EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2025, 26(12), 1–14
https://doi.org/10.12912/27197050/213432
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Received: 2025.10.15
Accepted: 2025.11.15
Published: 2025.12.01

Enhancing the control of Bayoud disease in date palm through the inhibitory effect of *Trichoderma asperellum* and endomycorrhizae

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ABSTRACT

Fusarium oxysporum f.sp. *albedinis* is the causal agent of vascular wilt of date palms (Bayoud). This study evaluated the effectiveness of a biological control approach combining arbuscular mycorrhizal fungi (AMF) and *Trichoderma asperellum* against this pathogen. The experiment was conducted in a randomized block design using two date palm varieties (Najda and Bouzekri), each subjected to four treatments: control, *Fusarium*-inoculated, *Fusarium* + *T. asperellum*, *Fusarium* + AMF, and *Fusarium* + *T. asperellum* + AMF. The results demonstrated that the combined treatment with AMF and *T. asperellum* significantly reduced disease severity, with stunting indices decreasing to 6.5–7.9% compared with 48–56% in the controls, while markedly enhancing plant growth. Successful mycorrhizal colonization was confirmed by the presence of characteristic symbiotic structures, with maximum colonization frequencies reaching 95% for the Najda variety and 80% for Bouzekri. The dual inoculation completely suppressed *F. oxysporum* in the stipes (0% re-isolation) and reduced its presence in the roots to 10–20%. This treatment also led to strong growth stimulation, with root length and leaf number increasing by 67–100% and total biomass multiplying by five. The slightly better performance of Najda (20% residual pathogens vs. 10% for Bouzekri) indicates varietal differences in response to the biocontrol agents. Overall, the synergistic action between AMF and *T. asperellum* offers an efficient, sustainable, and environmentally friendly strategy for controlling Bayoud disease and enhancing date palm vigor.

Keywords: arbuscular mycorrhizal fungi, date palm, *Fusarium oxysporum* f sp. *albedinis*, fusarium wilt (Bayoud), *Trichoderma asperellum*.

INTRODUCTION

Bayoud, or vascular wilt of date palm (*Phoenix dactylifera* L.), caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), is a devastating disease that has affected North African palm groves for over a century (Fernandez, 1995; Sghir et al., 2014; Smail et al., 2017; Abdellaoui et al., 2020). In Morocco and Algeria, it has destroyed millions of palms, reducing populations from approximately 15 million to less than 1.5 million trees (Toutain, 1967; Hamidi et al., 2017). The pathogen infects plants through the roots, colonizes the vascular system, and causes progressive wilting and death. Its persistence in the soil through resistant chlamydospores makes eradication particularly challenging.

In recent decades, several studies have emphasized the potential of biological control agents to mitigate soil-borne diseases in date palms and other crops. Arbuscular mycorrhizal fungi (AMF) enhance plant nutrition and induce resistance to root pathogens, while *Trichoderma* spp. act as both growth biostimulants and antagonists against numerous phytopathogenic fungi (Khattabi et al., 2004; Kribel et al., 2019; Adnani et al., 2024). Both microorganisms are environmentally friendly alternatives to synthetic pesticides and contribute to sustainable agriculture.

The objective of this study was to evaluate, for the first time, the combined effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on the growth and health of date palm vitroplants infected with *F. oxysporum* f. sp. *albedinis*. The work aimed to determine whether this dual inoculation could suppress Bayoud symptoms, enhance plant vigor, and provide a viable biological strategy for the sustainable management of date palm diseases in arid regions.

MATERIALS AND METHODS

Plant material

Date palm vitro-plantlets (*Phoenix dactylifera L.*) belonging to the Najda and Bouzekri varieties were provided by the experimental station of the Regional Office for Agricultural Development of Tafilalet. After acclimatization under aseptic conditions, the plantlets were transferred to plastic pots containing a sterile substrate composed of peat and sand (1:1, v/v), sterilized at 250 °C for 2 h. The plants were maintained in a greenhouse under a controlled microclimate and watered regularly with tap water.

Pathogen isolation

A composite inoculum of eight endomycorrhizal species originating from the rhizosphere of the carob tree (*Ceratonia siliqua*) was used. Barley seeds were disinfected (5% NaCl, 2 min) and germinated in sterile sand mixed with the inoculum. After 4 weeks of growth, the mycorrhizal roots were washed, cut into 1–2 mm fragments, and used as inoculum. The mycorrhization rate of barley roots was assessed according to the method of Phillips and Hayman (1970).

Mycorrhizal inoculation

The composite mycorrhizal inoculum used Figure 1 was a mixture of eight species of endomycorrhizal fungi, originating from the rhizosphere of the carob tree and known for their mycorrhization potential (*Ceratonia siliqua*) (Viterbo et al., 2002). Barley seeds were disinfected with 5% sodium hypochlorite for 2 min, then germinated in plastic pots filled with a mixture of sterile sand and endomycorrhizal inoculum (Hanson and Howell, 2004; Talbi et al., 2016).

After 4 weeks of culture, barley roots were excised, rinsed 3 times with distilled water and cut into fragments 1 to 2 mm long. These root fragments were used as endomycorrhizal inoculum. After 4 weeks of cultivation, the frequency and intensity of mycorrhization of barley roots were assessed using the method of Phillips and Hayman (1970). These mycorrhized roots were then used as inoculum for date palms.

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Inoculum of *Trichoderma asperellum*

A strain of *Trichoderma asperellum*, from the mycotheque of the Laboratoire des Productions Végétales, Animales et Agroindustrie, was used in this work. This strain has been tested in several works as a growth biostimulant or as a plant bioprotector against pathogens (Sghir et al., 2014; Qostal et al., 2020; El Kaissoumi et al., 2023). It is grown on PSA medium and incubated at 25 °C for seven days. The conidial suspension was prepared by washing the cultures and adjusted to 10^7 conidia/ml.

Mycorrhizal inoculation procedures

Inoculation of control plants with *F. oxysporum* – the roots of vitro-plants at the two-leaf stage were soaked for 30 minutes in Foa conidial suspension before being transplanted into Mamora forest soil.

Inoculation with mycorrhizae – mycorrhized barley roots were cut into 1 to 2 mm fragments, then incorporated into the growing medium (approx. 3 g per plant) around the roots of fusarium-damaged and/or treated seedlings prior to planting.

Inoculation with *T. asperellum*: The roots of vitro-plants inoculated with Fusarium were soaked for 30 minutes in the *T. asperellum* suspension.

