated a statistically significant at the 95% confidence level with the independent sample t test ( $t_{soil} (82) = -55.34$ ,  $p < 0.05$ ). As a result of the calculations made in terms of root colonization ratio (%) distribution, it was observed that the 1<sup>st</sup> and 4<sup>th</sup> fields among the infected samples had the highest AMF colonization values (lowest values in healthy samples), while in terms of the One-way ANOVA test and Tukey assumption analysis performed on the AMF root colonization ratio (%) changes in healthy and infected samples according to the fields, it was identified that there were significant differences between the healthy samples and that they were collected under two different groups ( $p < 0.05$ ) (Figure 3).



**Figure 3.** The AMF root colonization ratio (%) profiles according to fields and sample cases (\*: $p < 0.05$ ).

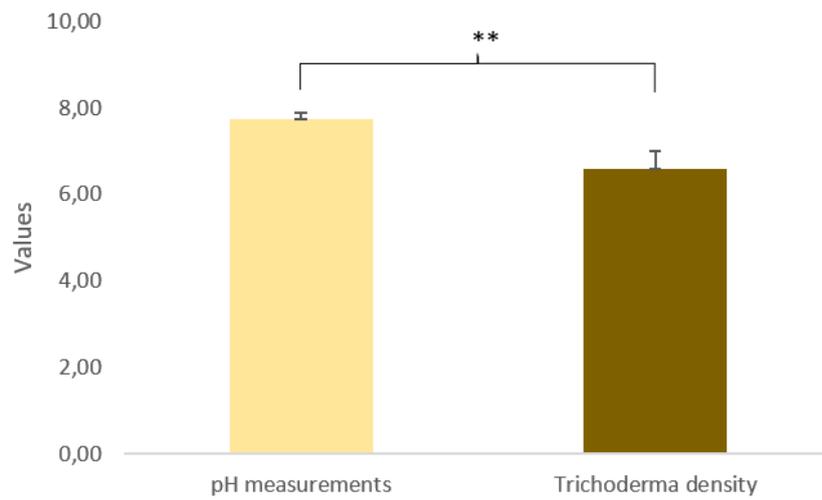
### Soil pH and Microbial Correlations

Soil pH values varied significantly between fields, with slightly higher pH levels observed in infected samples. A significant change was observed among healthy samples with the One-way ANOVA test and Tukey assumption performed to determine the difference that constitutes the significant change between the fields, and the difference was found to be significant at 95% confidence level and it was occurred that they were gathered under two different groups ( $p < 0.05$ ) (Figure 4).



**Figure 4.** The graph of pH values according to fields and sample cases (\*: $p < 0.05$ ).

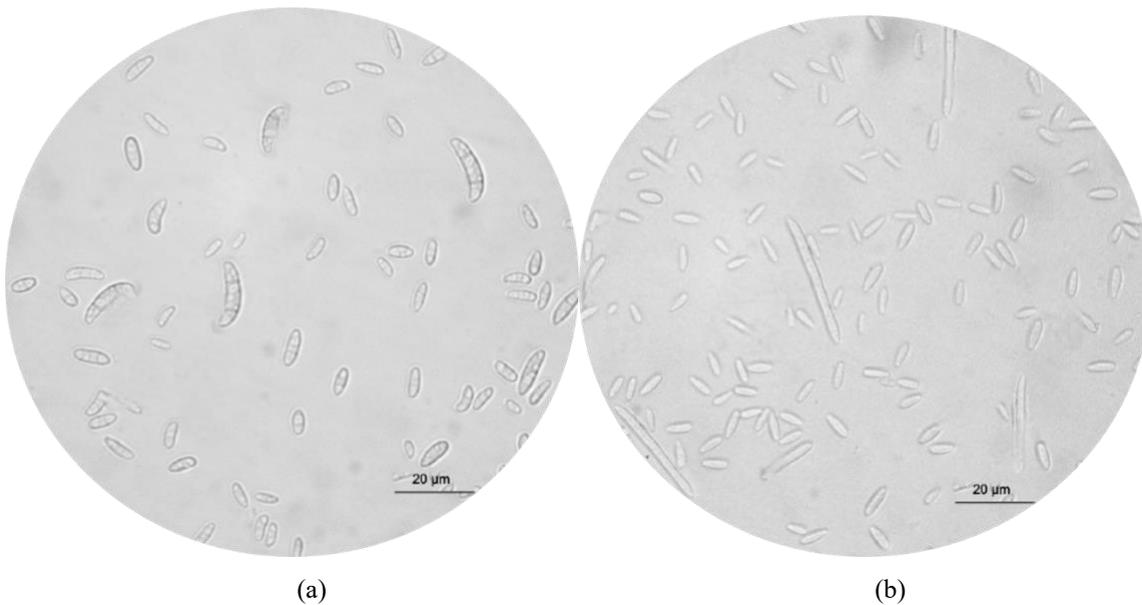
It was observed that there was a significant moderate and positive correlation between soil pH values and *Trichoderma* spp. soil spore density at the 99% confidence level (Pearson's correlation coefficient = 0.371 and  $p < 0.01$ ) (Figure 5). On the contrary, the correlation between this pair and AMF soil spore density and root colonization has a negative and medium feature (Pearson's correlation coefficient<sub>AMF soil density</sub> = -0.426 and Pearson's correlation coefficient<sub>AMF root colonization</sub> = -0.285 for pH, and Pearson's correlation coefficient<sub>AMF soil density</sub> = -0.355 and Pearson's correlation coefficient<sub>AMF root colonization</sub> = -0.640 for *Trichoderma* spp. soil density,  $p < 0.01$ ).



