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Reduced nitrogen and phosphorus fertilization combined with mycorrhizal inoculation enhance potato yield and soil mineral fertility

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Abstract

To improve sustainable agriculture it becomes crucial to minimize the use of chemical inputs and involve new practices that are more productive and healthier for humans and environment. Biofertilization with arbuscular mycorrhizal fungi (AMF), known for increasing the productivity of many crops including potatoes, could be considered as one of these sustainable practices. The purpose of this study was to determine whether mycorrhizal inoculation can minimize chemical fertilization for potato (*Solanum tuberosum* L.), one of the most demanding species in mineral elements. Thus, by studying the effect of chemical fertilization and mycorrhizal inoculant on potato yield and soil quality. Two doses of chemical fertilizer (NP) corresponding to 50 % and 100 % of the recommended dose has been applied in the presence or not of the mycorrhizal inoculant. The results have shown that adding arbuscular mycorrhizal fungi in combination with 50 % of the chemical fertilizer dose gives the best effects. A significant increase in yield, root colonization, and P soil content has been observed ($P < 0.001$). Thus mycorrhizal inoculation could reduce the application of synthetic fertilizers and thus improve yield while preserving the environment.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.,) is considered as the most important non-cereal (Ezekiel et *al.* 2013) and tuber crop in the world (Hassan, 2003). It is cultivated in more than 150 countries and eaten by more than 1 billion people every day (FAO, 2020). This crop could play a significant role in poverty eradication in the world (Lutaladio and Prakash, 2010) and malnutrition and hunger alleviation in Africa having a production rate around 26 million tons in 2018 (FAO, 2020). In Tunisia, potato crop has a significant contribution to the agricultural outputs and exports of the country. Approximately, 17 % of the Tunisian cultivated lands are used for potato production. In 2021, potato production in Tunisia was about 450 thousand metric tons (Ministry of Agriculture, Water Resources and Fisheries of Tunisia, 2022; FAOSTAT, 2022).

However, potato plants are high consumers of chemical fertilizers (Adavi and Tadayoun, 2014) due to their shallow and underdeveloped root system. Furthermore, the excessive use of chemical fertilizers could have adverse effects on the environment and human health (Savci, 2012) and did not present a sustainable solution for maintaining maximum yields and improving soil fertility (Rivera-Becerril et *al.* 2017). In addition, potato crop faces various biotic stresses affecting its production such as pests and pathogens. Nowadays, there is a growing interest for integrating potato cropping practices permitting the reduction of mineral fertilizers use (Maynard and Hochmuth, 2007) and enhance resistance to pests and soil-borne diseases (Al-Karaki, 2006).

Among these practices, biofertilization with arbuscular mycorrhizal fungi (AMF) can present a promising solution. In fact, mycorrhizal inoculation had taken the intention of scientists (Adesemoye and Kloepper, 2009) for its role in increasing plant growth and mineral uptake, especially phosphorus, thanks to the extra-radical fungal hyphae (Nurbaity, 2014; Smith and Read, 2008), enhancing soil quality (Junior et *al*. 2018) and improving plant tolerance to *Fusarium*, *Verticillium* and Bacterial wilts (Boutaj et *al.* 2022).
Few studies (Hijri, 2006; Douds et *al*. 2007) have shown the efficiency of arbuscular mycorrhizal fungi (AMF) inoculation for potato crop. The objectives of this study were to investigate the effect of mycorrhizal biofertilization on potato yield and quality and soil properties under complete and reduced chemical fertilization program as well as to mitigate the infection caused by the soil born fungal pathogen *Fusarium solani*.

2. MATERIAL AND METHODS

2.1. Experimental design

The experiment was carried out under greenhouse conditions in the Center of Biotechnology of Borj Cédria- Tunisia (23° 14' 28.846" N lat., and 16° 52' 30" E long). The period of cultivation was run in 2020 from February 24th to June 15th. The substrate used for the experiment is a sandy garden soil. It was distributed in 20 liters pots. The physicochemical properties of the soil have been determined in the Laboratory of Horticultural Sciences (LSH) at the National Agronomic Institute of Tunisia (INAT) (Table 1).

Table 1. Physical and chemical properties of the substrate used during the experiment.