Experimental device

The experiment was conducted under a randomized block design involving four treatments, each consisting of three replicates (pots)

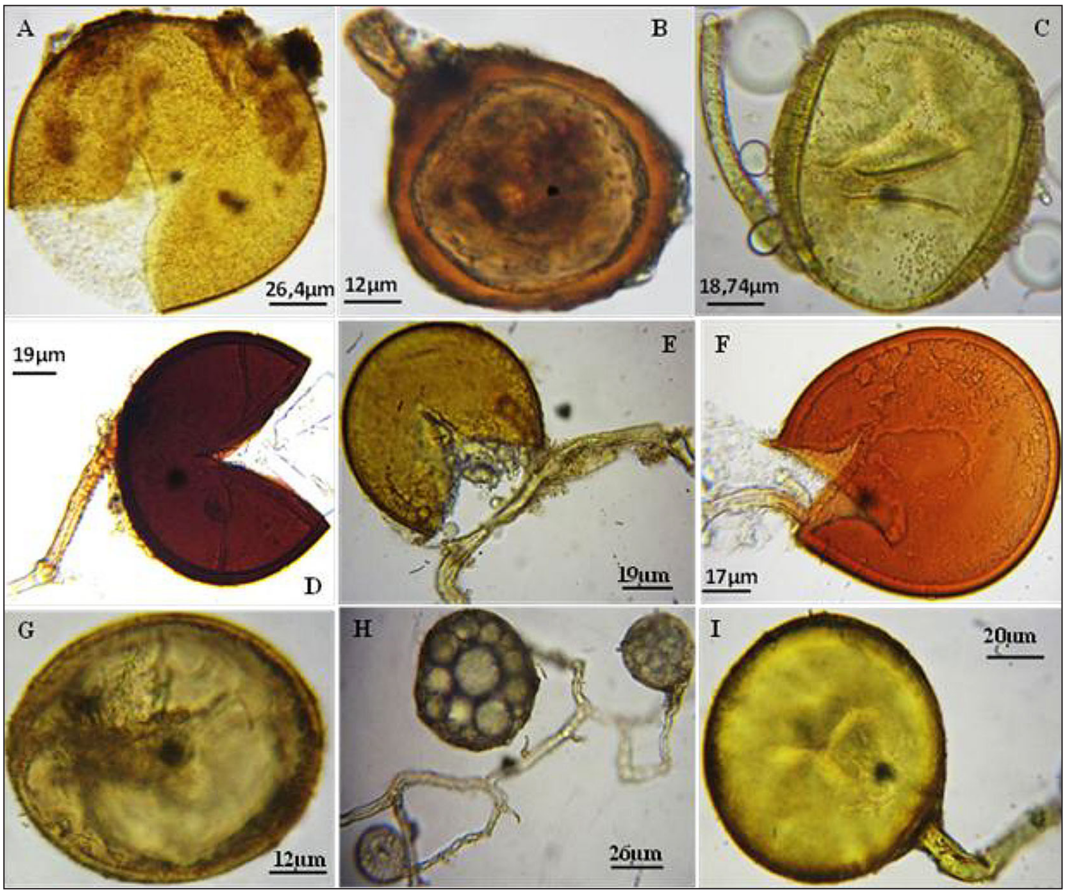

Figure 1. Arbuscular mycorrhizal fungi forming the composite inoculum, originating from the rhizosphere of *Ceratonia siliqua:* [A] *Glomus fecundisporum*, [B] *G. macrocarpum*, [C] *G. calospora*, [D] *G. deserticola*, [E] *G. fasciculatum*, [F] *G. aggregatum*, [G] *Acaulospora gedanensis*, [H] *G. clarum,* [I] *G. etunicatum* (Viterbo et al., 2002)

containing four date palm vitro-plants per replicate, for a total of 96 plants (2 varieties × 4 treatments × 3 replicates × 4 plants). The treatments were as follows:

- Lot 1 (Tf) – control plants inoculated only with *Fusarium oxysporum* f. sp. *albedinis* (Foa).
- Lot 2 (F + Ta) – plants inoculated with Foa and *Trichoderma asperellum*.
- Lot 3 (F + My) – plants inoculated with Foa and arbuscular mycorrhizal fungi (AMF).
- Lot 4 (F + My + Ta) – plants inoculated with Foa, *T. asperellum*, and AMF.

For the fungal inoculations, *F. oxysporum* suspension (10^6 conidia/mL, 10 mL per plant) was applied to the root zone one week after transplanting. The *T. asperellum* inoculum consisted of a 5 mL conidial suspension (10^7 spores/mL) applied directly to the substrate during planting. AMF inoculation was performed by incorporating 10 g of colonized root fragments (1–2 mm) into the peat–sand substrate (1:1, v/v) before transplanting.

All pots were maintained in a greenhouse under controlled conditions (18–25 °C, natural daylight) for seven months, and watered daily with tap water without additional fertilizers.

After the growth period, plants were carefully uprooted for morphological and physiological evaluation. Root and stem samples were subjected to fungal re-isolation assays on PDA medium to confirm the persistence or elimination of *F. oxysporum* within plant tissues. Mycorrhizal colonization was assessed microscopically after staining according to Phillips and Hayman (1970).

Evaluation of agronomic parameters

Growth parameters

After 7 months of cultivation, the roots of all date palm plants were washed with tap water and dried on absorbent paper overnight under ambient laboratory conditions. The following growth parameters were determined: plant height, number of leaves, shoot biomass, root biomass and stipe diameter.

Indices of stunting and leaf deterioration

The degree of leaf deterioration is graded according to the Douira et al. (1993) and Asrar et al. (2014). Modified scale of leaf appearance: 0 – healthy appearance, 1 – yellowing, 2 – wilting, 3 – necrosism, 4 – falling.

The scores, divided by the number of leaves, make up the leaf decay index (FDI), calculated according to the formula below. An average index is then calculated for each batch of plants.

$$IAF = \frac{[\sum(i \times x i)]}{6 \times NtF} \tag{1}$$

where: *IAF* – leaf deterioration index, *i* – leaf appearance scores 0–4, *xi* – number of leaves with rating *i*, *NtF* – total number of leaves.

The stipe size of all plants is measured at the end of the trials, and the stunting index, corresponding to the reduction in size of inoculated plants compared with fusarium-damaged control plants, is calculated according to the following relationship (Douira and Lahlou, 1989 modified):

$$IR = \frac{M - X}{M} \tag{2}$$

where: *IR* – stunting index, *X* – stipe size of inoculated plants, *M* – average size of fusarium-affected control. plants for each treatment.

Evaluation of mycorrhization parameters

Mycorrhization of date palm seedling roots

After seven months of cultivation, AMF colonization of date palm plant roots was carried out using the root staining technique of Phillips and Hayman (1970). Roots were removed from the substrate and washed with tap water. On the same day, the finest roots were cut into 1 cm-long fragments and heated in a 10% KOH solution with a few drops of hydrogen peroxide (H_2O_2) at 90 °C for 45 min, then rinsed with running water. The root fragments were then reheated in 0.05% cresyl dye at 90 °C for 15 min.

Randomly selected fragments were used for microscopic observation and calculation of mycorrhization parameters, i.e. mycorrhization frequency (%F), mycorrhization intensity (%M), arbuscular content (TA) and vesicular content (TV) according to the mycorrhization index of Trouvelot et al. (1986).

Extraction of spores

Spores were extracted using the wet sieving method described by Gerdemann and Nicolson (1963). In a one-liter beaker, 100 g of AMF-inoculated soil was immersed in 500 ml of tap water. The suspension was stirred for one minute with a spatula. After settling for 10–30 seconds, the supernatant was filtered through a four-layer sieve, decreasing the mesh size by 500, 200, 80 and 50 microns. This operation was repeated twice.

The filtrates obtained by sieving at 200, 80 and 50 microns were divided into two tubes each and centrifuged for 5 minutes at 2000 rpm. The supernatant was discarded and a viscosity gradient was created by adding 20 ml of a 40% sucrose solution to each centrifuge tube (Walker et al., 1982).

The mixture was returned to the centrifuge for 1 min at 3000 rpm and then filtered, but only through the 50-micron sieve. The resulting substrate was rinsed with distilled water to remove sucrose, then disinfected with streptomycin solution. The spores were then collected in an Erlenmeyer flask with a little distilled water. Spores density was calculated using filter paper and a binocular magnifying glass. Species identification was carried out by observing macroscopic and microscopic morphological characters and referring to the determination key by Schenk and Perez (1990).

Re-isolation of microorganisms

Roots and stipes from inoculated plants were disinfected, transplanted onto PSA medium and incubated to check for the presence of *T. asperellum* and *F. oxysporum*.

