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Research Article
Arbuscular Mycorrhizal Symbiosis Decreases Sclerotinia Caused by *Sclerotium rolfsii* Sacc. in Tomato (*Solanum lycopersicum* L.)

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Abstract

Background and Objective: Tomato (*Solanum lycopersicum* L.) domestic production is largely below requirements in Côte d'Ivoire because of numerous abiotic and biotic constraints, especially sclerotinia caused by *Sclerotium rolfsii*. The present study was initiated to test the antifungal activity of a complex consisting of propagules of 6 different species of arbuscular mycorrhizal fungi (AMF) on *S. rolfsii*. **Materials and Methods:** Inoculations with *S. rolfsii* sclerotia were performed. Green house tomato plants of the 45 day old Lindo F1 variety were transplanted into pots of 297 cm^3 volume. The impact of inoculation was assessed at planting and at the end of the experiment on plant height growth, collar diameter, number of functional leaves and number of flowers. **Results:** The results revealed that the mycorrhization of nursery plants with the CMAs used has antifungal action on *S. rolfsii*. Mycorrhization had a beneficial effect on growth, especially in the early phase, when mycorrhizal plants appeared to be better developed than non-mycorrhizal plants. The incidence of dry rot in non-mycorrhizal plants is 2.5 times higher than the incidence of mycorrhizal plants. **Conclusion:** Mycorrhization may be advisable for growers in tomato growing areas where sclerotinia is more prevalent as an alternative to the over use of synthetic fungicides.

Key words: Tomato, arbuscular mycorrhizal fungus, sclerotinia, antifungal, *Sclerotium rolfsii*, propagules, incidence

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), native to the Andes in south America, would have been domesticated and cultivated for consumption[1]. Today it represents one of the most profitable vegetable crops[2]. Indeed, its importance as food and the numerous therapeutic virtues make it, the first vegetable-fruit in the world. In Côte d'Ivoire, it is the most consumed vegetable because cooked in all sauces and all forms of raw. But the sector is mostly held by women in the production areas. The national yearly production between[3,4] 22,000 and 35,000 t is supplemented by a very high import to satisfy one third of the demand[5]. While at the global level the average yield of this crop is about[6] 25 t ha^{-1}, in sub-saharan Africa it is only[7] 10 t ha^{-1}. Growing tomato remains difficult given its sensitivity to various biotic stresses (pests, diseases, weeds) and abiotic (drought).

Among the different biotic constraints, root dry rot, caused by *Sclerotium rolfsii*, is one of the most restrictive fungal diseases to Solanaceae culture in Côte d'Ivoire[8,9]. The fight against this constraint is mainly carried out by the use of synthetic pesticides or the use of prophylactic measures. The potential dangers to the environment and to humans, linked in particular to the bioaccumulation of these synthetic pesticides, motivate to be more agro-ecosystem friendly.

To limit the use of these hazardous products, the use of antagonistic biological agents of the micro-organisms responsible for this damage is increasingly underway[10,11] with more or less success. The mycorrhizal symbiosis, especially that of arbuscular, whose beneficial effects on growth and stress tolerance of the majority of economically important plants are allowed[12], proposes a pathway of exploration and could participate in the determination of credible alternatives to synthetic chemical inputs.

An effective fight against tomato dry rot due to *S. rolfsii* mycorrhizal plants, remains accessible to farmers and could significantly contribute to improving yield. The objective of this work was to evaluate in the greenhouse, the capacity of tomato mycorrhizal plants to develop on a soil inoculated with *S. rolfsii*, pathogen responsible for the sclerotinia or dry rot of the tomato.

MATERIALS AND METHODS

Research work took place from June-October, 2017, at University Félix Houphouët-Boigny scientific pole in Bingerville (Côte d'Ivoire) for green house activities and for labwork, in the Laboratory of Plant Physiology of the same university at Cocody, Abidjan (Côte d'Ivoire).

Materials

Tomato cultivar used: The *in vivo* evaluation of the antifungal activity of arbuscular mycorrhizal fungi (AMF) was performed on tomato seedlings of the F1 Lindo variety. The choice is focused on this variety because of its high sensitivity to the sclerotinia and also because of its availability in the trade. The seeds were obtained from a seed company in Abidjan (Côte d'ivoire).