Soil properties	Value
Physical :	
Sand (%)	88
Silt (%)	9.27
Clay (%)	2.73
Textural Class	Sandy
Chemical :	
pH	7.69
Electrical Conductivity (dS /m)	0.25
Organic carbon (%)	0.24
Organic Nitrogen (%)	0.69
Available Phosphorus (ppm)	145

Germinated potato tubers, variety Spunta, were used for plantation. The mycorrhizal inoculum applied is composed of a mixture of spores, hyphae and fragments of roots colonized by the two AMF species *Glomus deserticola* and *Glomus sp*., provided by the LSH-INAT. For each inoculated pot, 50 g of inoculum was placed directly below the sprouted tuber. After planting, all pots were irrigated twice a week with tap water at a rate of 800 mL/pot.

For the experiment design, a completely randomized block design was followed, composed of three blocks and each block contains four treatments. Fifteen replicates per treatment and per block were applied.
The treatments are: T1: Non-inoculated tubers receiving 100 % of the recommended dose of chemical fertilization (NP) and not infested by the pathogen "*Fusarium solani*"; T2: Non-inoculated tubers with 100 % of the recommended dose of chemical fertilization and infested by the pathogen "*Fusarium solani*"; T3: Inoculated tubers with the mycorrhizal biofertilizer and fertilized with 50 % of the recommended dose of chemical fertilization (NP) and not infested by the pathogen "*Fusarium solani*" and T4: Inoculated tubers with the mycorrhizal biofertilizer and 50 % of the recommended dose of chemical fertilization (NP) and infested by the pathogen "*Fusarium solani*".
The quantities of mineral fertilizers introduced during the trial were applied according to the recommended fertilization program by the Potato and Artichoke Technical Center in Tunisia (CTPTA) and are listed below (Table 2)
The total quantity of phosphate fertilizer was added to the soil three days before plantation. Nitrogen and potassium fertilizers doses were distributed as follows: the first doses were added 21 days after plantation, the second at 28 days, the third at 54 days, the fourth at 72 days, and the fifth at 88 days after plantation.

Table 2. Potato crop recommended doses of chemical fertilizers (CTPTA, 2020).

	Phosphate fertilizer (Super45)	**Nitrogen fertilizer (Ammonium Nitrate)**	**Potassium fertilizer (Solupotasse)**
Quantity (Kg/H)	200	480	380

2.2. Soil analyses

For each treatment, representative soil samples were taken before crop establishment and after harvest, air-dried and conserved until use for soil analyses. Soil properties including pH, electrical conductivity (EC) (dS /m), organic carbon contents (%), N (%), and P were determined. Soil pH was determined in water (1:2.5) and KCl solution (1:1) using a pH meter. Electro-conductivity (EC) measured in a 1:5 (soil: water) suspension using the electrometric method (Chapman, 1965). The soil organic matter was determined according to the Walkey and Black (1934) method. The available phosphorus (P) was extracted using Olsen's extract and P in the extract was determined by using a spectrophotometer. The organic nitrogen (N) was estimated according to the Kjeldhal method (Pauwels et *al.* 1992).

2.3. Mycorrhizal colonization rate

Root samples collected from harvested plants were washed with distilled water, then stained following the technique of Phillips and Haymann (1970). The root samples were placed in a 5 % KOH solution at 90°C for 20 minutes, then washed and incubated in Trypan blue. Mycorrhization rates were determined under microscope using the method described by McGonigle et *al.* (1990).

2.4. Potato production and quality determination

The potato tubers were harvested at 112 days after plantation. Six plants were selected from each treatment to calculate the number of tubers/plant and the weight of tuber/plant. The quality of produced tubers was estimated by measuring the size of their maximum width.

2.5. Statistical analyses

Statistical analyses were carried out using R statistical software version 3.5.2. The average value of all measured parameters (at least three replicates) was compared by one-way analysis of variance (ANOVA) using the least significant difference (LSD) at the probability level of 0.05.

