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Impact of Arbuscular Mycorrhizal Fungi and Fertilization Levels on biochemical changes in potato (*Solanum tuberosum* L., Solanales Solanaceae)

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ABSTRACT Experiments have been carried out to evaluate the potential biochemical mycorrhizal benefit of potato (*Solanum tuberosum* L., Solanales Solanaceae) grown in northeastern Algeria. Three doses of chemical fertilizer (NPK) corresponding to 0, 50 and 100% of the recommended dose have been applied in presence or not in presence of the mycorrhizal inoculant. The results have revealed that the additional contribution of arbuscular mycorrhizal fungi combined with 50% chemical fertilizer gives the best results. A significant increase has been observed at the root colonization and chlorophyll content level. In terms of tuber quality, there is a significant increase in starch and protein content. However, the application of mycorrhizae alone will not compete with synthetic fertilizers in vegetable gardening, but could reduce their applications and thus improve yield while preserving the environment.

KEY WORDS Commercial AMF; Biochemical contents; Potato; Fertilization; vegetable crops.

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INTRODUCTION

The potato (*Solanum tuberosum* L., Solanales Solanaceae) is the most important tuber crop in the world, grown in more than 125 countries and consumed almost daily by more than 1 billion people (FAO, 2013) and is among the main vegetal products which can make it possible to combat poverty in the world (Lutaladio & Prakash, 2010).

It is one of the most demanding crops of fertilizers, due to its shallow and less developed root system that makes its area of exploration and nutrient mining limited. Potato crops need adequate strategies for disease control and fertilization to maximize yield. All of these chemicals could have adverse effects on the environment and health. There is a growing need for integrated potato cropping systems that combine different management strategies with reduced use of mineral fertilizers and pesticides (Maynard & Hochmuth, 2007).

According to the researchers, the shift towards more productive and less phosphate-based chemical inputs cannot be achieved without better management of biological interactions in agroecosystems, such as mycorrhizae (Plenchette et al., 2005; Adesemoye & Kloepper, 2009). They are root-associated symbiotics and show strong growth-promoting effects for most plants, including almost all edible crops, by increasing the contribution of available phosphorus from soil (P) and other nutrients. Im-

mobile minerals essential for plant growth, they are key players in the sustainable management of agricultural systems (Smith & Read, 2008). In several countries, inoculation practices have proved to be a very effective solution for increasing crop yields and reducing their need for phosphate mineral inputs (Hamel & Plenchette, 2007).

The use of arbuscular mycorrhizal fungi (AMF) to improve potato growth has been a focus of several studies (Hijri, 2006, Douds et al., 2007; Wu et al., 2013) which have shown the efficiency of these microorganisms for this culture. In Algeria, the potato sector in all its aspects of seeds and consumption now holds a strategic position in the new policy of agricultural and rural renewal, where its culture remains among the market gardening species which are fundamental for feeding strategy (FAO, 2015). It would be interesting to study the potential benefits of commercial mycorrhizal inoculants in this crop that could open up a very attractive market for these products.

In real field cropping conditions, the aim of this project is to determine the effect of mycorrhizal inoculation on certain biochemical parameters of potato plants for a better quality of tubers and to study the possibility of reducing the applications of chemical fertilizers for a better environment.

MATERIAL AND METHODS

Study area

The experiment has been carried out in a private agricultural field situated in the locality of Drean, Taref wilaya, Algeria (36°41'00"N, 7°45'00"E). The climate is of the Mediterranean type. The period of cultivation which runs from 13th January 2014 to 13th June 2014 is characterized by average maximum temperatures of 21 °C and minimum of 9 °C, total precipitation being 255 mm. The substrate chosen for the implementation of the experiment is a clayey soil having the preceding crop as wheat. These characteristics have been determined from a composite sample of 0-20 cm taken from deep soil. The physicochemical properties of the soil have been determined in the agricultural laboratory of FERTIAL (Annaba, Algeria): 28% sand, 44% clay, 24% silt; pH 7.1; 13.60 ppm available P; 0.76% total C; 0.99 meq / 100 g K; 35.35 meq / 100 g Ca; 5.22 meq / 100 g Mg; 0.87 meq / 100 g Na; 0.952% organic matter.

Vegetable material

The plant material consists of potato tubers (*Solanum tuberosum* L., Solanales Solanaceae), a Spunta variety fast-growing, giving large-sized tubers.