Mycorrhizal inoculum: Biological control material consists of propagules (spores, mycelium fragments and small fragments of mycorrhizal roots) of six different species of Arbuscular Mycorrhizal Fungi (AMF) in a mixture of inert substrates, amended with bio-additives promoting the development of the mycorrhizal symbiosis. The inoculum was provided for free by Inoculum plus of Technopôle Agro-environment. AMF involved are presented in Table 1.

Fungal material: *Sclerotium rolfsii* has been the pathogenic fungus used for this study. The strain originates from the Laboratory of Plant Physiology of Université Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire). It has been maintained on the PDA medium (potatoes Dextrose Agar) by subculture. The pathogen was originally isolated from tomato plants with rotting symptoms[11].

New subcultures were carried out in sterile Petri dishes containing the PDA medium in order to obtain the necessary sclerotia for the experiment (Fig. 1).

Methods

Obtaining tomato plants for inoculation: Tomato seeds (*Solanum lycopersicum*) of the F1 Lindo variety were sown in two plastic bins containing a culture substrate. The substrate is composed of sterile sand/soil mixture in the 6/1 proportions. In the first bin, mycorrhizal inoculum was added to the substrate prior to sowing. Thus, 150 g of the inoculum is rigorously mixed with the substrate in order to obtain mycorrhizal plants (M). In the second bin, there was no contribution of mycorrhizal inoculum. Tomato plants

Table 1: Fungi forming the arbuscular mycorrhizal fungi complex inoculated with tomato

Number	Denomination
1	*Claroideoglomus etunicatum*
2	*Glomus microaggregatum*
3	*Rhizophagus intraradices*
4	*Claroideoglomus claroideum*
5	*Funneliformis mosseae*
6	*Funneliformis geosporum*

Minimum number of fungal propagules: 1 million/kg (evaluated according to the Most Probable Number test)

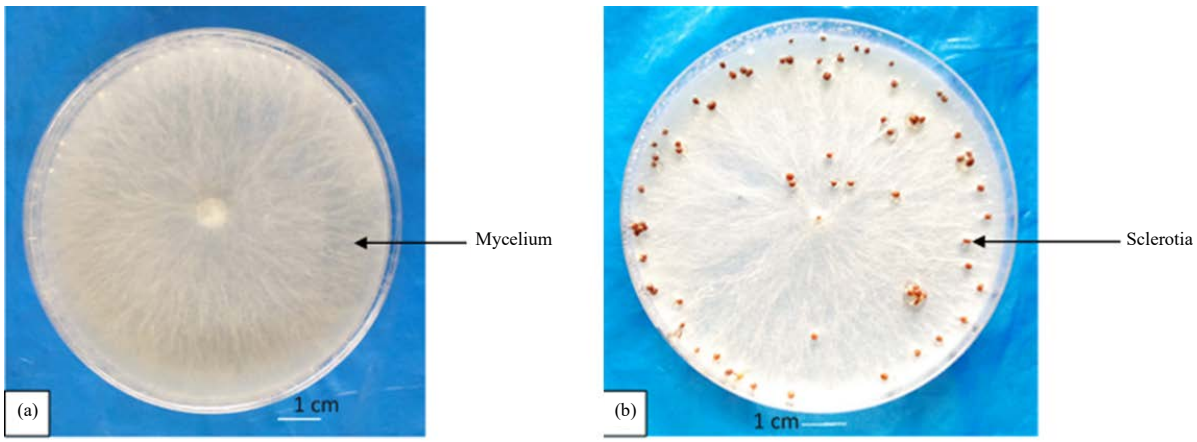

Fig. 1(a-b): Macroscopic characteristics of *Sclerotium rolfsii*, (a) Mycelium appearance 5 days after cultivation and (b) Sclerotia apparition 15 days after culture