3. RESULTS AND DISCUSSION

3.1. Mycorrhizal infection rate

The AMF structures such as arbuscules and vesicles were observed in all potato's roots (Fig. 1). Potato plants had formed a symbiotic intercation in all the treatments even in non-inoculated pots which presented a mycorrhizal infection rate of 5.55% and 3.70% respectively in T1 and T2 (Table 3). This result could be explained by the presence of native arbuscular mycorrhizal fungal community in the substrate (garden soil), as it was not sterilized before planting. These results highlight that potato is a mycotrophic plant.

Furthermore, inoculated plants showed the highest mycorrhizal colonization rates with 13.33 % in T4 and 30.37 % in T3. The results showed a

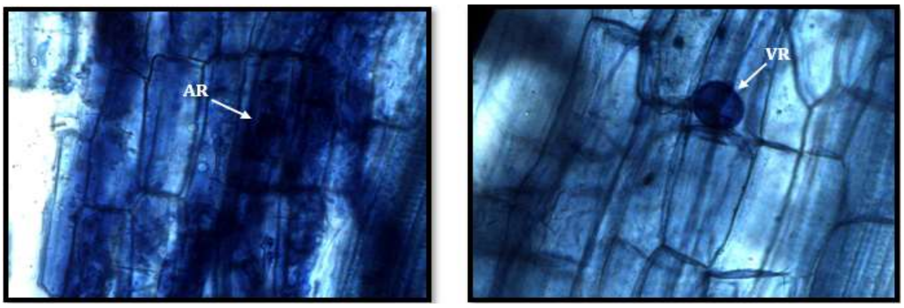

Fig. 1. Microscopic observation of potato (*Solanum tuberosum L.*) roots after harvesting and staining with Trypan blue. Arbuscular mycorrhizal structures: Vesicles (VS) (×400); Arbuscules (AR) (×100).

Table 3. Effect of mineral fertilization and *Fusarium solani* infection on root mycorrhizal colonization of potato (*Solanum tuberosum* L.).

Treatments	**Root Colonization**		
	Mycorrhizal Rate (%)	**Vesicles (%)**	**Arbuscules (%)**
T1	5.55 ± 2.33c	1.85 ± 1.67bc	2.59 ± 2.60c
T2	3.70 ± 1.81c	0.74 ± 1.81c	2.22 ± 1.99c
T3	30.37 ± 3.34a	10.74 ± 1.67a	23.70 ± 3.63a
T4	13.33 ± 1.40b	3.70 ± 1.14b	10.00 ± 2.33b
F-value	161.86	47.52	82.68
Significance	***	***	***

Values represent means of 6 replications per treatment ± SD. The Least Significant Difference (LSD) interaction was calculated with the Fisher's protected LSD test at α = 0.05. NS, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

significant decrease in the mycorrhizal infection rate when applying the total mineral fertilization (Table 3). The study of Martin et *al.* (2011) demonstrated that chemical fertilizer, at high levels, reduced mycorrhizal infection rate and the number of spores. In addition, Getman- Pickering et *al*. (2021), reported in field and pot experiments that several nitrogen fertilizers can decrease mycorrhizal colonization of tomato plants. Thus, a high application of chemical fertilizers decreases colonization while low to medium levels can increase colonization (Lin et *al.* 2020).

The infection of potato plants with *F. solani*, decreased the mycorrhizal colonization rate in non-inoculated and mycorrhiza inoculated plants (Table 3). In agreement, Aysan and Demir (2009) and Spagnoletti et *al.* (2017), had reported in common bean and soybean that pathogen infection reduced mycorrhizal colonization rate. However, some other researchers such as Spagnoletti et al. (2021) reported that the AMF *Rhizophagus intraradices* a biocontrol agent against *Fusarium pseudograminearum* in wheat plants gave a high rate of mycorrhizal colonization when comparing with the non-mycorrhized and pathogen infested plants. Thus, suggesting that the presence of the pathogen stimulated the mycorrhizal colonization. McAllister et *al.* (1994), García-Romera et *al.* (1998) and Fracchia et *al.* (2000) have found a stimulation of mycorrhizal colonization by other *Fusarium* species, such as *F. oxysporum* or *F. solani* for soybean, pea, and sorghum.