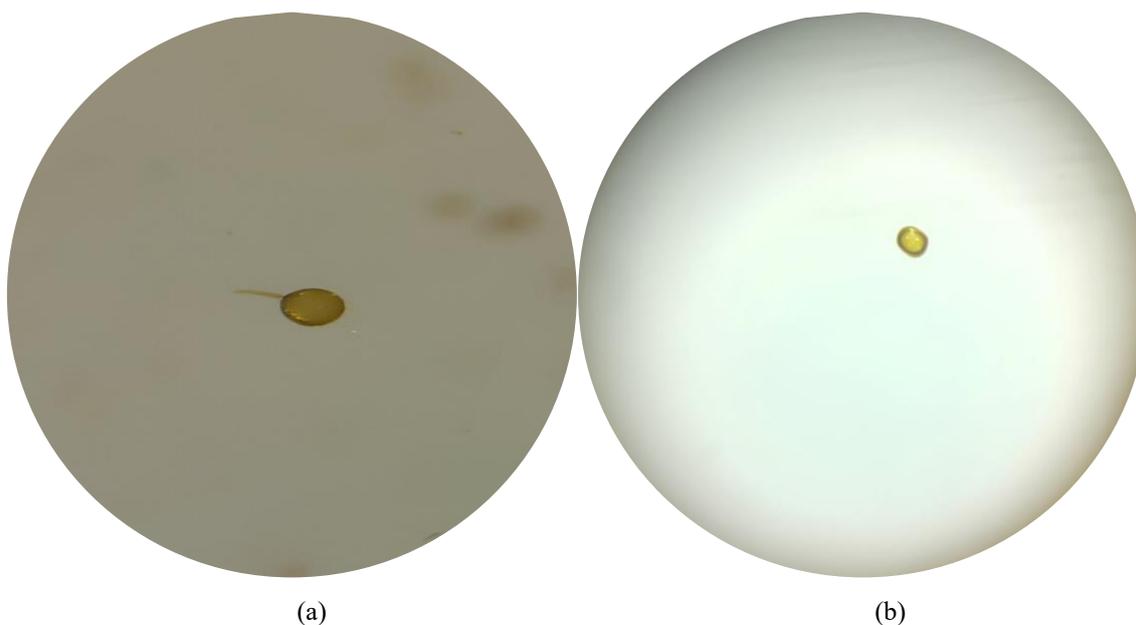
**Figure 5.** The correlation graph between pH measurement and *Trichoderma* soil spore density (\*\*: $p < 0.01$ ).

From all this information and data, both microbial and chemical parameters differed significantly between healthy and infected fields.