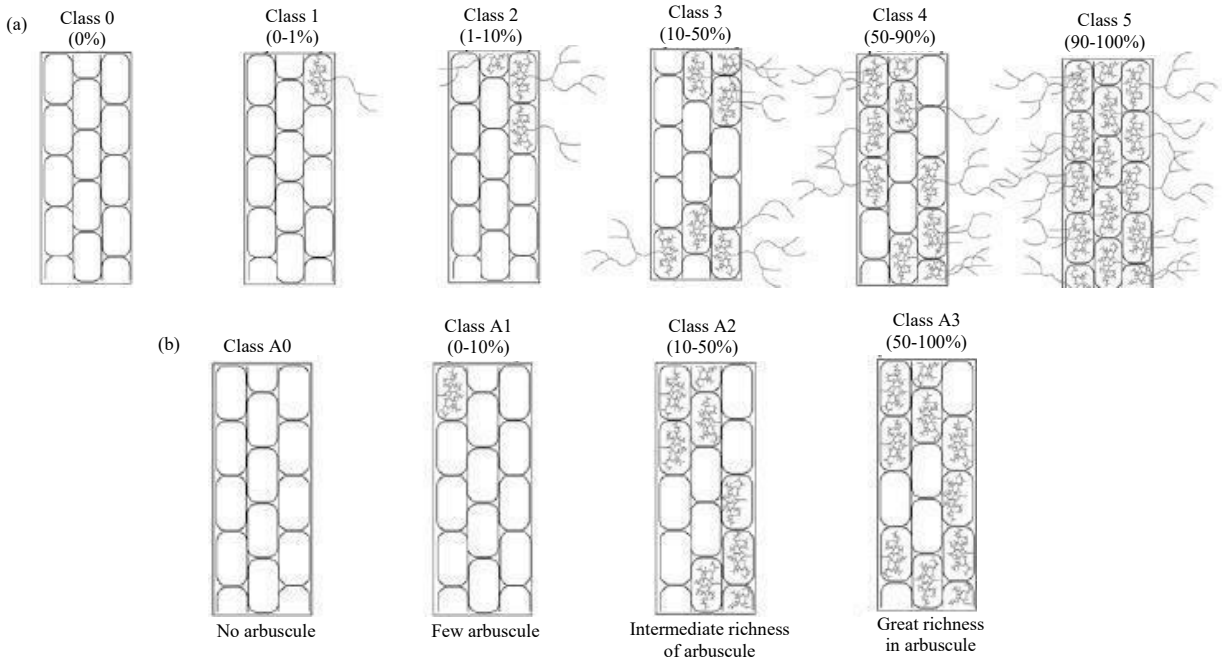

Fig. 2(a-b): Notation of mycorrhizal infection and richness in arbuscular, (a) Mycorrhizal infection (classified from 0-5) and (b) Wealth in arbuscular (classified from A0-A3)

from this container are non-mycorrhizal plants (NM). Bins are kept under greenhouse and the substrate is regularly watered to keep it moist continuously.

After germination, plants have been exposed to ambient light only and watered daily to field capacity. No amendment has been made.

Observation of hyphae, arbuscules and vesicles: The identification of the endo-mycorrhizal infection is achieved by the roots staining method[13].

Determination of the mycorrhization rate: For each plant, 10 root fragments, each of 1 cm length have been observed and evaluated under a microscope[14]. With this method, infection frequencies are classified from 0-5 and arbuscular contents from A0-A3 (Fig. 2). Then, from the MycoCalc software the following values have been calculated[14]:

- The infection frequency F (%) or percentage of the number of endo-mycorrhizal root fragments

- The relative mycorrhization intensity M (%) corresponding to the proportion of the colonized cortex
- The absolute mycorrhization intensity m (%)
- The relative arbuscular content of infection A (%) corresponding to the proportion of the root cortex containing arbuscular
- The absolute arbuscular content of infection a (%)

Before transplanting: Tomato plants inoculated with AMF were used to determine the frequency mycorrhization, its intensity and arbuscular content. First, at the time of sub-culturing (40 days after seedlings), five plants that have received mycorrhizal inoculum have been randomly selected for roots staining[13]. Finally, mycorrhization rate is determined[14]. The same treatment is performed with five non-mycorrhizal plants (control plant).