3.2. Potato production

The results showed that the number and the weight of tubers per plant varied significantly with the treatment (Table 4). The highest tuber number and weight were registered in T3 and T4, where the arbuscular mycorrhizal biofertilization was combined to 50% of the recommended chemical fertilization (Table 4). According to Maboko et *al.* (2013), tomato production and the product quality were enhanced with mycorrhizal inoculation under reduced fertilizer applications (25% and 50% reduction of recommended doses). Different studies demonstrated that mycorrhizal inoculation improves plant productivity and yield even under stress conditions. Abdel-Latef and Chaoxing (2011) had shown that mycorrhization mitigated the salt-induced reduction in fruit fresh weight and yield of tomato through the increase of plant nutrient acquisition. In addition, according to Mäder et *al.* (2011) mycorrhizal inoculation could present an interesting option for sustainable high quality wheat production in low-input areas of India, promising to improve the nutritional status of plants, as well as the yield and the quality of wheat grains. Indeed, mycorrhizal inoculation increased wheat yields by 30 and 50% compared to non-inoculated plots. As well, Mathimaran et *al.* (2020) pointed out an increase in cereal yield and showed also that reduction chemical fertilizers in combination with biofertilizers can reduce soil degradation. Furthermore, similar results were obtained on sugarcane where the AMF inoculation and the application of half of the fertilizers dose gave the highest values of leaf area, height and diameter of plants (Juntahum et *al.* 2021). Moreover, Jaffri et *al.* (2021) showed that soil inoculation with AMF could reduce the required chemical fertilizer inputs by half and even increase pineapple yield and quality compared to the use of full dose of the chemical fertilizers. It was demonstrated that AMFs mobilize and deliver nutrients to pineapple to achieve the best results in terms of fruit size and quality with half fertilizers dose.

This study showed also that AM inoculation improved potato production under *F. solani* infection, demonstrating an increase of plant tolerance to this pathogen. The infection of plants with *F. solani* decreased both the number and the weight of tubers in comparison to non infected plants (Table 4). The inoculation of plant with the mycorrhizal inoculum combined with a reduced

Table 4. Effect of mineral fertilization, *Fusarium solani* infection and mycorrhizal inoculation on potato (*Solanum tuberosum*) tuber's number and weight per plant.

Treatments	**Tuber production**	
	Number/Plant	**Weight(g)/Plant**
T1	7.00 ± 0.89 b	203.42 ± 33.92 c
T2	5.66 ± 1.03 c	178.91 ± 42.86 c
T3	9.50 ± 1.38 a	421.08 ± 48.64 a
T4	6.83 ± 1.17 b	358.25 ± 24.88 b
F-value	3.51	55.83
Significance	.	***

Values represent means of 10 replications per treatment ± SD. The Least Significant Difference (LSD) was calculated with the Fisher's protected LSD test at α = 0.05. NS, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

chemical fertilization increased potato production by 2 folds in comparison to full chemical fertilized potato plants both in *F. solani* infected and non-infected plants (Table 4). In addition, the mycorrhizal inoculation increased the tuber weight per plant under the pressure of *F. solani* infection (T4) in comparison to both non-infected (T1) and infected (T2) plants which did not received AM inoculums (Table 4).

These results are similar to those reported by Ismail and Hijri (2012) when studying the impact of *Glomus irregulare* on disease severity caused by *Fusarium sambucinum* on potato plants. They found that disease severity of *F. sambucinum* was significantly reduced in mycorrhized plants compared to controls. In addition, they reported that the co-inoculation of *G. irregulare* and *F. sambucinum* reduced the negative effects of the *F. sambucinum* pathogen and significantly improved potato tuber yield compared to non-mycorrhized and infected plants. Also, Spagnoletti et *al.* (2017) demonstrated that AMFs can increase the yield and growth of *Macrophomina phaseolina*-infected soybean plants compared to non infected plants.

For our study, the quality of production was estimated by measuring the tuber size (Table 5). Our findings revealed that there is no difference between treatments for small tuber sizes (<35). However, we observed a significant difference between treatments for medium (35-45) (p < 0.01) and especially large size tubers (>45) (p< 0.001). The large tuber sizes (>45) are mainly recorded in the mycorrhizal treatments which demonstrate the effect of mycorrhizal inoculation on improving the marketable potato tubers.