Statistical analysis

All experimental data were expressed as means ± standard deviation (SD). Before statistical comparisons, data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test.

For normally distributed data, one-way analysis of variance (ANOVA) was performed to assess the effect of treatments (*Tf*, *F+Ta*, *F+My*, *F+My+Ta*) on each growth or health parameter within each date palm variety. When ANOVA indicated significant differences ($p < 0.05$), Duncan's multiple range test was applied to separate means.

For variables that did not meet normality assumptions, a non-parametric Kruskal–Wallis test was used, followed by pairwise Mann–Whitney U tests with Bonferroni correction where applicable. In addition to statistical significance, biological relevance was considered when interpreting results. Differences were discussed not only based on *p*-values (threshold of significance set at $p < 0.05$), but also on effect magnitude (percentage variation from control) and consistency across replicates and varieties. Parameters showing moderate statistical significance but high biological impact (e.g., marked increases in biomass or pathogen suppression) were highlighted as biologically meaningful effects. All analyses were performed using SPSS version 25.0.

RESULTS

Statistical analyses revealed highly significant effects ($p < 0.05$ to $p < 0.001$) of the different treatments on all measured parameters, confirming that the observed variations were not due to random factors.

Disease severity indices

The stunting index (SI) and leaf deterioration index (LDI) showed very highly significant differences ($p < 0.001$) among treatments for both Najda and Bouzekri varieties. Compared with the Fusarium-inoculated control (Tf), treatments with T. asperellum (F+Ta), mycorrhizae (F+My), and especially the combined treatment (F+My+Ta) resulted in a sharp reduction of SI (from 48–56% to 6.5–7.9%).

Post-hoc Duncan's test indicated that the F+My+Ta group formed a distinct statistical subset ("group c"), significantly lower than all other treatments. These results confirm a strong synergistic interaction between *T. asperellum* and AMF in limiting wilt symptoms and restoring plant growth.

Growth parameters

Plant height and root length showed highly significant differences ($p < 0.01$), while shoot and root biomass exhibited extremely significant differences ($p < 0.001$) among treatments. In the Najda variety, the combined treatment (F+My+Ta) induced a 204% increase in plant height and a 323% increase in root length compared with controls, whereas Bouzekri recorded increases of 152% and 183%, respectively. These statistically significant differences also reflect a biologically meaningful improvement, associated with better nutrient uptake and enhanced root development.

The analysis of variance also revealed a significant treatment × variety interaction ($p = 0.032$), indicating that varietal response modulates the effectiveness of biological agents. Najda was more responsive to dual inoculation, while Bouzekri showed a steadier but moderate improvement.

Biomass accumulation

Shoot biomass differed significantly among treatments ($p < 0.001$). The mean value increased from 6.2 g in controls to 34 g under F+My+Ta for Najda, and from 4.3 g to 31.3 g for Bouzekri. According to Duncan's grouping, the following hierarchy was established: F+My+Ta > F+Ta ≈ F+My > Tf, confirming that the combined inoculation significantly outperformed single treatments.

Root biomass followed the same trend ($p < 0.01$), reflecting a harmonious and functional root system, characteristic of improved stress tolerance and reduced pathogen colonization.

Mycorrhizal colonization and pathogen re-isolation

Mycorrhizal colonization frequency was significantly influenced by treatment ($p < 0.05$). Maximum colonization rates were recorded in AMF-only treatments (95% for Najda, 80% for Bouzekri). Co-inoculation with *T. asperellum* slightly reduced these values ($p = 0.041$) without suppressing symbiosis, indicating good compatibility between the two microorganisms.

Re-isolation rates of *F. oxysporum* decreased sharply ($p < 0.001$) following biological treatments. The pathogen was completely eliminated from the stipes (0%) and reduced to 10–20% in roots under F+My+Ta, compared with 85–100% in untreated plants, demonstrating a strong fungistatic and antagonistic effect of the combined treatment.

Biological interpretation

Overall, all parameters showed statistically and biologically significant improvements under the combined F+My+Ta treatment. Beyond the p-values, these results represent functional biological effects, including enhanced growth, improved nutrient assimilation, strengthened root defenses, and reduced pathogen load. Thus, dual inoculation with *T. asperellum* and AMF constitutes an integrated and sustainable biocontrol approach to Bayoud disease, capable of improving date palm vigor and resilience under biotic stress.

Effect of *T. asperellum* and endomycorrhizae on agronomic parameters of fusarium-damaged date palm seedlings

Indices of stunting and leaf deterioration

Table 1 compares the stunting index and leaf impairment index (FAI) of date palm seedlings subjected to the different treatments. The results show significant differences between treatments, with a particularly marked effectiveness for the combined F+Ta+My treatment. Fusarium-affected control plants (Tf) showed the highest values, with an IR of 56% for the Najda variety (N) and 48% for the Bouzekri variety (B), as well as an IAF of 0.86% [N] and 0.91% [B], confirming their high susceptibility to weathering in the absence of treatment.

Application of the F+My and F+Ta treatments results in a significant reduction in indexes compared with the fusarium control. For the Najda variety, the RI rises to 12.1% with F+My and 11.98% with F+Ta, while for the Bouzekri variety, the values reach 12.55% and 10.03%, respectively. Similarly, IAF drops to 0.31% [N] and 0.29% [B] for F+My, and 0.21% [N] and 0.19% [B] for F+Ta, indicating a significant improvement in leaf health.

The combined F+Ta+My treatment stands out as the most effective, with significantly lower IRs: 6.5% for the Najda variety and 7.91% for the Bouzekri variety. The IAF are also the lowest 0.09% for [N] and 0.08% for [B], underlining a positive synergy between the different agents applied.

The F+Ta+My combination offers the best protection against stunting and leaf deterioration, followed by the F+Ta and F+My treatments. These results suggest that the combination of these agents potentiates their effect, particularly for the Najda variety, which shows a more marked response than the Bouzekri variety.

Plants inoculated with My+Ta stood out for their vigour, with a highly developed root system and strong suppression of symptoms linked to infection by *Fusarium oxysporum* f.sp. *albedinis* (Plate 1 and 2). These results underline the beneficial effect of the combination of mycorrhizae and T. asperellum in alleviating the symptoms of fusarium wilt in date palms and stimulating plant growth.

Two results in the same column, followed by the same letter, do not differ significantly at the 5% threshold according to the ANOVA test.

Growth parameters

Table 2 shows the average lengths of the aerial part (in cm) of date palm seedlings for two varieties (Najda and Bouzekri) according to the different treatments applied. The results show a significant improvement in the growth of treated plants compared with untreated control plants (Tf), with performance varying between treatments and varieties.

Untreated plants showed the lowest lengths, with 4.3 cm for the Najda [N] variety and 6.1 cm for Bouzekri [B], reflecting limited growth in the absence of treatment.

The application of F+Ta and F+My treatments induced a significant increase in growth. For the Najda variety, length reached 11.4 cm with F+Ta and 12.2 cm with F+My, while for Bouzekri, values were 7.9 cm [F+Ta] and 9.3 cm [F+My]. These results suggest that F+My is slightly more effective than F+Ta, particularly for the Najda variety.

The combined F+My+Ta treatment showed the best performance, with an average length of 13.3 cm for Najda, confirming a synergistic effect of the agents applied. For Bouzekri, the length remained stable at 9.3 cm, identical to that obtained with F+My alone, indicating that the addition of Ta brings no further improvement for this variety.