Transplant and inoculation: Tomato mycorrhizal plants (M) or not (NM) have been transplanted after 45 days in polyethylene pots of 297 cm^3 of volume, perforated at the base. The substrate is composed of the sterile sand/soil mixture, in the 5/1 proportions.

Sclerotia inoculation has been made to the subculturing, as follows: Ten Sclerotia were set to 1 cm deep, two to two in 5 holes in the form of a pentagon 2 cm around each plant. The sclerotia have then been covered by the substrate. Thus, are performed:

- About 3 repetitions of 10 non-mycorrhizal plants: The 25 inoculated by *Sclerotium rolfsii* (NMi) and 5 uninoculated (NM)
- About 3 repetitions of 10 mycorrhizal plants: The 25 inoculated by *S. rolfsii* (Mi) and 5 uninoculated (M)

Seedlings have been watered every morning using a squeeze. The experiment was repeated 3 times over time.

Assessment of the effect of inoculation on tomato plants growth: In order to assess the impact of sclerotia inoculation on seedling growth, stem height measurements, number of functional leaves, seedlings collar diameter and the number of flowers have been determined at seedlings transplant, inoculation and at the end of experimentation. These measurements were performed on 25 non-mycorrhizal plants inoculated with *Sclerotium rolfsii* (NMi), 25 mycorrhizal plants and inoculated with *S. rolfsii* (Mi) and 10 control plants not inoculated with *S. rolfsii* including 5 non-mycorrhizal and 5 other mycorrhizal. Seedlings were dug up 60 days after transplanting and weighed to determine the fresh mass.

Mycorrhization evaluation at the end of the experiment: The evidence of the mycorrhizae have been carried out[13]. Five pre-inoculated seedlings have been used to determine the rate of mycorrhization. These plants have been randomly selected from plants that have received mycorrhizal inoculum before being in contact with the pathogen.

Dependence on mycorrhization: Unearthed seedlings were put in an oven at 70°C for 3 days for dry mass determination. The values obtained have been used to calculate the dependence on mycorrhization (DM)[15]. The DM is determined by the difference in the biomass of mycorrhizal plants compared with non-mycorrhizal plants reported to the dry mass of non-mycorrhizal seedlings[15], according to the following equation:

$$DM = \frac{\text{Dry mass of mycorrhizal plants} - \text{dry mass of non} - \text{mycorrhizal plants}}{\text{dry mass of nonmycorrhizal plants}} \times 100$$

Evaluation of mycorrhization effect on the incidence of root dry rot due to *Sclerotium rolfsii*: The assessment of symptoms is based on a symptom rating scale[16]:

0 : Healthy plant
1 : Light yellowing, slight rot of the pivot and secondary roots and snare rot
2 : Yellowing of leaves with or without wilting or stunting of plants, important rot of the collar and browning of the stem vessels
3 : Death of the plant

The values obtained were used to calculate the incidence of the disease[17], according to the equation:

$$\text{Incidence} = \frac{\Sigma Vi \times Ni}{\text{Highest value} \times \text{Plants total number}} \times 100$$

where, Vi = Grade i attributed according to symptoms rating scale and Ni = Plants with the grade i.

Statistical analysis: Collected data were subjected to an analysis of variance to one classification criterion (ANOVA I) using statistica, version 7.1. A post ANOVA analysis was carried out for averages comparison using the Newman-Keuls' least significant difference test ($p<5\%$).

RESULTS

Growth parameters

Measuring growth parameters at subculturing: At the time of transplantation (Fig. 3), statistically, there was a significant difference between mycorrhizal and non-mycorrhizal plants at all agronomic parameters measured with $p \leq 0.001$.

Plants height evolution over time: Overall, the height of plants has increased over time (Fig. 4). Since sub-culturing, the height of mycorrhizal seedlings (Mi) has remained significantly higher than that of non-mycorrhizal plants inoculated with *Sclerotium rolfsii* (NMi) or not (NM) with $p \leq 0.0001$.