Fungal material

The fungal material used is a commercial mycorrhizal inoculum called Symbivit®, produced by a French company specialized in the development and distribution of mycorrhizogenic products (INOCULUM plus, France). It consists of a natural clay base and propagules of 6 species AMF (*Claroideoglomus etunicatum*, *C. claroideum*, *Glomus microaggregatum*, *Rhizophagus intraradices*, *Funneliformis mosseae*, *F. geosporum*).

Experimental device and processing of treatments

The complete random block device has been used, it comprises three blocks corresponding to the number of repetition, each block contains all the treatments, the distribution of the treatments within the same block was made randomly by drawing by lot. In a block there are six associated plots in pairs, each consisting of 4 rows which are 75 cm apart and six rows with a spacing of 20 cm between the plants. The surface area of each plot was 2.25 m^2 (2.2 m wide and 1 m long).

The treatments that have been applied are:

- inoculation with Symbivit and not (control) on plots without performing chemical contribution (0% NPK)
- inoculation and not on plots having been enriched in doses of localized fertilizers (50% NPK) corresponding to 30 g / plant
- inoculation and not with 100% (NPK), ie 60 g/plant.

The quantities of mineral and organic fertilizers introduced during the trial recommended in the Dréan area for potato production are: nitrogen in the form of amnonium nitrate (N33%:100 kg/ ha $^{-1}$) Phosphorus in the form of triple superphosphate

(P_2O_5 45%: 150 kg/ ha $^{-1}$) and potassium in the form of K_2SO_4 sulphate (K_2O 54%: 400 kg/ ha $^{-1}$).

Data collection

After three months of cultivation in the vegetative phase, 5 plants have been selected from each plot and the concentration of chlorophyll has been measured with a SPAD meters.

The rate of root colonization has been estimated and staining has been done by the technique of Phillips & Haymann (1970). The root samples are mixed and placed in 10% KOH solution, in a Marie bath at 90 °C for 1 hour, then rinsed and stained with 0.05% Trypan blue solution for 15 minutes. The roots have been observed on 5 replicates of root fragments of 1 cm length mounted between the blade and the slide in a drop of glycerol. The annotation has been made according to the method described by Trouvelot et al. (1986), using the MYCOCALC software (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

The protein content of the potato tubers has been estimated after harvest using the technique of Lowry et al. (1951), the absorbance readings have been taken at 660 nm.

The total amount of starch present in the potato tubers has been estimated using the anthrone method proposed by Hedge & Hofreiter (1962). Absorbance readings have been taken at 630 nm.

Methods of data statistical analysis

The description of the different studied characteristics of the plant is made by calculating the mean (m), the standard deviation (s) and the minimum (Xmin) and maximum (Xmax) values for each treatment.

The variance analysis (ANOVA) to a classification criterion of the Minitab software for the data statistical analysis (Minitab Inc, 2014) was used to compare the averages of the three doses of the fertilizer (NPK) for each studied characteristic (Dagnelie, 2009).

The TUKEY test (Dagnelie, 2009) made it possible to determine the groups of homogeneous doses by plant characteristics (Minitab Inc, 2014).

The DUNNETT test (Dagnelie, 2009) was used to compare the mean of the control dose with each of the averages of the other doses, for each parameter of the plant (Minitab Inc, 2014).

The STUDENT T test compared the averages of the two treatments using data from two independent samples (Dagnelie, 2009).

RESULTS

Table 1 illustrates the values of the statistical parameters obtained through characteristics and per dose of the fertilizer according to treatment (inoculated/non-inoculated) for the plant. The mean values and the standard deviations are represented graphically by histograms in figures 1–4.

The results of the analysis of variance (ANOVA) are presented in Table 2, showing a highly significant effect on the chlorophyll content of the potato leaves between the averages of the three doses of the fertilizer, either for the inoculated and non-inoculated plants.

A very highly significant difference has been found for the starch and protein content of inoculated tubers, and highly significant for non-inoculated tubers. As for the root colonization rate, it shows a significant difference for mycorrhizal plants and not significant for non-mycorrhizal plants.

The TUKEY test, following the rejection of ANOVA's hypothesis of equality of means, shows that there are two groups of homogeneous doses for chlorophyll (leaves), starch and protein (tubers) content for the inoculated or non-inoculated plant. For the root colonization rate there is a single group for the mycorrhizal plants and two groups for the non-mycorrhizae. The alphabetical letters (a, b) for the inoculated plants and (a ', b') for the uninoculated plants designate these groups (Figs.1–4).