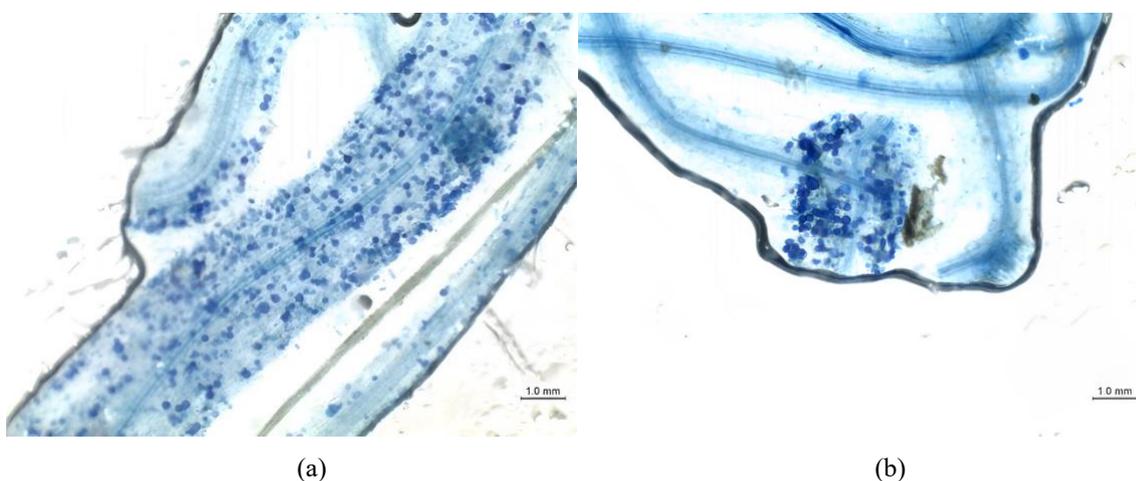
#### Supplementary material



**Supplementary Figure 1.** *Fusarium* spp. spores in infected fields (a) *Fusarium oxysporum* and (b) *Fusarium proliferatum*.



**Supplementary Figure 2.** AMF spore in soil (a) infected field and (b) healthy field.



**Supplementary Figure 3.** AMF root colonization (a) infected field and (b) healthy field.

## DISCUSSION

This study, microscopic examination of together with AMF species in the soil rhizosphere and root tissues, *Trichoderma* species in the soil rhizosphere, of garlic plants grown in Tut district of Adiyaman province, revealed the effects of regional microbial diversity on plant health.

The findings show that *Trichoderma* spp. and AMF species in particular form different distributions between infected and healthy (sample cases) and that these distributions are related to environmental factors such as soil pH, which is a chemical parameter.

On the other hand, in the scope of the study conducted by Dedecan (2024), which supports the importance of the study, comprehensive garlic disease investigations were carried out in 5 provinces (Gaziantep, Adiyaman, Şanlıurfa, Kahramanmaraş, and Hatay) where intensive garlic production is carried out in the Southeastern Anatolia and Mediterranean Regions. In Adiyaman, one of the provinces included in the study, 5 out of 6 fields were determined to be diseased and the disease prevalence rate caused by basal root rot was determined as 83.33%, disease incidence as 23.74% and disease severity as 12.60%. On the contrary, in Gaziantep province, where the most intensive production is made, the rates were determined as 76.32%, 12.43% and 4.89%, respectively. With this study, it was reported that although Gaziantep has intensive cultivation and production, it can be affected by the disease, but this is lower (76.19%) than the diseased field percentage in Adiyaman (83.33%). This suggests that the result is due to intensive commercial chemical pesticide application and poses a serious threat to the effectiveness of soil microorganism viability. Moreover, it is a known fact that soil structure, along with changing geographical features, contributes to the change of microorganisms. According to Dedecan (2024), despite the 12.6% disease severity observed in Adiyaman due to root rot, the determination of a proportional distribution of 4.89% severity in Gaziantep supports the claim of intensive chemical use. In this respect, it has been concluded that *Fusarium*-

related disease observation and control, especially due to less chemical use, are much more important for Adıyaman province and the microorganism content of the soil can be protected in this study.

In this study similarly Dedecan (2024) molecular data, *Fusarium proliferatum* and *F. oxysporum*, which are the agents of FSC, were microscopically identified in Adıyaman. In addition, the same study, examining basal root rot caused by *Fusarium* spp., Adıyaman was ahead of Gaziantep in terms of disease incidence and prevalence, which draws attention to the investigation of soil-borne diseases and emphasizes the importance of protecting root systems. Especially, the fact that Adıyaman is among the top 3 in terms of disease incidence despite the low disease severity rate once again reveals the importance of the studies to be carried out in Adıyaman. In this respect, testing integrated innovative disease prevention systems within the scope of this study will provide basic information for future studies.

In terms of *Trichoderma* spp. and pathogen suppression potential, the higher abundance of *Trichoderma* spp. in uninfected-healthy samples suggests that these species may play a pathogen suppressive role in the rhizosphere. It has been reported in the literature that *Trichoderma* species have antagonistic effects against soil-borne pathogens such as *Fusarium* spp. and activate plant defense mechanisms (Abd-Elaziz et al., 2021; Chen et al., 2021). The high level of *Trichoderma* spore density in fields where infection was not observed supports the biological control potential of the soil microbial content.

In contrast, the higher AMF spore density and root colonization ratio (%) rates in infected fields can be explained by several possible mechanisms. First, pathogen pressure often alters root exudation patterns, which can stimulate the germination and colonization of AMF spores, a phenomenon described as stress-induced symbiotic activation (Smith and Read, 2009; Wahab et al., 2023). Second, *Fusarium* spp. infection weakens root tissues and creates new ecological niches that facilitate AMF entry and colonization (Chauhan et al., 2022). Finally, soil chemical properties, particularly pH, play a decisive role; the slightly alkaline conditions observed in infected sites are known to enhance sporulation and colonization of certain AMF species (Zhu et al., 2007; Djekiref et al., 2025). Taken together, these findings suggest that AMF may play an active role not only in healthy plants but also in stressed plants, potentially establishing healing symbiotic relationships that contribute to resilience under biotic stress.