All treatments improved plant growth compared with the untreated control, with maximum effectiveness for F+My+Ta in Najda. The Bouzekri variety seems less responsive to combinations, reaching its optimum with F+My alone. These inter-varietal differences could guide the choice of treatments according to the cultivars used.

On the other hand, the data reveal significant variations in leaf development depending on the treatments applied and the varieties considered. The control treatment shows baseline values with 3 leaves for the Najda variety and only 2 for the

Table 1. Stunting index and leaf impairment index of date palm seedlings subjected to different treatments

Treatment	Stunting index (%)		Leaf deterioration index (%)	
Tf	56a (N)	48a (B)	0,86a (N)	0,91a (B)
F+My	12,1b (N)	12,55b (B]	0,31b (N)	0,29b (B)
F+Ta	11,98b (N)	10,03b (B]	0,21b (N)	0,19b (B)
F+Ta+My	6,5c (N)	7,91b (B)	0,09d (N)	0,08b (B)

Notes: Th – *T. asperellum*, My – Mycorhizes, F – *F. oxysporum* f sp. *albedinis*, N – Najda, B – Bouzekri.

Bouzekri variety, indicating weaker initial leaf growth in the latter.

Application of the individual treatments produced contrasting effects: the F+Ta treatment led to an unexpected reduction at 2 leaves for Najda (-33%), while it improved it for Bouzekri at 3 leaves (+50%). The F+My treatment maintains N at its initial level (3 leaves) while increasing B to the same level (3 leaves), suggesting differential efficacy between varieties.

The combined F+My+Ta treatment showed optimum performance, with record values of 5 leaves for N (+67% compared with the fusaried control) and 4 leaves for Bouzekri (+100%). This spectacular improvement, particularly marked for N, demonstrates a clear synergistic effect of the treatment components.

Data analysis reveals distinct leaf development dynamics between the two date palm varieties in response to different treatments. The Najda variety showed particular sensitivity to interventions, with a paradoxical response to the F+Ta treatment (33% decrease in leaf number) contrasting with an exceptional response to the combined F+My+Ta treatment (67% increase). This bipolarity suggests the existence of complex physiological mechanisms, where the Ta component may exert an inhibitory effect in isolation, but prove synergistic in combination.

Bouzekri showed a more linear progression, with a gradual improvement leading to a doubling in the number of leaves under combined treatment. Although less spectacular than for Najda, this sustained improvement indicates a different capacity for adaptation, possibly linked to more stable metabolic pathways or less sensitivity to individual component variations.

The systematic superiority of the F+My+Ta protocol for both cultivars (5 leaves for N, 4 for B) highlights a biologically significant synergy phenomenon. This observation argues in favor of a positive interaction between the different agents, creating a favorable environment for leaf development that transcends varietal specificities. Inter-cultivar differences could be explained by distinct assimilation capacities, differential sensitivities to active components, or intrinsic growth strategies. Table 3 shows significant variations in root and stem development of the two date palm varieties, depending on the treatments applied. Untreated plants show the lowest values, with root lengths of 7.2 cm for Najda and 10.0 cm for Bouzekri, and stipe diameters of 0.5 cm and 0.2 cm respectively.

Application of the treatments resulted in a marked improvement in these parameters. The F+Ta treatment already delivers a substantial increase in root length (19.3 cm for N and 21.2 cm for B), while F+My gives even better results (26.3 cm for N and 27.0 cm for B). The combined treatment F+My+Ta achieves optimum performance with 26.4 cm for N and 28.3 cm for Bouzekri.

With regard to stipe diameter, results varied from one cultivar to another. For Najda, all treatments show similar efficacy (1.1–1.4 cm). For Bouzekri, however, only the combined F+My+Ta treatment produced a significant improvement (1.2 cm vs. 0.3–0.7 cm for the other treatments).

Table 4 shows the significant impact of the different treatments on the biomass production of the two date palm varieties studied. Untreated plants (control) show the lowest biomass, with only 6.2 g (aerial) and 3 g (root) for Najda, compared with 4.3 g and 3.4 g for Bouzekri. Application of the treatments produced remarkable increases, particularly for F+Ta, which multiplied Najda's above-ground biomass by more than 5 (33.3 g) and Bouzekri's by almost 7.5 (32.2 g). The F+My+Ta treatment combines the best performances with 34–34.3 g of above-ground biomass and 25.4–27 g of root biomass.

Comparative analysis between varieties reveals differentiated behavior to treatments. While Bouzekri shows a better response to F+Ta for above-ground biomass, Najda generally presents slightly higher values. As regards root biomass, F+My gives particularly interesting results (24 g for Najda, 23 g for Bouzekri), although the combined F+My+Ta treatment achieves optimum

Table 2. Average length of aerial part (in cm) and average number of leaves of date palm plants in the two date palm varieties (Najda and Bouzekri) according to the different treatments applied

Treatment	Average length of aerial part [cm]		The average number of leaves	
Tf	4.3 (N)	6.1 (B)	3 (N)	2 (B)
F+Ta	11.4 (N)	7.9 (B)	2 (N)	3 (B)
F+My	12.2 (N)	9.3 (B)	3 (N)	3 (B)
F+My+Ta	13.3 (N)	9.3 (B)	5 (N)	4 (B)

Table 3. Average root length [in cm] and average stipe diameter of date palm seedlings for two varieties (Najda and Bouzekri) according to the different treatments applied

Treatment	Average root length [cm]		Average diameter of date palm stipes [in cm]	
Tf	7.2 [N]	10.0 [B]	0.5 [N]	0.2 [B]
F+Ta	19.3 [N]	21.2 [B]	1.4 [N]	0.7 [B]
F+My	26.3 [N]	27.0 [B]	1.1 [N]	0.3 [B]
F+My+Ta	26.4 [N]	28.3 [B]	1.3 [N]	1.2 [B]

performance. These observations underline the importance of adapting the choice of treatment to production objectives, with the F+My+Ta protocol appearing to be the most complete for harmonious development of the whole plant.

Reisolation of *T. asperellumet* and *F. oxysporum* f sp. albedinis

All tested plants showed the presence of *Trichoderma* in the lower parts of the plants, whereas the pathogenic fungus was detected only in specific tissues of the tested plants (Tf).

Table 5 shows the marked effectiveness of the various treatments in reducing the pathogens *F. oxysporum* f sp. *albedinis* (F) and *T. asperellum* (T) in the two date palm varieties. Untreated control plants showed high re-isolation rates (85–100% for F and 70–100% for T), confirming the high natural presence of these pathogens. Application of the treatments shows a spectacular reduction in these rates, particularly for the combined F+My+Ta treatment, which reduces the presence of F in the roots to 0–20% and completely eliminates (0%) the pathogen in the stipes. A similar trend was observed for *T. asperellum*, with significant reductions under the effect of the treatments.

Comparative analysis between varieties and plant tissues shows that Najda, N and Bouzekri, B respond similarly to treatments, although there are some minor variations (e.g. 10% F in Bouzekri roots versus 20% for Najda under F+My+Ta). Overall, stipes appear to be better protected than roots, with re-isolation rates frequently reduced to 0%. Among the individual treatments, F+Ta appears more effective against *F. oxysporum*, while F+My shows better action against *T. asperellum*. These results clearly demonstrate the effectiveness of treatments, and particularly of the F+My+Ta combination, in controlling these major date palm pathogens.

Evaluation of the effect of *T. asperellum*, mycorrhizae and *F. oxysporum* albedinis on the mycorrhization parameters of date palm plants

Analysis of the rhizosphere soil of various date palm plants inoculated with AMFs revealed the presence of endomycorrhizal species used as inoculum in the varieties tested. Species of the Glomus genus were the most dominant, with 60% in the rhizosphere of the Najda variety and 54% in the Bouzekri variety.

