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Ability of arbuscular mycorrhizal to protect tomato (*Solanum lycopersicum*) seedlings from *Fusarium oxysporum*

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ABSTRACT

Present experiments were conducted in the microbiology laboratory, Department of Environmental and Agronomic Sciences and in a private nursery in the Tassoust region of Jijel during 2021 and 2022. This study was carried out on the use of strains of arbuscular mycorhizal fungi (*Acaulospora* sp. and *Glomus* sp.) as a means to control *F. oxysporum* effect. After 15 days of inoculation, several measurements of the lengths roots, stem and leaves were noted and symptoms of *Fusarium* disease were also recorded. The results revealed that *F. oxysporum* is a pathogen for tomato (*Solanum lycopersicum* L.) plants (super strain) variety, causing *Fusarium* disease of this host, which manifests by yellowing, wilting and necrosis of aerial parts, and reduced growth parameters in plants. Therefore, the disease incidence after 15 days of inoculation is estimated at 75 and 50% for (T_1 and T_3) respectively. In addition, our results revealed that the percentage of incidence was 100 and 25% respectively for T_1 and T_3 after 22 days. Whereas, the results showed that mycorhizal fungi are associated with the roots of tomato plants. They reduced the incidence rate of *Fusarium* disease by 50% (T_3) and improved the growth of tomato seedlings (Super strain) which manifests itself by a vigorous root system and a very important development of the aerial parts. These results indicate that arbuscular mycorhizal fungi can effectively contribute to the ecological management of soil-borne fungal disease.

Keywords: Biological control, *Fusarium* disease, Growth, Incidence, Wilting

Tomato (*Solanum lycopersicum* L.) historically went from being a merely ornamental plant to being one of the most important vegetables today in terms of consumption and production (Aydi-Ben-Abdallah *et al.* 2020), as well as, is a model organism to genetic and molecular studies related with plant defense response. Low yield of tomato is attributed to its susceptibility to several pathogenic fungi, bacteria etc. which are major constraints to tomato cultivation such as *Fusarium* wilt and damping off. *Fusarium oxysporum* is an important, soil-inhabiting ubiquitous fungus, known for its phylogenetic diversity (Nicholas *et al.* 2017, Xiong and Zhan 2018). *Fusarium oxysporum* is a worldwide spread and phylogenetically diverse species and is considered as the most frequent species causing wilts, as well as crown and root rot, in different crops. *F. oxysporum* is a soil-borne fungus that infects plants via the roots and colonizes the xylem vessels. *F. oxysporum* infection causes wilt symptoms.

Biological control, based on the use of natural antagonistic organisms against plant pathogens, could be a promising solution. This preventive strategy consists in stimulating the natural defenses of plants by mycorrhization. In Algeria, the study of the biodiversity of mycorrhizal fungi remains poor in relation of microbial ecology. Moreover, the relative agronomic value of native isolates is unknown. Despite the ubiquity of arbuscular mycorrhizal (AM) symbioses in our natural environment; they remain insufficiently used in modern agriculture. AM fungi is reputed to control a number of plant diseases, especially soil-borne diseases (Kabdwal *et al.* 2019) and it is known that they have an impact on plant community structure and diversity by altering inter or intra-specific competitive situations (Singh *et al.* 2020). The symbiotic group of plants with AM fungi favour water and mineral nutrition and decrease abiotic and biotic stresses. It is in this context that the present study aims to identify an effective biological control method that is an alternative that raises more hope in the fight against soil pathogens.

MATERIALS AND METHODS

The experiments were conducted in two stages; part one of the experiments was conducted in the microbiology laboratory, Department of Environmental and Agricultural Sciences and the second was carried out in a private nursery in the Tassoust region of Jijel 2021 and 2022.

Plant materials: Seeds of tomato variety Super strain (Agreto vegetable seeds) were obtained from seed distributors. Seeds were surface sterilized and washed thrice

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with sterile distilled water as per Benhamou *et al.* (1997). The first treatment contained only (positive control (T_0), while the second treatment contained only *Fusarium oxysporum* (negative control (T_1), the third treatment contained only *Arbuscular mycorrhizal* fungi (AMF) (T_2) and the last one contained the test (T_3, treatment with AMF and inoculated by *F. oxysporum*).

The arbuscular mycorrhizal fungi consists of two strains: strain 1 (*Glomus* sp.) and strain 2 (*Acaulospora* sp.) extracted from soil of the agricultural region of Bouhamdoune, identified at quality control laboratory, University of Jijel. According to Haro (2011), the inoculum multiplication is obtained after two months (a mixture of spores, mycorrhized roots fragments of *Zea mays* and soil.