Evolution of collar diameter over time: Since transplanting, the collar diameter of mycorrhizal seedlings (Mi) has remained higher than that of non-mycorrhizal plants inoculated with *Sclerotium rolfsii* (NMi) or not (NM) (Fig. 5). This difference was significant with $p = 0.0428$ at the end of the experiment.

Evolution of the number of functional leaves: For the duration of the experiment, the number of functional leaves of the Mi seedlings remained higher than that of the NMi and NM plants (Table 2). The difference was significant between the different treatments at the end of the experiment ($p = 0.0232$) (Fig. 6).

Study of the mycorrhization: All seedlings that developed on the substrate that received mycorrhizal inoculum did not form mycorrhiza with the propagules of fungi present in the mycorrhizal inoculum (Table 2). Parameters of mycorrhization remained low but increased between sub-culturing and the end of the experiment (Table 3).

Dependence on mycorrhization: Fresh and dry masses of the mycorrhizal plants have been superior to the fresh and dry masses of the non-mycorrhizal plants. The dependence on mycorrhization has increased by 33.16% (Fig. 7).

Assessment of symptoms: The first symptom observed has been the aged leaves yellowing observed 2 weeks after seedlings planting. This yellowing has extended after few days to the whole leaf system. About 2-3 days later, white sclerotia at the beginning and then brown to blackish in maturity were formed at the collar of tomato plants in culture. Aqueous lesions of light brown color at the collar, sometimes on the stem are followed by thinning of the infected area. The stem rot have started at the collar level and the plant have dried out completely after 3-4 days (Fig. 8).

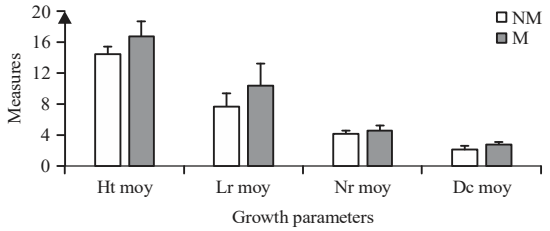

Fig. 3: Histogram of averages of stem height, root length, number of functional leaves and collar diameter
Ht: Height of the stem, Lr: Length of the root, Nf: Number of functional sheets, Dc: Collar diameter, moy: Average

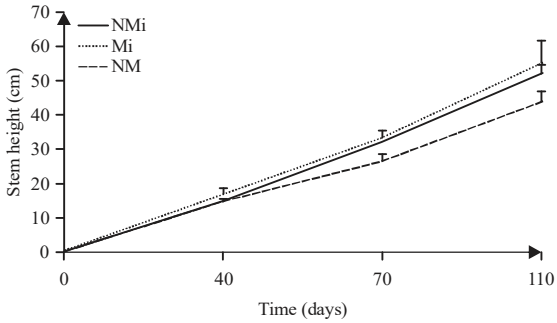

Fig. 4: Evolution of the stem height of tomato plants as a function of time and different treatments
NMi: Not mycorrhizal inoculated with *Sclerotium rolfsii*, NM: Not mycorrhizal, Mi: Mycorrhizal inoculated with *Sclerotium rolfsii*, 0: Semi (start), 40: Subculturing 40 days after semi, 70: Inoculation 30 days after subculturing, 110: Harvesting of plants (end)

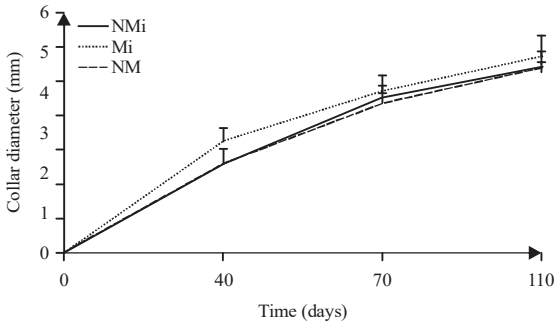

Fig. 5: Evolution of the collar diameter of tomato plants as a function of time and treatments
NMi: Not mycorrhizal inoculated with *Sclerotium rolfsii*, NM: Not mycorrhizal, Mi: Mycorrhizal inoculated with *Sclerotium rolfsii*, 0: Semi (start), 40: Subculturing 40 days after semi, 70: Inoculation 30 days after subculturing, 110: Harvesting of plants (end)