Microscopic analysis revealed complete root colonization by endomycorrhizal fungi in all inoculated plants. A variety of characteristic fungal structures were observed, including arbuscules, intracellular hyphae, lipid vesicles, spores and endophytes (Figure 2). These structures testify to the establishment of a functional symbiosis between the fungus and the host plant.

The impact of the various treatments on the symbiosis is clear from the results. Co-inoculation resulted in a significant but uniform reduction in shrub formation.

These observations highlight the predominant influence of inoculation type on symbiosis characteristics. The cultivated variety plays a secondary role in modulating this interaction. The study thus confirms the robustness of the mycorrhizal association, even in the presence of other microorganisms,

Table 4. Effect of different treatments on average above-ground biomass and average root biomass of date palm seedlings for two varieties (Najda and Bouzekri)

Treatment	Average above-ground biomass [g]		Average root biomass [g]	
Tf	6.2 [N]	4.3 [B]	3 [N]	3.4 [B]
F+Ta	35.3 [N]	33.2 [B]	26 [N]	22 [B]
F+My	18 [N]	13 [B]	27 [N]	23 [B]
F+My+Ta	34 [N]	31.3 [B]	23 [N]	19 [B]

Table 5. Average percentages of re-isolation of *F. oxysporum* f. sp. *albedinis* (F) and *T. asperellum* (T) from stipes and roots of date palm seedlings in Najda and Bouzekri varieties

Treatment	Agent	Variety			
		Najda		Bouzekri	
		Root	Stipe	Root	Stipe
Tf	F	100	90	100	85
F+Ta	F	40	0	45	0
F+My	F	50	10	46	12
F+My+Ta	F	20	0	10	0
Tf	T	100	70	90	90
F+Ta	T	22	0	20	0
F+My	T	46	7	45	20
F+My+Ta	T	35	0	25	0

while demonstrating their modulating effect on certain parameters of the symbiosis.

The mycorrhization frequency of fusarium-damaged date palm plants varied according to treatment, and to a lesser extent according to variety (Table 6). Maximum values for this parameter were recorded for plants inoculated solely with mycorrhizae, respectively 95% for the Najda variety and 80% for the Bouzekri variety. Co-inoculation of dishes with Trichoderma and mycorrhizae reduced the mycorrhization intensity of both varieties at similar mycorrhization frequencies.

Overall, the results demonstrate that combining *T. asperellum* with AMF provides integrated protection to date palm plants by both reducing pathogen development and enhancing plant vigor. The synergistic interaction promotes improved root architecture, biomass accumulation, and physiological resistance, highlighting the potential of this biocontrol strategy as an eco-sustainable alternative to chemical methods for managing Bayoud disease.

DISCUSSION

The use of endomycorrhizal fungi (AMF) is an effective approach for improving the tolerance of date palms (*Phoenix dactylifera L.*) to infections caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), the causal agent of Bayoud. In this work, the combined effect of *Trichoderma asperellum* and a composite endomycorrhizal inoculum on the growth of date palm seedlings showed a positive effect on all seedling growth parameters and on reducing the development of Fusarium head blight. A significant reduction in disease symptoms, such as stunting and leaf damage, was observed, as well as an improvement in growth parameters in plants treated and inoculated with F. oxysporum. In this sense Sghir et al. (2014) reported that double inoculation [endomycorrhizae + *Trichoderma harzianum*] had a significant effect on the growth of date palm plants and on the installation of Fusarium in date palm plants and the development of symptoms.

The results obtained are in line with those highlighting the role of AMFs in modulating soil microbial interactions and stimulating plant defense mechanisms against root pathogens (Smith, 2009; Asrar et al., 2014; Gianinazzi et al., 2010; Pozo and Azcón-Aguilar, 2007). According to these authors, plant inoculation and root colonization by endomycorrhizae confirm the positive impact of these fungi on plant resistance to pathogens. In particular, mycorrhizal inoculation induces the activation of molecular signaling pathways involved in root defense, thus promoting greater tolerance to fungal infections. Several studies have demonstrated that

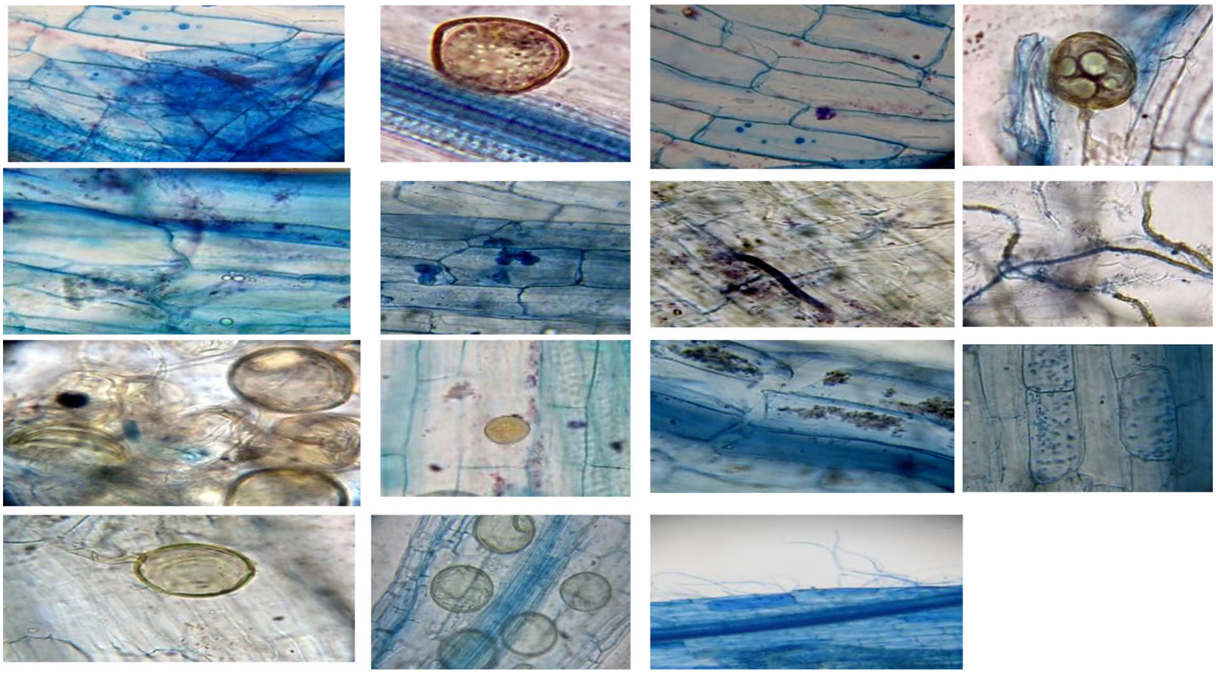

Figure 2. Structures of arbuscular mycorrhizal fungi in treated date palm seedlings

Table 6. Frequency of mycorrhization of date palm seedling roots in Najda and Bouzekri seedlings according to the different treatments tested

Treatment	Frequency of root mycorrhization [%]	
Tf	0.0 [N]	10.0 [B]
F+Ta	0.0 [N]	21.2 [B]
F+My	95 [N]	80 [B]
F+My+Ta	28.3 [N]	24.6 [B]

mycorrhization stimulates the expression of genes encoding defense proteins, notably PR (pathogenesis-related) proteins, thus enhancing plant protection mechanisms against root pathogens (Pozo and Azcón-Aguilar, 2007).