Pathogen: The isolate of *F. oxysporum* was obtained on the basis of an isolation made of the palms of the palm tree. Date palm shows symptoms of *Fusarium* disease, palms which are taken from Oumeche region. Biskra. The identification of the pathogen was carried out microscopically, according to the characteristics of macroconidia, phialides and chlamydospores (Nelson *et al.* 1983).

Concentration conidia were evaluated using a Thoma chamber, the concentration was adjusted to 1×10^6 conidia/ml. Before inoculation, the roots were slightly severed (wounded) by inserting a needle, 1 cm away from the stem. Root severing was done to ensure pathogen penetration through roots. Observations were recorded on wilt symptoms for up to two weeks.

Determination of disease incidence: The disease incidence was recorded on a scale of 0–4 referring to the degree of wilt as reported by Song *et al.* (2004) where scale zero refers to healthy plant without any wilt symptoms. On the other hand, scale four refers to complete wilted plants. The scale 1- slight yellowing, slight rot of the pivot and secondary roots and crown rot 1–20%; scale 2- significant yellowing of leaves with or without wilting, stunting of plants 21–40%; scale 3- significant yellowing of leaves with or without wilting, stunting of plants, crown rot and browning of stem vessels 41–60%; scale 4- is when all leaves become yellow as an indication of complete infection (plant mortality). Disease incidence is a parameter which includes number of disease plants divided by total number of plants considered for observation, disease intensity.

$$\text{Disease incidence (\%)} = \frac{\text{n scale} \times \text{number of plants infected}}{\text{Highest scale} \times \text{total number of plants}}$$

The importance of mycorhization is understood using the following parameters (Giovannetti and Gianinazz-Pearson 1994):

$$\text{Frequency of mycorhization (F\%)} = (\text{number of mycorrhizal fragments/total number}) \times 100$$

$$\text{Overall intensity of mycorhization (I\%)} = (95n5+70n4 + 30n3 + 5n2 + n1)/\text{total number of fragments}$$

n5, n4, n3, n2 and n1: represent the number of mycorrhizal fragments denoted respectively 5,4,3,2 and 1.

Measurement of growth parameters of tomato plants (Super strain): Different growth parameters were measured (size, number leaves and leaflets). These measurements were made on one tomato plant per pot and four pots by condition (healthy negative control and with AMF) (Caron *et al.* 2002). The size was measured from the crown to the apex of the last leaf of the main strand. As well as measuring the length of the roots and leaves and their width (cm). The dry and fresh weights (g) of the aerial parts and roots of each pot were weighed before and after drying in the oven at 60°C for 48 h and 4 days. The dry matter percentage for the aerial and root systems was calculated as:

$$\text{Per cent dry matter} = 100 - [\text{fresh weight (g)–dry weight (g)/fresh weight (g)}] \times 100$$

Statistical analysis: The data collected were subjected to statistical analysis by analysis of variance method (ANOVA) using the general linear model (GLM) procedure of the R4.1.2 software version 9.1. The pair comparisons were done by Turkey Multiple Range Test. ANOVA is suitable to complete the experiment in the field. Significant differences among treatments were based on the F-test in ANOVA and treatment means were compared using the least significant difference at ($P<0.05$).

RESULTS AND DISCUSSION

Development of disease after 15 days of inoculation: The main objective of this study is to evaluate the AMF effect on the growth parameters of tomato seedlings (Super stain) variety and their power to control *F. oxysporum*. So, our results come close to Agrios (2005), depending on the plant, the symptoms are very variable. They are manifested by a thinning of the vein, necrosis and chlorosis of the leaves followed by their drying out. In a severe attack, most plants wither and die quickly, while the least affected plants become stunted and unproductive. It should be noted

Table 1 Symptoms that appear on plants after 15 days of infection with *F. oxysporum* (original)

Inoculation	Symptom
T_0	Normal size and green colour.
T_1	Plants are low weight (g) with a small stem size with a dark red colour at the collar. The 1st symptom of *Fusarium* disease is manifested by yellowing of the lower leaves, it has been revealed that their surfaces are reduced (Comprising 3 plants per pot showed the symptoms).
T_2	Plants are well developed with normal stems and leaves are dark green.
T_3	Stage of 7 leaves, the stem has a medium size (cm), the leaf surface is normal; the symptoms appear first on the lower leaves which manifest as dark brown necrosis spots, the central veins are red in colour and the formation of weedy roots is distinguished.