Lombardo et al., (2020) recorded a significant improvement of marketable yield (+25%) in potato plants inoculated with AMF and grown under calcareous soils compared with non-inoculated potato plants. In addition, they outlined a significant increase of potato marketable tubers when applying the mycorrhizal inoculation with halved fertilizers dose (F50+M). Also, Saini et al., (2021) had registered that potato tuber width was increased by 143.58% in plants treated with *Glomus mosseae* and 75% of the recommended dose of Superphosphate as compared to control. Furthermore, Ismail et al. (2011) attributed the enhancement in potato tuber production by AMF inoculation to the increased nutrient uptake, mainly P due to the ability of mycorrhizal fungal hyphae to acquire P well beyond the limits of the rhizosphere.

3.3. Chemical soil quality

The soil physio-chemical properties, determined after potato harvest, had shown significant effects of treatments on soil available P amounts (p< 0.001), organic N (p< 0.001), pH (p< 0.01) and EC (p< 0.001) (Table 6). The most significant effects were recorded for the inoculated treatments. These results were also reported by Fall et *al.*

Table 5. Effect of mineral fertilization, *Fusarium solani* infection and mycorrhizal inoculation on potato (*Solanum tuberosum*) classification of tuber size.

Treatments	**Tuber maximum width (%)**		
	Small (< 35)	**Medium (35-45)**	**Large (> 45)**
T1	33.71 ± 19.01a	54.64 ± 22.28ab	11.65 ± 18.06b
T2	20.93 ± 13.57a	73.92±18.65a	5.15± 12.62b
T3	14.88 ± 4.18a	33.75± 24.17bc	51.37± 23.88a
T4	15.82 ± 5.48a	19.58± 16.76c	64.60± 20.16a
F-value	3.04	7.96	14.05
Significance	NS	**	***

The Least Significant Difference (LSD) was calculated with the Fisher's protected LSD test at α = 0.05. NS, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Table 6. Effect of fertilization management on soil chemical properties after potato harvest.

Treatments	**Soil Chemical Properties (After Harvesting)**			
	pH	**CE (dS /m)**	**N (%)**	**P (ppm)**
T1	7.06 ± 0.12c	0.62 ± 0.01b	0.27 ± 0.01b	144.14 ± 12.67c
T2	7.26 ± 0.13b	0.40 ± 0.03d	0.21 ± 0.02d	120.65 ± 11.10d
T3	7.35 ± 0.20ab	0.71 ± 0.05a	0.32 ± 0.02a	392.48 ± 28.12a
T4	7.44 ± 0.11a	0.49 ± 0.04c	0.23 ± 0.01c	304.93 ± 19.48b
F-value	7.70	87.80	64.33	280.04
Significance	**	***	***	***

The Least Significant Difference (LSD) was calculated with the Fisher's protected LSD test at α = 0.05. NS, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

(2022) which proved that mycorrhizal association have a key role in improving the availability of macro-nutrients in the soil, particularly phosphorus and nitrogen. According to Zhu et *al.* (2018), arbuscular mycorrhizal fungi are an important tool for improving soil P availability. They play a role in hydrolyzing the soil P pool to make it available in the soil for plant uptake. AMFs also release molecules such as glomalin, a glycoprotein secreted by AM hyphae and spores. When released into the soil, glomalin facilitates the uptake of nutrients such as phosphorus, which is difficult to dissolve (Emran et *al.* 2017). Indeed, through a mechanism related to the production of enzymes called phosphatases (Tarafdar and Marschner, 1994), AMFs can hydrolyze organic P into inorganic phosphorus (Shen et *al.* 2011). In addition, AMFs convert insoluble P to soluble forms through their production of acids during their metabolic activities (Kalayu, 2019).

Behera et *al.* (2014) explained that AMFs can also solubilize inorganic phosphate into soluble forms through processes of acidification, chelation, exchange reactions, and production of organic acids, H^+ and metabolites. In addition, AMFs can establish associations with phosphate soluble bacteria (PSBs) to convert insoluble phosphates to available forms. However, this association can indirectly reduce rhizosphere pH and increase P levels by affecting the root system and, consequently, increasing root exudates. Since root exudates contain organic acids, they can increase the rhizospheric P availability (Jones and Oburger, 2011). Nitrogen (N) is also essential for plant life and it is present in the soil in organic and mineral form. Lambers et al. (2008) showed that AMF can ensure the decomposition and mineralization of plant organic matter which increase N bioavailability in the soil.