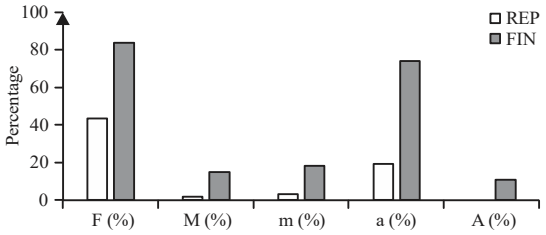

Fig. 6: Mycorrhization settings at sub-culturing and end of experiment

F: Frequency of infection, M: Relative mycorrhization intensity, m: Absolute mycorrhization intensity, a: Absolute arbuscular content of the infection, A: The relative arbuscular content of the infection, REP: Sub-culturing, FIN: End of experience

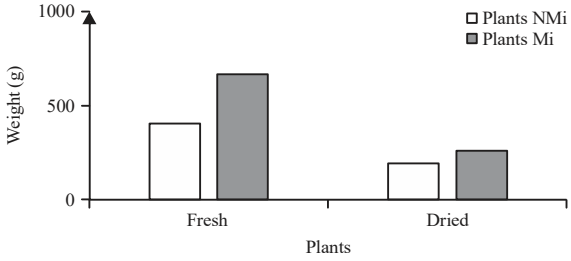

Fig. 7: Fresh and dry weight of mycorrhizal plants and non-mycorrhizal plants

NMi: Not mycorrhizal inoculated with *Sclerotium rolfsii*, Mi: Mycorrhizal inoculated with *Sclerotium rolfsii*

Table 2: Evolution of the number of functional leaves based on treatment over time

	Temps (jours)			
Traitements	0	40	70	110
NMi	0.00±0.00[a]	4.20±0.41[a]	10.40±0.50[a]	5.20±3.01[b]
Mi	0.00±0.00[a]	4.63±0.56[a]	10.80±0.61[a]	7.24±2.20[a]
NM	0.00±0.00[a]	4.20±0.41[a]	10.20±0.45[a]	7.80±1.09[a]

In the same column, the numbers assigned to the same letter are identical to the 5% threshold of the Newman-Keuls test, NMi: Not mycorrhizal inoculated with *Sclerotium rolfsii*, NM: Not mycorrhizal, Mi: Mycorrhizal inoculated with *Sclerotium rolfsii*, 0: Semi (start), 40: Sub-culturing 40 days after semi, 70: Inoculation 30 days after sub-culturing, 110: Harvesting of plants (end)

Table 3: Mycorrhization parameters to transplanting

Parameters	Plante 1	Plante 2	Plante 3	Plante 4	Plante 5
F (%)	0.00	60.00	50	70.00	40.00
M (%)	0.00	4.30	0.90	4.00	0.80
m (%)	0.00	7.17	1.80	5.71	2.00
a (%)	0.00	37.91	10.00	40.00	10.00
A (%)	0.00	1.63	0.09	1.60	0.08

F: Infection frequency, M: Relative mycorrhization intensity, m: Absolute mycorrhization intensity, a: Absolute arbuscular content of the infection, A: Relative arbuscular content of the infection

The disease was found to be more severe with NMi. The incidence of dry rot due to *Sclerotium rolfsii* in NMi plants has been 2.5 times greater than the incidence for Mi (Fig. 9).