Treatment details are given under Materials and Methods.

that, the tomato seedlings (Super strain) inoculated with *F. oxysporum* showed a difficult start (T_1). It therefore seems that *Fusarium oxysporum* can be considered as the main factor of the disturbances recorded in tomato seedlings. In addition, disease progression was faster and stronger at (T_1) compared to the other treatment (T_3) which had a low progression of disease. The negative control comprising seedlings previously soaked in sterile distilled water, showed no symptoms of disease on any part of the plants, which are therefore intact (Table 1).

The results obtained confirmed the pathogenicity of *F. oxysporum* which produces discoloration at the level of leaves and crown of tomato seedlings (Super strain). However, the disease incidence was significantly affected by inoculation. When comparing inoculated plants with *F. oxysporum* and the other treatments scored a significantly higher disease incidence. Similarly, Nelson *et al.* (1983) described the structural events in the infected plant correspond to the primary symptoms (root and crown rot), and will be responsible for secondary symptoms of wilting of the aerial parts of the plant. In addition, the results obtained revealed that the incidence of (T_1) disease is 75% higher after 15 days of inoculation. This incidence increases over time because the disease gradually progressing reached 100% after 37 days of inoculation. Some severely affected seedlings (T_3) showed root proliferation adventives; due to a defense mechanism put in place by the plant to replace its damaged root system and ensure its water supply and mineral salts (Fig 1). These results coincide with that observed by Hashem *et al.* (2021) who showed the role of arbuscular mycorrhizal fungi (AMF) against *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in tomatoes and explored in an inducing plant systemic defense. After inoculation by AMF, they revealed that AMF reduced the wilt disease within vascular tissue and *in vivo* production of fusaric acid was observed which may be responsible for reduced wilting.

While, measurements of growth parameters (length, width, fresh and dry weight, number of leaves and leaflets) show differences in plants response to *F. oxysporum* inoculum evidently when the effect on height and weight is examined. Statistical data showed that the stem, roots and leaves length significantly decreased in *Fusarium*-infected plants (T1) than the uninfected ones (healthy plants). While, the control plants have the highest growth and the width of the leaves with a number of leaves and leaflets (Fig 2). The average of stem, root and leaf lengths between treatments have significant differences because the calculated probability F is less than 0.05. It points out that the number of average leaves and leaflets between treatments does not have a significant difference because the calculated probability F is greater than 0.05. Supplementary Fig 1 shows the effects of *F. oxysporum* on fresh and dry root biomass after 5% ANOVA analysis. On the other hand, fresh and dry roots biomasses between treatments (T_1, T_0 and T_3) have a very highly significant difference because the calculated probability F is less than 0. 05. While, the biomasses of the fresh and dry aerial parts have highly significant differences because the calculated probability F is less than 5%. However, the treatments were differentiated into three groups, the first of which is represented by the T_1 treatment which has the lowest biomass, the second consists of the treatments T_0 and T_2 which are homogeneous, and the third is represented by the treatment T_3 which has the largest biomass. Braz *et al.* (2011) stated that the inoculation of tomato plants with mycorrhiza was found to be responsible for higher growth. Significantly higher shoot length, fresh weight and dry weight were recorded in mycorrhiza inoculated pots.

Estimation of the growth parameters of tomato plants (Super strain) mycorrhizae: The morphological growth parameters (the length of the roots, stems, leaf length and width, number of leaves and leaflets, wet weight and dryness of the roots and aerial parts) were significantly ($P<0.05$) higher in AMF inoculated (T_3) in comparison to control (T_0). Fig 3 shows the effects of treatments on the length of (stem, root, leaves and leaf width) per treatment obtained after a 5% threshold variance analysis. The length and width between treatments have very significant differences because the calculated probability is less than 0.05. However, the results obtained show a significant difference between the morphological aspects of the treatments. While, we noted in contrast an increase in roots and aerial parts fresh and dry weights was observed upon inoculation with AMF (Supplementary Fig 2). The same results are obtained by Shukla *et al.* (2015), who showed the interactions between

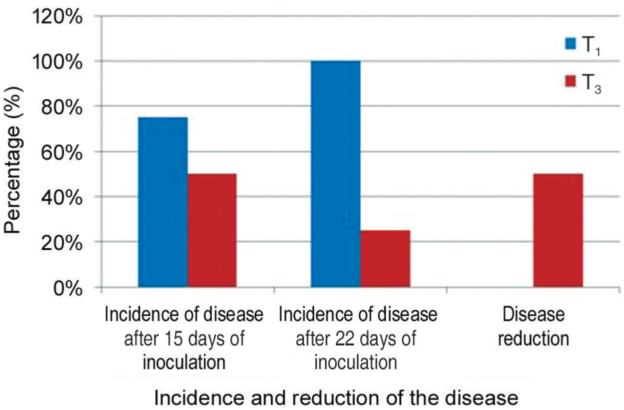

Fig 1 Incidence and reduction of the disease (original).

