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Index

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Introduction

Capsicum annum L pepper is one of the most widespread cultivars in Almeria (Spain). In this region, three main pepper varieties are cultivated: Italian Sweet Pepper, Lamuyo and California. They cover a greenhouse area of 7000 ha in this region. For achieving a good fruit yield and quality, an exacting treatment with phytosanitary treatments, good quality water, high nutrient concentrations and soils rich in organic matter (from 3 to 4%) should be guaranteed.

Products which optimize the water use efficiency and the plant nutrition and yield are increasingly used in current intensive agriculture. These inoculants include the arbuscular mycorrhizal fungi (AMF) which establish a symbiotic relationship with 85% of plants (Smith and Read, 1997) and improve their influence depending on the soil where hey are grown (Wu and coll., 2007; Herrera and coll., 2011). AM fungi are important in agriculture because of the benefits they provide such as the improvement of nutrient and water uptake and the increase of the plant's root system (Rivera and coll., 2005; Hamel and Plenchette, 2007).

Although mycorrhizal colonization provides a bioprotectional effect on plants, its commercialization is not yet a widespread practice in agriculture due to its inappropriate usage and the low resistance of the varieties planted following an agricultural system based on a nutrition intense management (Gadkar and coll., 2001; Herrera and coll., 2011).

The commercial mycorrhizal inoculant MycoUp, produced by Symborg S.L., should be applied by a drip irrigation system. This product contains an arbuscular mycorrhizal fungus, *Glomus iranicum var. tenuihypharum var. nova*, isolated from a sodium-saline soil and high production of external mycelium, intense root colonization and resistance to high nutrient concentrations (Fernández and Juárez, 2012).

The aim of this research is therefore to assess the influence of MycoUp in the nutrients of soils and plant yield and quality of a pepper cultivar.

Material and methods

Experimental conditions

The research was carried out at a greenhouse which covers an area of 1 ha located in El Ejido (Almería). The plant material of the experiment was a California pepper variety, Godzila. On July 23th 2011, pepper seedlings were sown following a 0.5 x 0.3 planting frame with rows separated at 1m (Picture 1). The irrigation consisted of a drip system with outlets of 2L H^{-1} discharge rate and spaced at 0.40 m. The water used has good quality and medium-low electrical conductivity (EC=1.5 dS/m). The sandy soil used for cultivation followed the cultural practices in Almeria.

Treatments and dosage

The experimental design consisted of two treatments: a T0 control treatment without inoculant and a T1 treatment with MycoUp applied at the first stages of the experiment (at a week after sowing) with a 3Kg/ ha dosage.

Picture 1: Pepper rows planted in greenhouse conditions

Mycorrhizal colonization measurements and leaf nutrient content

Samples of roots were collected at the beginning and the end of the experimental period (at 56 and 281 days after sowing –DAS-). Samples were cleaned with deionised water and clarified with a 10% KOH solution during 10 minutes at 90°C. After that, the samples were treated with HCl 2N during 10 minutes and stained with a 0.05 Lacto-glycerol solution, Trypan blue. The methodology followed in this investigation meant a change in the Phillips and Hayman (1970) guidelines. Root mycorrhizal colonization was evaluated by using the Giovannetti and Mosse's (1980) intersect method.

Leaf samples were also evaluated at the beginning and the end of the research in order to analyze the bioelement (macro and micro nutrients) content and the crop's nutritional status.

Plant water potential and gas exchange

Stem water potential (ψ_t) and gas exchange parameters (net photosynthesis Pn and leaf conductance g_s) were measured in 10 plants per treatment by using the Scholander pressure chamber PMS 600 (Corwallis, Oregon, USA) and the LI-6400 Portable Photosynthesis System (Lincoln, NE, USA). The measurements were taken at midday (12:00 GMT) selecting well developed leaves of the pepper plants during the first four months of the growing stage and every two months during the end of the cycle. The water use efficiency (WUE) was measured by using the Pn/g_s quotient.

Crop quality measurements and final production

Measurements of the total production and the crop quality from each treatment were taken. During the five cuts made from December until the end of April, the following fruit characteristics were taken into account: weight per unit, peel thickness, soluble solids content (Atago refractrometer), firmness (PCR PTR-200 penetrometer) and fruit color (Konica Minolta Sensing CR-10 colorimeter).

Results and discussion

Fungal colonization and water status

From the early stages of the experimental period, the root colonization of inoculated plants was positively influenced by the arbuscular mycorrhizal fungi (AMF) ranging from 17.5 to 50% at the beginning and at the end of the research, respectively. AMF inoculant affected the root system during the whole growing cycle. In the T0 control treatment, plants hardly had root colonization due to the soil disinfection and the low levels of native AMF. The values obtained in each treatment are shown in Table 1.