The negative correlation observed between *Trichoderma* spp. and AMF in soil microbial content indicates the presence of microbial competition in the rhizosphere. The rapid colonization ability and secondary metabolite production of *Trichoderma* spp. can suppress the germination of AMF species spores (Özkale, 2017). This situation reveals that species selection and combinations should be carefully planned in microbial-based applications. Moreover, when examining and interpreting microbial parameters between healthy and infected fields, the management history of the fields (e.g., previous crops, fertilization practices) should also be considered. Because of this can particularly affect both soil chemistry and microbial composition.

When the fields (1<sup>st</sup> and 4<sup>th</sup>) reported in the literature to be infected with *Fusarium* spp. are examined (Dedecan, 2024), the high level of AMF colonization coupled with the low spore density of *Trichoderma* species suggests that they may be beneficial together, becoming the focus of antagonistic interactions. This provides fundamental information for both maintaining soil health and increasing plant resilience through the formation of effective modulations in terms of plant protection responses. Additionally, in line with the findings of this study, which are particularly consistent with Yağmur et al. (2024), benefiting from the combined use of plant protection products like this in the *Allium* genus is successful in preventing or reducing the incidence of *Fusarium* spp.-based diseases.

According to an onion study (Yağmur et al. 2024), the combined or separate use of AMF and *Trichoderma harzianum* offers an effective strategy for the biological control of *Fusarium* root rot. AMF (commercial formulation of AMF -*Funneliformis mosseae*-), in particular, has shown promising results in terms of both disease suppression and plant growth. This shows that competition and antagonism can coexist (Turhan, 2010).

Considering soil microbial dynamics, a positive and moderately strong relationship was recorded between soil pH and *Trichoderma* spp. spore density, suggesting that pH may affect microbial abundance and activity (Okoth et al., 2009). Similarly, it was determined that both organisms (*Trichoderma* and AMF species) can affect rhizosphere pH, but this relationship occurs in opposite directions. This difference is based on the fact that AMF generally prefer neutral pH, while some *Trichoderma* species are much more active in acidic environments (Cabral-Miramontes et al., 2022). This indirectly explains how one organism prevents the colonization of another.

On the other hand, although P levels could not be directly measured in the present study, the role of soil pH in regulating P availability is well documented in literature. At higher pH values, P tends to precipitate with calcium and magnesium, thereby reducing its solubility and plant uptake (Hinsinger, 2001; Raghothama, 1999). This reduction in P availability may explain the increased colonization of AMF observed in infected fields with this study, as plants under nutrient stress often rely more heavily on symbiotic associations to enhance P acquisition (Smith and Read, 2009). Such findings are consistent with previous reports that AMF colonization is stimulated under conditions of limited P availability, particularly in alkaline soils (Plenchette et al., 1983). Therefore, the observed differences between healthy and infected fields should not be interpreted solely in terms of microbial composition, but also in relation to soil management history and nutrient dynamics. Integrating pH–P interactions into the analyses can provide a stronger ecological explanation for AMF proliferation under infection stress, highlighting the importance of considering both chemical and biological factors in garlic rhizosphere studies.

## CONCLUSION

This study highlights the strategic importance of local microbial diversity in garlic cultivation systems. The findings highlight that indigenous microbial populations can serve as valuable biological resources, offering environmentally friendly alternatives to chemical inputs and supporting resilience in traditional production areas. In this context, the research provides a foundation for integrating microbial-based approaches into agroecological strategies, reinforcing the necessity of natural and sustainable solutions in the face of climate change and increasing pathogen pressure.

Overall, the future studies should focus on the long-term monitoring, molecular characterization, functional genomics and mechanistic approaches, bioreactive product development, emphasis on the functional integration of these microbial populations into garlic production systems. Expanding such research across different agroecological zones will not only strengthen regional food security but also contribute to global strategies for climate-resilient and sustainable agriculture.

## Author Contributions

Ö. D. contributed to the conceptual design of the study, and helped laboratory analyses. Additionally, she performed statistical analyses, drafted the initial manuscript, illustrated all figures and graphs, and assisted in data interpretation, literature integration. H. G. conducted laboratory analyses and performed microscopic evaluations. Ö. D., H. G., and C. C. contributed to the final revision of the manuscript.

All authors reviewed and approved the final version of the manuscript.

## Conflicts of Interest

The authors declare that they have no competing interests.

## Data, Illustration and Deposited Material Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The deposited materials are not presented in the article.

All illustrations were created by Özge Demirel and are shared under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (©Illustrated by Özge Demirel - CC-BY-NC-SA 4.0). Microscope images were generated and visualized by the corresponding author (Hasret Güneş).

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