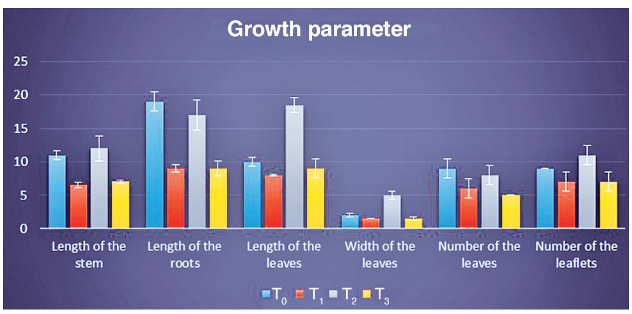

Fig 2 Growth parameters of tomato seedlings after 15 days of inoculation (original).

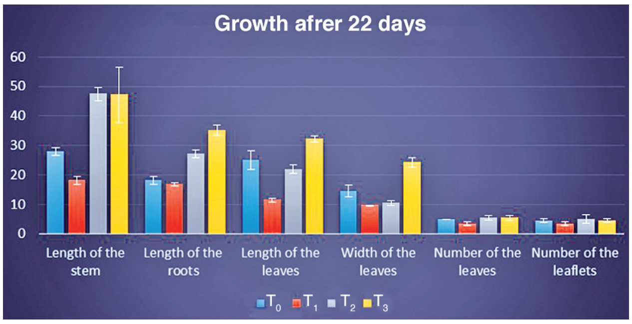

Fig 3 Tomato seedlings growth (length, width (and number) after 22 days of treatment (original).

arbuscular mycorrhizae and *Fusarium oxysporum* f. sp. *Ciceris* when the mycorrhizal suppresses wilt disease in *Cicer arietinum* L. and help the seedlings to improve their growth. According to Azcon and Barea (1996), AMF can intervene in the rhizosphere or in the root tissues to protect the roots against phytopathogens. Similarly, our study showed the efficacy of mycorrhizal fungi that manifest themselves by vigorous roots and width surface of the leaves, which demonstrate the resistance of tomato seedlings against *F. oxysporum* compared to healthy control. On other hand, Mishra *et al.* (2015) proved that interaction between plant-microbe lead to enhanced protection of plants against phytopathogenic agents. According to the results of statistical analysis, T_3 plants behave better in the face of *Fusarium* disease because they do not exhibit any apparent symptoms of *F. oxysporum* or have mild symptoms, compared to T_1 plants. In addition, aerial plant biomass is significantly higher in the presence of AMF unlike inoculated plants by *F. oxysporum*. Moreover, the roots biomass is significantly lower in plants inoculated (T_1) than in plants treated by arbuscular mycorrhizal. It would therefore be useful to use more effective mycorrhizal strains including ability to reduce the reproduction of *Fusarium* species.

Estimation of frequency and intensity of mycorhization: The frequency of mycorhization (F) is found to be 68.75%. When the intensity of colonization of the root cortex (I) is low, it is 26.75%. This is due to the short lifespan of the arbuscular fungi (one to three weeks) which then degenerate to be replaced by the hyphae and vesicles which persist until the death of the cell (Jain and Pundir 2019). The results revealed an effect of the sampling date for the frequency of root colonization. While, the highest mycorhization frequency was observed from the month. In addition, high mycorrhizal colonization was observed; manifested by the presence of hyphae, spores and shrubs in the roots of seedlings. This colonization stimulates the height, width, biomass of tomato seedlings and increases the number of leaves and leaflets per plant. Similar results were found by Hadou *et al.* (2020) which shows that mycorrhizal inoculation improves the biomass of *Mucuna pruriens* plants.

The purpose of this study is to contribute to the improvement of tomato production through the use of mycorrhizal fungi. It is aimed to evaluate the effects of AMF strains for the control of *F. oxysporum* on tomato on field trial. It has been noted that the incidence of the disease before the treatment is 50% and after the treatment is 25%.

Overall, it may be said that *F. oxysporum* is responsible for the *Fusarium* wilt. In addition, the results obtained showed the effect of this pathogen on the inoculated tomato plants, which resulted in reduced growth of the plants tested as well as low weight in different parts of plants. However, to be more affirmative, it would be more interesting to test this biocontrol agent in field to see their potential use as a control strategy against *F. oxysporum*. In addition, it is more advantageous to use AMF and to avoid those synthetic products which will pose harmful problems for the environment and human and animal health.

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