Table 3 shows the results of the DUNNETT test, which shows that the 50% and 100% fertilizer doses give different results from the 0% dose for chlorophyll, protein and starch content in inoculated plants. In the case of non-mycorrhizal plants, the 0% dose and the 50% fertilizer dose are the same for the chlorophyll and starch content, with respect to the protein content of no inoculated tubers, the samples from the 0% fertilizer dose are different from those from the 50% and 100%. The colonization rate for the three doses of the fertilizer is identical for the case of the inoculated plants. For non-inoculated plants, the D0 is different from the two other doses.

The results of the STUDENT t test show that the combination of the mycorrhizogenic inoculum with the various doses of chemical fertilizers has got variable effects on the studied biochemical parameters of the potato. In the absence of fertilizer (0% CF), the difference between the inoculated and non-inoculated plants, for the potato leaf chlorophyll content and the amount of protein in the tubers, is not significant. On the other hand, the starch content of the inoculated tubers increases significantly compared to non-inoculated controls. The mycorrhization of the plants with half the amount of the fertilizer D50 is sufficient to get very highly significant differences in chlorophyll leaf content and in the starch and protein concentration of the tubers, with an increase of about 37%, 32% and 27% respectively. The differences are also very highly significant between inoculated and non-inoculated plants for the full dose of D100 fertilizer.

Mycorrhizal inoculation significantly increases the rate of root colonization whatever the level of fertilization is. However, the treatment (I + 0% CF) shows the highest rate with 47%. The root colonization in uninoculated plants shows the lowest level with 100% CF (20%).

DISCUSSION

This research was carried out with the aim of studying the efficiency of mycorrhizal inoculation in the field on potato cultivation and to evaluate the potential of the commercial inoculum to reduce the inoculum fertilizers.

The results show that the chlorophyll content value is higher in the case of mycorrhizal plants, the treatment combining the inoculum with 50% of the recommended fertilizer dose gives good results, besides the plants that have not received a fertilizer supply have shown poor chlorophyll rate, probably due to nutrient deficiency in the soil (Soltner, 1981). The inoculated plants are more likely to produce food through photosynthesis. Ruiz et al. (1996), Wright et al. (1998) suggested that mycorrhizal colonization helps the absorption of inorganic nutrients. Eissenstat et al. (1993) observed an increase in leaf area by arbuscular mycorrhizal fungi and therefore an increase in photosynthetic activity which explains an increase in chlorophyll content in the inoculated samples.

The protein content in the tubers has shown higher values in the mycorrhizal plants than in the controls. Our results are similar to the results obtained by Lenin et al. (2010) when studying the effect of arbuscular fungi on biochemical parameters and the growth evolution of four different vegetable crops. The increasing levels of protein in inoculated plants could be due to either the presence of fungal proteins or infectious stimulation of protein synthesis in the host plant (Krishna & Bagyaraj, 1983). Mycorrhizal and non-mycorrhizal plants are recognizable by a difference in the biochemical constitution, in particular in the amino acid and protein fractions (Nemec & Meredith, 1981).

Starch is one of the main important biochemical components in a potato tuber. We have observed that the inoculated plants contain higher concentrations of starch, in particular those which have received 50% of fertilizer; Wu & Xia (2006) also obtained similar results when the amount of starch was observed to be higher in the mycorrhizal plants.

The root colonization rate was higher in the inoculated plants than in the controls, as a commercial inoculum contribution probably favored the development of the mycelial network related to the roots of the inoculated plants (Smith & Read, 2008). We have observed a decrease in colonization of non-inoculated roots, especially for the 50 and 100% doses. Al-Karaki (2013) showed that increasing soil fertilization may reduce colonization of roots AMF and spore density.

CONCLUSIONS

Observation of the data set reveals that the additional contribution of mycorrhizogenic arbuscular fungi taken as inoculum significantly improves the quality of the potato. Combined with the chemical fertilizer at 50% of recommended doses, this experience gives better results. However, mycorrhizae alone will not compete with chemical fertilizers. The best combination would therefore be to reduce the use of synthetic fertilizers and to provide an inoculum in addition to the mycorrhizal populations already existing in the soil. This suggests that the advised application for chemical fertilizers could possibly be reduced at least by half by applying mycorrhizal inoculum.