Table 1. Colonization values (%) in pepper plants of the T0 control treatment and T1 MycoUp treatment at 56 and 281 days after seeding (DAS).

Date	T0	T1
56 DAS	0.5a	17.5b
281 DAS	1.5a	50.0b

Colonization values followed by different letters show significant differences according to the Duncan test (P=0.05). These values are the average obtained from six samples tested during both harvests.

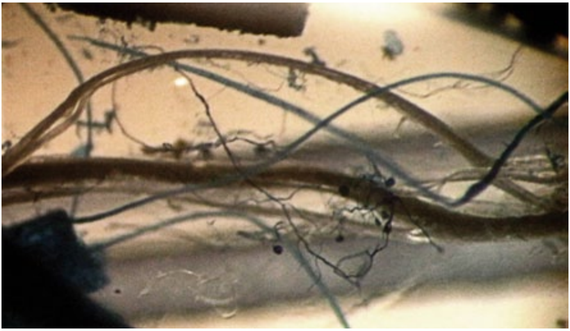
Water status values were better in inoculated plants due to the higher colonization percentage they reached when compared to T0 control treatment's pepper. The difference was higher at the end of the research (Table 2). These results are in accordance with published investigations which confirm that the mycorrhizal inoculants had a positive influence in the plant's water status (Ruiz Lozano and col., 2003; Augen, 2004; Sánchez-Blanco and coll., 2004; Aroca and coll., 2008).

Table 2. Stem Water Potential (ψ_t, MPa) in T0 control treatment and T1 MycoUp treatment pepper plants.

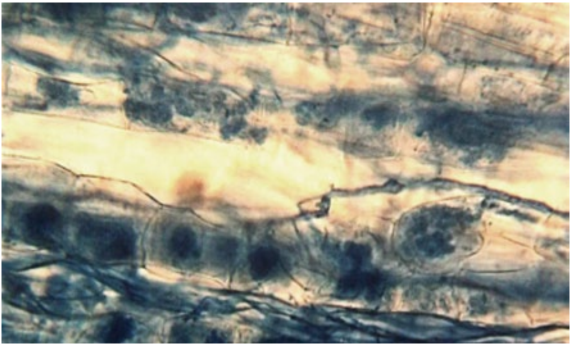
Date	T0	T1
30 DAS	-0.37±0.14a	-0.21±0.10b
59 DAS	-0.46±0.04a	-0.41±0.04a
93 DAS	-0.41±0.06a	-0.29±0.04b
150 DAS	-0.69±0.06a	-0.55±0.07b
205 DAS	-0.75±0.17a	-0.59±0.12b

ψ_t values followed by different letters show significant differences according to the Duncan test (P=0.05). These values are the average obtained from six samples tested.

In the following pictures, fungal colonization of pepper plants treated with MycoUp and arbuscules viewed under a microscope can be observed. Pictures were taken at 270 days after the inoculant treatment application (Pictures 2 and 3).



Picture 2: Detail of external mycelium,spores and roots of mycorrhizal plants.



Picture 3: Arbuscule of mycorrhizal pepper plant's root.

Nutrient content and leaf gas exchange

Leaf biolements values differed between treatments (Table 3). Samples taken at the beginning and at the end of the experimental period showed higher nitrogen and phosphorus (N and P) levels in inoculated plants, but no significant differences were found.

Table 3. Biolement content: macroelements (N, P, K, Ca and Mg, %) and microelements (Fe, Zn and Mn, ppm) in leaf dry matter of T0 control treatment and T1 MycoUp treatment plants.

Treatments	N	P	K	Ca	Mg	Fe	Zn	Mn
T0	4.2a	0.28a	4.0a	4.6a	1.0a	144a	104a	147a
T1	4.5a	0.30a	4.0a	4.5a	1.0a	175a	104a	134a

Values for the same type of bioelement and leaf followed by different letters are significantly different according to LSD $_{0.05}$ test.

Micronutrients levels (Fe and Mn) rose in peppers treated with the inoculant reaching a significant increase of Fe values. The rise of leaf nutrient content has been shown in previous literature (George, 1991; Ortas and Akpinar, 2006). It is important to mention that ion's acquisition in mycorrhizal colonization depends on the experimental conditions, type of fungus, soil, pH, nutrients and soil temperature (Treeby, 1992; Clark and Zeto, 1996; Raju and coll., 1990). Effectiveness achieved in this research was closely related to the AMF species used.

Gas exchange levels did not differ between treatments throughout the investigation (Table 4). However, these levels were significantly different during two periods: at 2 months after sowing and at the end of the research. In the early stages, the photosynthesis levels (Pn) increased by 11.1% in inoculated plants causing a rise of the water use efficiency (WUE) values. In the later stages, differences between treatments increased significantly reaching the highest levels of Pn and stomatal conductance (gs) in the inoculated peppers. The visual parameters of ageing during the coldest days in February had a higher influence on uninoculated plants than in plants treated with AMF due to a better defense system which enhance the resistance to stress conditions such as low temperatures (Xian-Can and col., 2010).