Endomycorrhizal fungi (EMF) don't just improve plant nutrition; they also play a key role in activating vitro-plant defense mechanisms, boosting resistance to root pathogens such as *Fusarium oxysporum* f.sp. *albedinis* (Foa). Mycorrhizal inoculation stimulates the production of defense enzymes, notably peroxidase and chitinase, which help strengthen cell walls and limit tissue invasion by pathogens. AMFs also modulate the chemical composition of the rhizosphere by inducing the production of antifungal metabolites, thereby reducing the infectious pressure exerted by soil-borne pathogens. These protective effects can be explained by complex interactions between AMFs and the soil microbiome, favoring the establishment of an environment less conducive to pathogen development (Pozo and Azcón-Aguilar, 2007; Asrar et al., 2014).

The interaction between endomycorrhizal fungi and *Trichoderma asperellum* is a promising approach for enhancing biocontrol of vitro-plants against *Fusarium oxysporum* f. sp. *albedinis* (Foa). This combination enhances plant protection through a dual mechanism: on the one hand, *T. asperellum* produces hydrolytic enzymes, such as chitinases and glucanases, which degrade the cell walls of pathogens, thus reducing their virulence; on the other hand, AMFs induce systemic immune responses in the plant, promoting the activation of defense pathways. Several studies have demonstrated that the joint application of these microorganisms results in a significant reduction in Fusarium head blight symptoms, while improving the growth and resistance of vitro-plants to biotic and abiotic stresses (Pozo and Azcón-Aguilar, 2007; Asrar et al., 2014).

Trichoderma, like arbuscular mycorrhizal fungi, are among the active species involved in the phosphate cycle. They are considered phosphate biosolubilizers (Kribel et al., 2019; Madiha and Allal, 2018; Azeddine et al., 2024; Madiha and Allal, 2018) and crop growth biostimulants (Sellal et al., 2020; Achajri et al., 2025). Trichoderma are also known to be a strong antagonist against various fungal pathogens (Azeddine et al., 2024), and their protective potential against these plant pathogens has been reported in the literature (Adnani et al., 2024). The results of this study showed that inoculation of plants of the two date palm varieties tested with *T. asperellum* stimulated growth parameters compared with the fusarium control. (Dubský et al., 2002).

This stimulation was reflected in an increase in plant length and fresh matter weight compared with the control. These results are in line with those of Mouria et al. (2007), who reported that inoculation of tomato plants with T. harzianum improved their growth parameters. Khaled et al., (2005) explained the stimulation of the development of a melon crop following the application of *T. harzianum* (Bal and Altintas, 2008) by the activation of the plant's defense system, an increase in chitinase and peroxidase activity and an increase in enzymatic activity in the leaves inducing systemic resistance in these plants. In contrast, Bal and Altintas (2008) reported no significant effect on the growth of lettuce plants inoculated with *T. harzianum*.

The work of Mouria et al. (2013) demonstrated the ability of certain strains of *T. harzianum* to stimulate the growth of tomato plants and suppress verticillium wilt, particularly with a strain isolated from compost. Most importantly, the inoculation of date palm plants with *T. asperellum* and AMFs produced a significant synergistic effect on plant growth parameters compared with other treatments, as evidenced by an increase in shoot length, number of leaves, shoot fresh weight, and stem diameter. This marked stimulation can be explained by the different modes of action of *Trichoderma* and AMFs and their interaction (Chliyeh et al., 2013). These findings are consistent with those of Calvet et al. (1992), who reported that *T. aureoviride* and *Glomus mosseae* have a synergistic effect on marigold growth. Likewise, a positive effect of dual inoculation between AMF and *Trichoderma aureoviride* Rifai has been observed in *Tagetes erecta* (Calvet et al., 1992), while improved growth was also reported in citrus reshni tanaka plants after inoculation with AMF (*Glomus* spp.) and *Trichoderma aureoviride* (Camprubi et al., 1995).

Additional evidence shows that double inoculation with a four-species mixture of *Glomus* spp. and *T. harzianum* also enhanced the growth of three balcony plant species (Verbena, Torenia, Diascia) (Sramek et al., 2000). Similarly, inoculation of cyclamen (*Cyclamen persicum* Mill) and *Euphorbia pulcherrima* Willd with a mixture of four *Glomus* spp. and *Trichoderma harzianum* had a significant effect on the growth and flowering of cyclamen, but only a minor effect on Euphorbia pulcherrima (Dubský et al., 2002). In our study, *T. asperellum* proved to be compatible with AMFs, showing no inhibitory effect on mycorrhizal plant growth. On the contrary, some studies have reported inhibition of mycorrhiza formation by volatile metabolites secreted by Trichoderma species, although others suggest that such inhibition may only be temporary (Schoebitz et al., 2013).

It has also been proposed that the ability of *Trichoderma* species to inhibit root colonization by AMFs depends on the order of inoculation; for example, reduced colonization by *G. mosseae* occurs when plants are inoculated with *Trichoderma koningii* before *Glomus mosseae*, or when *T. koningii* is introduced two weeks earlier. Conversely, no reduction in colonization occurs if Glomus mosseae is inoculated two weeks before *Trichoderma koningii* (McAllister et al., 1994). Overall, *Trichoderma* species may exert negligible effects, or they may stimulate root colonization by AMFs, as in the case of *T. harzianum* in maize (*Zea mays* L.) roots colonized by mycorrhizal species, or in the case of *G. mosseae* and *G. deserticola* (Vázquez et al., 2001). Likewise, *T. harzianum* had no significant effect on AMF colonization of soybean roots colonized by *G. mosseae*, whereas a positive effect was observed when plants were inoculated with *Trichoderma pseudokoningii* (Fracchia et al., 1998).

According to several authors, plants colonized by arbuscular mycorrhizal fungi also benefit from improved phosphorus uptake (Schoebitz et al., 2013; Boutasknit et al., 2020; Soufiani et al., 2023). In fact, fungal hyphae, through the production of various extracellular enzymes (phosphatase, phytase), are able to release phosphorus from complex organic and inorganic compounds in the soil (Plenchette et al., 2005; Gobat et al., 2003), before transferring it to the plant (Talbi et al., 2016; El Gabardi et al., 2021). In this way, the mobilized phosphorus is readily absorbed and transported through the hyphae to the arbuscules and subsequently to the host plant.

Furthermore, it has been reported that inoculation with AMF significantly stimulates the growth of date palm vitro-plants, which is an essential asset for preserving varietal authenticity while optimizing their development. Several studies highlight a marked increase in the number of leaves, stipe height, and root biomass in mycorrhized plants. This phenomenon is explained by the improved uptake of essential nutrients, particularly phosphorus and nitrogen (El Gabardi et al., 2019; Soufiani et al., 2023), promoted by the extension of the extraradical mycelial network of AMF and an increased tolerance to various stress factors (Boutasknit et al., 2020; Boutasknit et al., 2021; Soufiani et al., 2022). In addition, this mycorrhizal symbiosis plays a key role in enhancing plant water-use efficiency, thereby improving their adaptation to growing conditions. These findings confirm "the central role of AMF in promoting vigorous vitro-plant growth and their potential to improve yields in date palm groves (El Aymani et al., 2023).

It has been reported that AMF also enhance plant tolerance to abiotic stresses, which represents a crucial advantage for palms cultivated in arid environments (El Gabardi et al., 2021). According to our findings, mycorrhized seedlings display improved water uptake and optimized osmotic regulation, thereby mitigating the adverse effects of drought (Asrar et al., 2014). Moreover, studies on rice plants have shown that AMFs induce the production of heat shock proteins (HSPs), which stabilize cells under heat stress Jakobsen et al. (1992). In addition, a recent investigation on date palm seedlings revealed an increase in proline synthesis, reinforcing their resilience to salinity (Jansa et al., 2020).