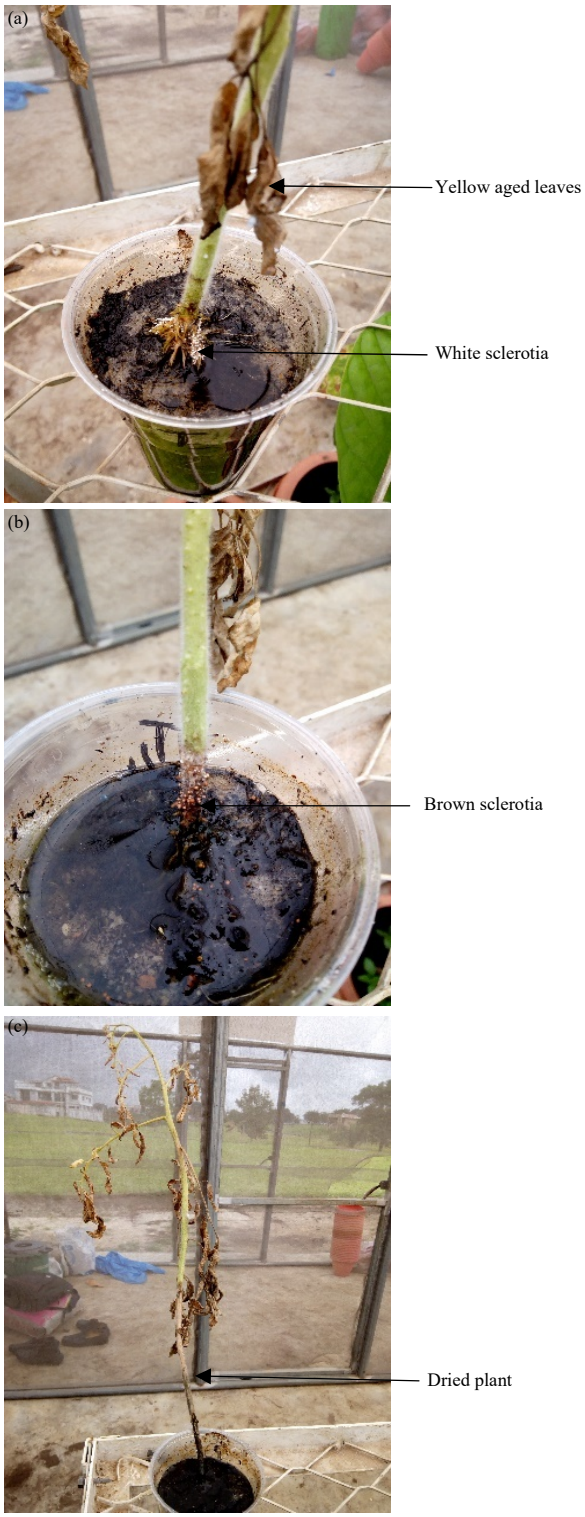

Fig. 8(a-c): Evolution of the symptoms of *Sclerotium rolfsii* on a tomato plant, (a) Yellowing of the aged leaves and appearance of white Sclerotia, (b) Browning of sclerotia at the collar and on the stem and (c) Drying of the plant

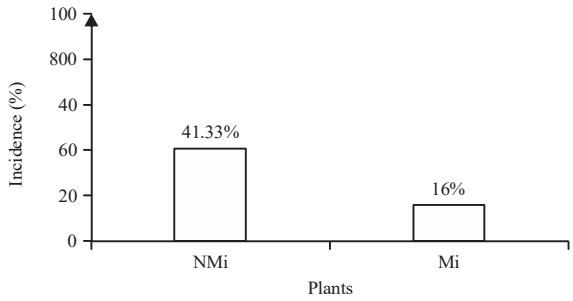

Fig. 9: Incidence of *Sclerotium rolfsii* in mycorrhizal plants and non-mycorrhizal plants
NMi: Not mycorrhizal inoculated with *Sclerotium rolfsii*, Mi: Mycorrhizal inoculated with *Sclerotium rolfsii*

DISCUSSION

The F1 Lindo variety formed mycorrhizae from the propagules contained in the inoculum brought. The symbiotic relationship thus established indicates that this particular variety and tomato in general is mycotrophe[18]. It is therefore, in symbiosis with at least one AMF contained in the mycorrhizal inoculum. Indeed, after the first physical contact between the hyphae of a AMF and the plant, the fungus forms a pre-colonization structure known as appressorium on the root surface by which it colonizes the intercellular space of the root cortex[19]. The plant cell forms a subcellular structure called a pre-penetration device that pre-determines the path of hypha growth through the plant cell. Differentiation of this cytoplasmic bridge allows the fungal hypha to penetrate the host cell[19] to develop intracellular hyphae. The fungus then crosses the outer cell layers, propagates longitudinally in the inner cortex and forms dichotomous branched hyphae within the cortical cells, called arbuscules[20,21].