Treatment	variables	Doses (NPK)	n	m	s	X_{min}___X_{max}
Inoculated	Chlorophyll	D_0	15	40.360	6.127	33.670 ___55.650
		D_{50}	15	56.868	3.029	46.650 ___ 57.300
		D_{100}	15	59.999	4.494	45.720 ___ 56.890
	Proteins	D_0	15	0.952	0.177	0.150 ___1.890
		D_{50}	15	1.977	0.364	1.780 ___ 2.890
		D_{100}	15	2.238	0.4982	2.470 ___ 2.960
	Starch	D_0	15	13.673	1.65	10.580 ___ 16.890
		D_{50}	15	18.738	2.551	14.360 ___ 19.650
		D_{100}	15	20.110	3.012	16.480 ___ 20.740
	Root mycorrhization rate	D_0	15	47.026	6.484	33.670 ___55.650
		D_{50}	15	41.099	7.239	46.650 ___ 57.300
		D_{100}	15	38.206	4.112	45.720 ___ 56.890
No inoculated	Chlorophyll	D_0	15	31.144	7.329	29.890 ___ 50.100
		D_{50}	15	35.670	7.256	23.760 ___ 46.780
		D_{100}	15	42.327	7.107	29.800 ___ 49.980
	Proteins	D_0	15	0.683	0.116	0.450 ___ 1.780
		D_{50}	15	1.424	0.167	1.780 ___ 2.120
		D_{100}	15	1.494	0.301	1.250 ___ 2.660
	Starch	D_0	15	10.820	1,487	10.850 ___ 16.360
		D_{50}	15	12.725	1.595	11.320 ___ 18.360
		D_{100}	15	15.704	2.597	15.210 ___ 20.120
	Root mycorrhization rate	D_0	15	29.216	3.206	18.250 ___ 30.450
		D_{50}	15	23.712	3.90	17.63 ___ 32.36
		D_{100}	15	20.275	3.620	16.23 ___ 30.960

Table1. The values of the basic statistical parameters calculated on potato plant samples; legenda: number of samples (n), mean (m), standard deviation (s), minimum values (Xmin), and maximum values (Xmax).

Treatment	variables	Source of variation	ddl	SCE	CM	F	P
Inoculated	Chlorophyll	doses	3	218.77	109.39	5.90	0.006**
	Proteins	doses	3	81.386	40.693	31.89	0.000***
	Starch	doses	3	160.814	80.407	28.70	0.000***
	Root mycorrhization rate	doses	3	37.39	18.69	0.26	0.023*
No inoculated	Chlorophyll	doses	3	112.88	56.14	1.26	0.007**
	Proteins	doses	3	181.456	90.728	42.58	0.003**
	Starch	doses	3	169.453	84.727	27.69	0.002**
	Root mycorrhization	doses	3	12.88	6.44	0.50	0.610 n.s

Table 2. Results of the analysis of variance (ANOVA) to a criterion. Legenda: number of degrees of freedom (ddl), sum of the squares of the deviations (SCE), mean square (CM), observed value of the Fisher F variable (Fobs.) and probability (P). N.S: there are no significant differences; *: there are significant differences ($p \leq \alpha = 0.05$); **: there are some highly significant differences ($p \leq \alpha = 0.01$); ***: there are very highly significant differences ($p \leq \alpha = 0.001$).

Treatment	variables	Means and doses identical to the control dose		
Inoculated	Chlorophyll	D_0	D_{50}	D_{100}
		40.36	56.86	59.99
	Proteins	D_0	D_{50}	D_{100}
		0.952	1.97	2.23
	starch	D_0	D_{50}	D_{100}
		13.67	18.73	20.11
	Root mycorrhization rate	D_0	D_{50}	D_{100}
		47.02	41.09	38.20
No inoculated	Chlorophyll	D_0	D_{50}	D_{100}
		31.14	35.67	42.32
	Proteins	D_0	D_{50}	D_{100}
		0.68	1.42	1.49
	Starch	D_0	D_{50}	D_{100}
		10.82	12.72	15.70
	Root mycorrhization rate	D_0	D_{50}	D_{100}
		29.21	23.71	20.27

Table 3. Results of the DUNNETT test. The averages of the underlined doses are identical to the mean of the control dose (D0).