It has also been demonstrated that mycorrhization alters the structure of the soil microbiome around plants and limits the proliferation of Foa. Our results confirm that inoculated soils harbor higher microbial diversity, generating competitive interactions that hinder pathogen development (Asrar et al., 2014, Singh et al., 2023). Furthermore, AMF-based inocula have been shown to protect plants against several diseases, including tomato verticillium wilt (Mouria et al., 2013; Madiha and Allal, 2018; Ruiz-Lozano, 2018), strawberry, olive (Ahmad et al., 2024, Singh et al.,2023), date palm fusarium wilt (Manzoor et al., 2024, Ahmad et al., 2024), and coffee (Vannier et al., 2019).

In addition,"the association of AMF with plants such as banana and tomato has been shown to promote the production of antimicrobial metabolites, offering a potentially applicable strategy for date palms against *Fusarium oxysporum* f.sp. *albedinis* (Foa) (Vannier et al., 2019).

It has been observed that the results obtained on controlled plant material open up promising prospects for improving the efficacy of mycorrhizal inoculations in palm groves (Pozo and Azcón-Aguilar., 2007; Gianinazzi et al., 2010). These findings encourage us to undertake more in-depth studies aimed at combating Bayoud disease, which has caused the disappearance of two-thirds of Moroccan palm groves (Fernandez, 1995). Indeed, the damage caused by Bayoud is not limited to the genetic erosion resulting from the loss of many of the best varieties, but also contributes to increasing desertification and the impoverishment of palm growers (Chen and Wu, 2021).

In light of these observations, the potential benefits of mycorrhization and *T. asperellum* on the growth of date palm seedlings were discussed. Thus, inoculation with AMF in combination with *T. asperellum* can be applied in biotechnology to optimize nutrient exploitation from substrates, leading to greater improvements in date palm growth.

It is also acknowledged that the use of AMF on vitro-plants represents a sustainable alternative to pesticides, preserving varietal authenticity while reducing environmental impacts. Our findings demonstrate that AMFs, whether used alone or in combination with Trichoderma asperellum, can effectively protect date palms against *Fusarium* head blight while maintaining their genetic integrity. These results highlight the importance of adopting an integrated approach to growth-promotion protocols, while stressing the need to adapt strategies to the genetic specificities of each variety.

CONCLUSIONS

This study successfully evaluated the effectiveness of a combined inoculation of *Trichoderma asperellum* and AMF against Bayoud disease in date palm, caused by *Fusarium oxysporum* f. sp. *albedinis*. The dual treatment significantly reduced disease symptoms, such as stunting and leaf deterioration, and decreased the presence of the pathogen. Simultaneously, it enhanced plant growth in the Najda and Bouzekri varieties. The novelty of this work lies in demonstrating a clear synergistic effect between the two microorganisms, showing that their combined application provides superior pathogen suppression and plant vigor compared to their use alone, thereby filling a critical knowledge gap in the biological control of this disease.

The findings present a promising sustainable agriculture strategy, particularly for arid and semi-arid regions. By leveraging two naturally occurring and environmentally safe microorganisms, this approach could be developed into biofertilizer or bioprotective formulations. Adopting such inoculants would reduce reliance on synthetic fungicides and chemical fertilizers, improve soil health, and increase plant resilience. This strategy is readily scalable through nursery inoculation programs or seedling pre-treatment, offering significant potential to enhance the productivity and economic sustainability of date palm plantations, which are vital to North African economies.

For the widespread adoption of this biocontrol consortium, future research should focus on validating these results through large-scale field trials across different genotypes and agroecological conditions. It is also crucial to optimize application protocols, assess the long-term persistence of the inoculated microbes, and investigate the molecular mechanisms behind their synergistic interaction. This study ultimately lays the groundwork for using this microbial consortium as a viable and eco-friendly alternative to manage soil-borne diseases and improve the health of date palm groves.

Acknowledgments

The authors would like to express their sincere gratitude to all contributors for their valuable efforts in the conception, execution, and writing of this work. Their collaboration and commitment were essential to the successful completion of this study.

REFERENCES

1. Abdellaoui, S., Aissami, A.E., Benkhemmar, O., Arahou, M., Touhami, A.O., Douira, A., n.d. Parasitic and pathogenic effects of *Fuarium oxysporum* f. sp. albedinis isolates on okra and collard plants. *Plant Cell Biotechnology and Molecular Biology*, 2020, 21 (5-6), 16-23.
2. Achajri . N et al., Effects of a composite endomycorrhizal inoculum on mycorrhization susceptibility and the growth of Iris germanica aromatic and medicinal plants. In: J. Mabrouki (Ed.) *Technical Innovation and Modeling in the Biological Sciences*, 2025, pp. 117–136.
3. Asrar, A.-W.A., Abdel-Fattah, G.M., Elhindi, K.M., Abdel-Salam, E.M., 2014. The impact of arbuscular mychorrhizal fungi in improving growth, flower yield and tolerance of kalanchoe (Kalanchoe blossfeldiana Poelin) plants grown in NaCl-stress conditions. *Journal of Food, Agriculture & Environment*, 12(1), 105-112.
4. Boutasknit, A., Baslam, M., Ait-El-Mokhtar, M., Anli, M., Ben-Laouane, R., Ait-Rahou, Y., Mitsui, T., Douira, A., El Modafar, C., Wahbi, S., Meddich, A., 2021. Assemblage of indigenous arbuscular mycorrhizal fungi and green waste compost enhance drought stress tolerance in carob (*Ceratonia siliqua* L.) trees. Sci. Rep. 11, 22835. https://doi.org/10.1038/s41598-021-02018-3
5. Camprubi A.. Calvet C., Estaún V. 1995. Growth enhancement of *Citrusreshni* after inoculation with *Glomus mossae and Trichoderma aureoviride* and associated effects on microbial populations and enzyme activity in potting mixes. *Plant and Soil*, 173, 233–238.
6. Calvet C., Barea J.M., Pera J. 1992. *In vitro* interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates. *Soil Biology & Biochemistry*, 24(8), 775-780.
7. Chen, X., & Wu, Y. (2021). Sustainable practices for palm growers: Enhancing productivity and environmental health. *Journal of Agricultural Sciences*, 159(3), 345-356.
8. Chliyeh, M. Ouazzani Touhami, A. FilaliMaltouf, A. El Modafar, C. Moukhli, A. Oukabli, A. Benkirane, R. Douira, A. Phytophthora palmivora: A new pathogen of olive trees in Morocco. Atlas Journal of Biology, 2013, 2(2): 130–135.
9. Douira A., El Oirdi M., Benkirane R., KheÛabi N. et Elhaloui N. E. (1993). Etude de I'efTcacité in vitro de quefques fbngicides sur la croissance de quelques souches marocaines de Pyricularia oryzae. Joutnéei nationales àe protection des plantes, Rabat, l-2 Décembre, p: 33.'
10. El Gabardi, S., Mouden, N., Chliyeh, M., Selmaoui, K., Ouazzani Touhami, A., Filali-Maltouf, A., Ibnsouda Koraichi, S., Amir, S., Benkirane, R., El Modafar, C., Douira, A., 2021. Effect of phospho-compost and phosphate laundered sludge combined or not with endomycorrhizal inoculum on the growth and yield of tomato plants under greenhouse conditions. *Acta Biol. Szeged*. 64, 221–232. https://doi.org/10.14232/abs.2020.2.221-232
11. El Gabardi S., Chliyeh M., Mouden N. et al., 2019. Study of the effect of different phospho-compost doses on the growth and development of bean plants. *Plant Cell Biotechnology and Molecular Biology*, 20(15–16), 710–725.
12. Fernandez, D. 1995. Étude génétique et pathologique de *Fusarium oxysporum* f. sp. albedinis [Thèse de doctorat, Université Montpellier II].
13. Fracchia S., Mujica M.T., García-Romera I., García-Garrido J.M., Martín J., Ocampo J.A., Godeas A. 1998. Interactions between *Glomus mosseae* and *Arbuscular mycorrhizal* sporocarpassociated saprophytic fungi. *Plant Soil*, 200, 131–137.
14. Gerdemann, J.W and Nicolson, T.H. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, 46, 235-244. http://dx.doi.org/10.1016/S0007-1536(63)80079-0
15. Gianinazzi, S., Gollotte, A., Binet, M.-N., Van Tuinen, D., Redecker, D., Wipf, D., 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza*, 20, 519–530. https://doi.org/10.1007/s00572-010-0333-3