The observed difference in initial growth between mycorrhizal plants and seedlings that did not receive mycorrhizal inoculum indicated that mycorrhization had a beneficial effect on growth. This benefit may be due to better nutrition of mycorrhizal plants compared to non-mycorrhizal plants. In fact, the development of mycelial hyphae following root infection by mycorrhizal fungi indirectly increases the volume of soil accessible by the host plant resulting in a better mobilization of soil nutrients and improving water nutrition[22-24]. This improvement in the quality of seedlings observed through growth parameters of mycorrhizal plants compared with non-mycorrhizal plants indicated that mycorrhizal seedlings improved their vigour, strength and development and therefore; their transplant performance before transplanting. This bio-augmentation due to AMF has also been reported to improve seedling qualities in nurseries[25,26] and more specifically tomato seedlings[27].

At the time of inoculation of sclerotia (70 days after sowing), there was a significant difference in the height of the seedlings between the different treatments (Mi and NMi). However, this difference was not significant in terms of the number of functional leaves and the diameter of the collar. This means that NMi plants had a higher rate of growth than Mi plants during the period between transplanting and inoculation of sclerotia of *Sclerotium rolfsii*. It has been indicated that changes in the response to mycorrhizal colonization occurred with plant developmental stages[28]. Similarly, increased vegetative growth at the early vegetative growth stage in Gladiolus varieties inoculated with AMF has also been reported[29]. In addition, sub-culturing in small volume pots (297 cm^3) did not provide mycorrhizal plants (M) with a source of nutrients and water that would be inaccessible to non-mycorrhizal (NM) plants. Thus, the NM plants were able to use their roots alone to prospect the same volume of soil as the M plants.

The observed root colonization of 15.14% of the F1 Lindo variety is low. It is lower than that obtained by researchers with tomato cultivated in soils[30] or 35.99%. This weakness could be due to the inoculum quality, the experimental conditions but also the fact that when the mycorrhizal symbiosis is established, it does not necessarily associate the most efficient fungal partner.

The value of mycorrhizal dependence measures seedling growth responses to AMF colonization[31]. The result of 33.16% confirmed the high dependence of tomato on mycorrhizae, which according some results[32] is even 59%. This high dependence on mycorrhization results in better resistance to collar dry rotting due to *S. rolfsii*, resistance 2.5 times higher than NM seedlings. Arbuscular mycorrhizae contribute to reducing plant infestation by pathogens at the root level[33]. Some researchers also showed that the roots of mycorrhizal tomato plants reduced the severity of tomato grey rot caused by attacks of *Botrytis cinerea*[34]. This resistance to *S. rolfsii* could be the result of local competition-related action for the occupation of sites of fixation between AMF and the pathogen[35], a mycorrhizosphere (root physiology and exudates) whose consequence is the enhanced protection of the plant against root pathogens[36], an improvement in nutrition to compensate for the damage caused by the pathogen[37] in relation to high DM[22] stimulation of plants natural defenses by a local and systemic induction of the expression of defense genes[38-40].

CONCLUSION

This study has demonstrated inhibitory effect of mycorrhizaes on *Sclerotium rolfsii.* Mycorrhization has a

significant effect on sclerotinia or tomato dry rot incidence. Thus, it reduces fungicides and phytoregulators use while minimizing production costs as well as negative impacts on consumer and environment. Ultimately, arbuscular mycorrhizal fungi allow faster growth of tomato plants and are bioprotective against *S. rolfsii* development.

Mycorrhization may be advisable for growers in areas where sclerotinia is more prevalent as an alternative to the overuse of synthetic fungicides.

SIGNIFICANCE STATEMENT

This study discover the faster growth induced and bioprotective effect of mycorrhiza against *Sclerotium rolfsii* that can be beneficial to tomato production. This study will help the researcher to uncover the critical areas of plant-AMF symbiosis bio-protective effect that many researchers were not able to explore. Thus a new theory on biological control of *Sclerotium rolfsii* may be arrived at.

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