Treatment	variables	Dose averages and homogeneous groups			Number of groups
Inoculated	Chlorophyll	$\overline{D_0}$ 40.36	$\overline{D_{50}}$ 56.86	$\overline{D_{100}}$ 59.99	2
	Proteins	$\overline{D_0}$ 0.952	$\overline{D_{50}}$ 1.97	$\overline{D_{100}}$ 2.23	2
	Starch	$\overline{D_0}$ 13.67	$\overline{D_{50}}$ 18.73	$\overline{D_{100}}$ 20.11	2
	Root mycorrhization rate	$\overline{D_0}$ 47.02	$\overline{D_{50}}$ 41.09	$\overline{D_{100}}$ 38.20	1
No inoculated	Chlorophyll	$\overline{D_0}$ 31.14	$\overline{D_{50}}$ 35.67	$\overline{D_{100}}$ 42.32	2
	Proteins	$\overline{D_0}$ 0.68	$\overline{D_{50}}$ 1.42	$\overline{D_{100}}$ 1.49	2
	Starch	$\overline{D_0}$ 10.82	$\overline{D_{50}}$ 12.72	$\overline{D_{100}}$ 15.70	2
	Root mycorrhization rate	$\overline{D_0}$ 29.21	$\overline{D_{50}}$ 23.71	$\overline{D_{100}}$ 20.27	2

Table 4. Result of the TUKEY test. Search for homogeneous dose groups.

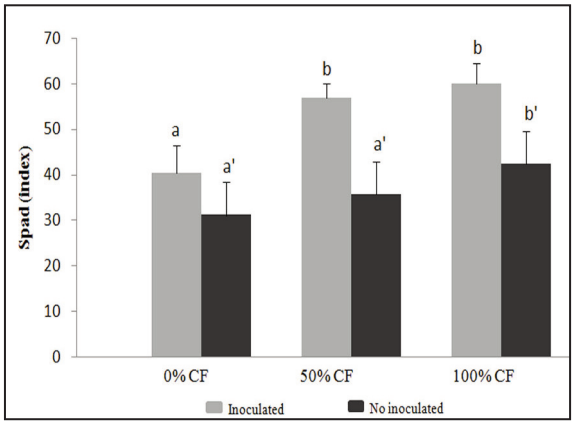

Figure 1. Chlorophyll content of potato leaves in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

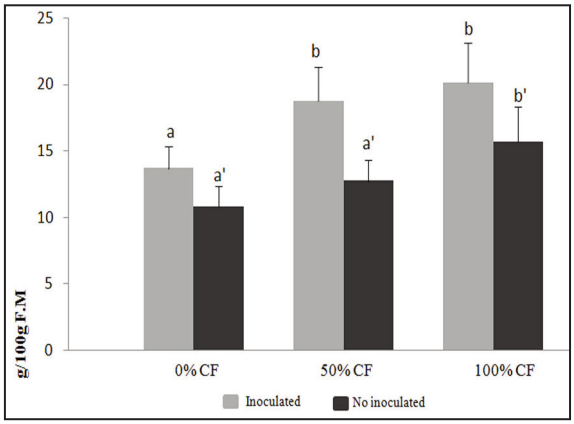

Figure 3. Starch content of potato tubers in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

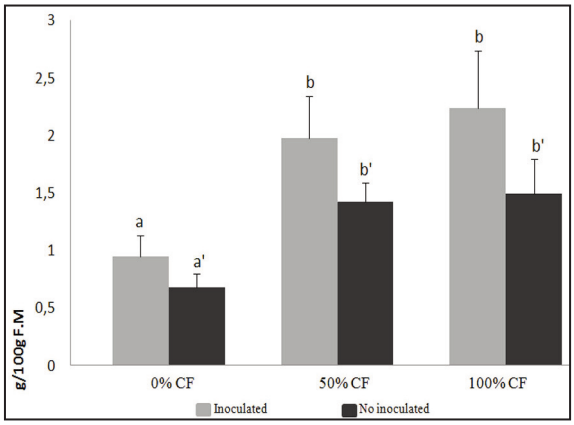

Figure 2. Protein content of potato tubers in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

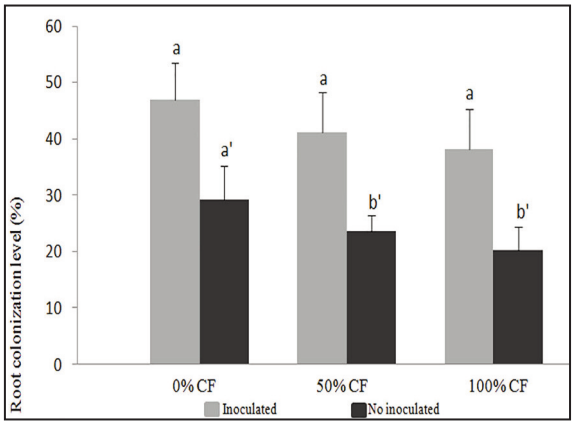

Figure 4. Total, arbuscular and vesicular colonization rates of potato roots in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

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