16. Gobat, J.-M., Aragno, M., & Matthey, W. 2003. *Le sol vivant: Bases de pédologie, biologie des sols.* Presses Polytechniques et Universitaires Romandes (PPUR).
17. Hamidi, O., Talbi, Z., Chliyeh, M., Touhami, A., Selmaoui, K., Benkirane, R., Douira, A., 2017. Effect of endomycorrhizal inoculation on the young cork oak plants (*Quercus suber*) *Growth. Annu. Res. Rev. Biol.,* 13, 1–11. https://doi.org/10.9734/ARRB/2017/33873
18. Jakobsen I, Abbott LK, Robson AD. 1992a. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifotium subterraneum*. I. Spread of hyphae and phosphorus inflow into roots. *Netv Phytologist*, 120, 371-380.
19. Jansa, J., Bukovská, P., & Gryndler, M. 2020. Mycorrhizal hyphae as ecological niches – nutrient transport and interactions with soil biota. *Frontiers in Plant Science*, 11, 536.
20. Khaled H., Daami-Remadi, M., Khiar, H., & El Mahjoub, M. 2005. Effet inhibiteur in vitro et in vivo du Trichoderma harzianum sur *Fusarium oxysporum* f. sp. radicis-lycopersici. *Biotechnologie, Agronomie, Société et Environnement*, 9(3), 163–171
21. Khattabi, N., Ezzahiri, B., Louali, L., Oihabi, A., 2004. Antagonistic activity of Trichoderma isolates against *Sclerotium rolfsii*: Screening of efficient isolates from Morocco soils for biological control. In: Phytopathologia mediterranea, Firenze University Press.
22. Madiha, B., Allal, D., 2018. The effect of an arbuscular mycorrhizal inoculum on the growth and fruiting of *Fragaria ananassa* (Weston) Duchesne Ex Rozier (Rosaceae) in Morocco. *Annu. Res. Rev. Biol.* 29, 1https://doi.org/10.9734/ARRB/2018/44956.
23. Manzoor, M., et al. (2024). Fusarium wilt of date palm: Recent advances in understanding and management. *Journal of Plant Pathology*, 106(1), 1-10.
24. Martínez-Medina A., Pascual J.A., Lloret E., Roldan A. 2009. Interactions between arbuscular mycorrhizal fungi and *Trichoderma harzianum* and their effects on Fusarium wilt in melon plants grown in seedling nurseries. *Journal of the Science of Food and Agriculture*, 89|(11), 1843-1850.
25. McAllister C.B., García-Romera I., Godeas A. and Ocampo J.A. 1994. Interaction between Trichoderma koningii, *Fusarium solani* and *Glomus mosseae*: Effect on plant growth, arbuscular mycorrhizas and the saprophytic population. *Soil Biol. Biochem*. 26, 1363–1367.
26. Mouria et al., 2013. Mise en évidence d'une variation intraspecific chez B. cinerea et lutte biologique in vitro par l'extrait de compost. *Journal of Applied Biosciences* 64: 4797–4812.
27. Mouria et al., 2007. Effect of trichoderma strains on the growth of tomato plants in greenhouses and their aptitude to colonize roots substrate. *Phytoprotection*, 88(3), 103–110.
28. Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55, 158-IN18. https://doi.org/10.1016/S0007-1536(70)80110-3
29. Plenchette, C., Clermont-Dauphin C., Meynard J.M., Fortin J.A., 2005. Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal of Plant Science*, 85(1):31-40. https://doi.org/10.4141/P03-159.
30. Pozo, M. J., & Azcón-Aguilar, C. 2007. Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology,* 10(4), 393-398.
31. Qostal et al., 2020. Management of wheat and barley root rot through seed treatment with biopesticides and fungicides. *Plant Cell Biotechnol. Mol. Biol.*, 21(36), 129–143.
32. Qostal et al., 2020. Biostimulant effect of trichoderma on the development of wheat and barley plants and its survival aptitudes on the roots. *Plants Archives*, 20(2), 7829-7834.
33. Ruiz-Lozano, J.M., Aroca, R. 2018. Molecular and physiological responses of mycorrhizal plants to abiotic stress. *Environmental and Experimental Botany*, 155, 313-325.
34. Singh, A., Kumar, V., Sharma, S. 2023. Heat shock proteins induced by CAM enhance thermotolerance in rice vitro-plants. *Plant Physiology and Biochemistr*y, 184, 56-65.
35. Schenck, N.C., Perez, Y. 1990. A manual for identification of vesicular arbuscular mycorrhizal fungi. INVAM, 3rd Ed. Gainsville, Florida: University of Florida, 1990.
36. Sellal et al., 2020. Effect of seeds treatment with Trichoderma harzianum on argan plants growth. *Plant Cell Biotechnol. Mol. Biol.*, 21(11-12), 69-77.
37. Smail, A., Aicha, E., Omar, B., Touhami, A., Benkirane, R., Douira, A., 2017. Study of the differentiation of fusarium oxysporum f.sp. albedinis chlamydospores on different culture media. *Annu. Res. Rev. Biol.* 18, 1–9. https://doi.org/10.9734/ARRB/2017/35653
38. Soufiani et al. 2022. Effectiveness of indigenous arbuscular mycorrhizal consortium on the growth and mineral nutrition of *Argania spinosa* (L.) skeels. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 156(6), 1365-1372.
39. Sramek, F., Dubsky, M., Vosartka, M., 2000. Effect of arbuscular mycorrhizal fungi and Trichoderma harzianum on three species of balcony plants. *Rostlinná Výroba*, 46(3), 127-131.
40. Toutain, 1967. Mission Bayoud en Tunisie. D.R.A.M., 17 p.
41. Trouvelot, A., Kough, J.L. et Gianinazzi-Pearson, V. 1986. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation et de signification fonctionnelle. In: Gianinazzi-Pearson V. and Gianinazzi, S., (Eds), Physiological and Genetical Aspects of Mycorhizae, INRA, Paris, 217-221.
42. Viterbo, A., Ramot, O., Chernin, L., Chet, I., 2002. Significance of lytic enzymes from Trichoderma spp. in the biocontrol of fungal plant pathogens. *Antonie Van Leeuwenhoek* 81, 549–556. https://doi.org/10.1023/A:1020553421740.
43. Walker, H.G.; Kohler, G.O.; Garrett, W.N., 1982. Comparative feeding value of alfalfa press cake residues after mechanical extraction of protein. *J. Anim. Sci.*, 55(3), 498-504.