Soil electrical conductivity increased significantly in all treatments. This may be due to the high dose of chemical fertilizer in T1 and T2 or the role of AMF in mineral decomposition in the soil in combination with chemical fertilization (T3 and T4). These results are in agreement with those found by Huanshi et *al.* (2011). They found that *Glomus aggregatum* and *Glomus mosseae* significantly improved the electrical conductivity of soils at different salinities. In contrast to Giri et *al.* (2003) who reported that mycorrhiza inoculation significantly reduced soil EC.

3.4. Bioprotective effect of Mycorrhization

The inoculation of potato plants with mycorrhizae before *F. solani* infection increased potato production in comparison to plants infected only with the pathogen, along with an increasein the percentage of large size tubers indicating an overall increase in plant tolerance to this pathogen (Table 4). Several researches showed that mycorrhizal treatment had a positive effect on potato tolerance to pathogens. In fact, Ismail and Hijri (2012) evaluated the impact of AMF *Glomus irregulare* on disease severity (wilting and yellowing) caused by *Fusarium sambucinum* and on plant growth. They found that disease severity with *F. sambucinum* was significantly reduced in mycorrhized plants compared to controls. Furthermore, they reported that the inoculation with *G. irregulare* reduced the negative effect of the *F. sambucinum* and significantly improved potato biomass production (root and shoot) and tuber yield compared to non-mycorrhized infected plants. In other experiments, the effect of Fusarium dry rot on postharvest potato was reduced by 20% to 90% on potato minitubers in the presence of AM fungi. In 2002, a work by Yao et al., showed that inoculation of potato with AMFs reduced the severity of shoot and crown rot disease caused by *Rhizoctonia solani,* while increasing nutrient uptake and fresh weight of tubers. Larkin (2008) observed similar effects when a mixture of AM fungi reduced stem canker and black scurf in the potato crop. According to El Hazzat et *al*. (2019), on chickpea plants inoculated with *F. solani*, the symptoms of dry rot decreased when the substrate contains a mycorrhizal inoculum. However, mycorrhization improves the growth of the aerial part and that of the root part, the number of leaves and pods, as well as decreasing leaf deterioration. Similar results were found by (Chliyeh et *al*. (2014) and Sghir et *al*. (2016)) on tomato and eggplant crops inoculated with *F. solani* et *F. oxysporum*. AM fungi were described as containing silencing RNA, allowing down-regulation of pathogen gene expression and thus protecting plants (Silvestri et *al.* 2020). The mycorrhizal plant/pathogen relationship has also been explained by Mukerji et *al*. (1999), as a series which start with the perception of arbuscular mycorrhizal fungi, signal transduction and subsequent activation of defense genes in the host plant. Indeed, according to this author, upon the first contact between AMF and the plant roots, defense mechanisms are induced by an important secretion of phenolic compounds that strengthen plant cell wall against pathogen invasion. Moreover, Karagiannidis et *al.* (2002) showed that the effect of AMF could be explained by an acquired resistance, probably due to the pre-activation of phenolic compounds, notably phytoalexins, flavonoids and associated isoflavonoids, secreted by these fungi at the root level and which block, according to Tahmatsidou et *al*. (2006), the entry points at the root level, thus suppressing the development of the pathogen by antibiosis. It seems that the response of AM fungi to stress is to improve the general health of the host plant through various processes.

4. CONCLUSION

The current study demonstrated that inoculating potato plants with AM fungi under reduced nitrogen and phosphorus fertilization (50% of the recommended dose) increased total potato yield, enhanced the proportion of mycorrhizal infection rates and improved soil fertility. Furthermore, the application of mycorrhizal inoculant showed a bioprotective effect against the soil borne pathogen *F. solani* the causal agent of wilting disease in potato. This study supports that mycorrhizal biofertilisation is an environmentally sustainable practice for increasing potato yield and health, as well as the fertility of the soil which constitutes the basis of a sustainable agriculture system.

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