Table 4. Gas exchange parameters: net photosynthesis (Pn, µmol m-2 s-1), stomatal conductance (g_s, mmol m-2 s-1) and water use efficiency (WUE, µmol CO2 mol-1 H2O) in T0 control treatment and T1 MycoUp treatment's leaves.

Date	Treatment	Pn	Gs	WUE
30 DAS	T0	10.8a	450.4a	24.0a
	T1	11.1a	449.9a	24.7a
59 DAS	T0	18.1a	575.9a	31.4a
	T1	20.1b	547.8a	36.7b
93 DAS	T0	19.8a	445.0a	44.5a
	T1	18.7a	479.8a	39.0a
142 DAS	T0	13.6a	276.1a	49.4a
	T1	13.8a	270.3a	51.1a
205 DAS	T0	4.3a	164.9a	25.8a
	T1	8.7b	282.7b	30.7a

Values of each column followed by different letters show significant differences according to the multilevel Duncan test (P=0.05). These values are the average of 10 sample tests.

Crop yield: fruit quality and productivity

Cultural practices of the area consist of keeping the fruits on the plants until mid winter (February), when the product reaches the highest prices in the market (during the 2011 season, pepper's price increased to 1.5 €/kg). For this reason, although a cut of few kilos was made in mid-December, most of the peppers (64% of the total) were harvested in midwinter (from February to mid-Mars). During the investigation period, 40 plants per treatment were randomly selected and studied through two replications in different areas of the greenhouse.

Inoculation with MycoUp resulted in a gradual growth of plants throughout the experiment, reaching the 48% during the third harvest (Table 5) which meant an average growth of 15.29%. The final yield increased from 7.39 kg/m2 in T0 control treatment to 8.52 Kg/m2 in MycoUp treatment. These results were in accordance with previous findings conducted into lettuce crops (Vicente-Sánchez, 2011), rice (Fernández and coll., 2010), tomato, strawberry plants, aubergines, cucumber, melon and other fruits (data not shown). Besides the conclusions achieved in this investigation, previous literature confirms the role played by inoculants in the increase of productivity when the symbiotic relationship is functional and efficient, moreover with low inputs and extensive farming (Terry and coll., 1998; Fernández and coll., 2005; Hamel and Plenchette, 2007; Herrera and coll., 2011).

As for fruit quality, no significant differences between harvests were found. The firmness, soluble solids content, thickness and color parameters (HUE, Chroma and L*) were similar in both treatments. The weight per unit rose however 5.4% in inoculated pepper plants during the first and third cuts (data not shown).

Table 5. Average production values (kg/m^2) and increase (%) during the different harvest periods in T0 control treatment and T1 MycoUp treatment.

Date	Treatment	Production	Increase
13th December 2011 (142 DAS)	T0	0.64	10.9
	T1	0.71	
14Th February 2012 (205 DAS)	T0	2.81	1.42
	T1	2.85	
7th Mars 2012 (227 DAS)	T0	1.19	21.8
	T1	1.45	
23rd Mars 2012 (243 DAS)	T0	0.75	48.0
	T1	1.11	
30th April 2012 (281 DAS)	T0	2.00	20.00
	T1	2.40	
Total	**T0**	**7.39**	**15.29**
	T1	**8.52**	

Conclusions

Arbuscular mycorrhizal *Glomus iranicum var. tenuihypharum* contained in MycoUp influenced significantly the plant physiology (better water status and higher gas exchange levels) and the productivity of the pepper crop cultivated under greenhouse conditions and following the cultural practices of the area, El Elejido (Almería). Inoculant not only was efficient at early stages but throughout the whole experiment, enhancing the nutrient uptake and physiological characteristics and improving the plant's defense system to stress conditions such as the low winter temperatures.

Acknowledgements

The investigation's authors gratefully acknowledge the scientific support and collaboration of Juan José Vargas during all the experimental process in El Ejido, Almeria.

References

Aroca, R, Vernieri P, Ruiz-Lozano JM. 2008. Mycorrhizal and non-mycorrhizal Lactuca sativa plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. Journal of Experimental Botany, Vol. 59, No. 8, pp. 2029–2041.

Augé RM. Arbuscular mycorrhizae and soil/plant water relations. 2004. Canadian J Soil Sci. 84: 373-381.

Berta G, Fusconi A, Trotta A, Scannerini S 1990. Morphogenetic modifications induced by the mycorrhizal fungus Glomus strain E3 in the root system of *Allium porrum* L. New Phytology 114:207–215

Clark RB, Zeto SK 1996. Iron acquisition by mycorrhizal maize grown on alkaline soil. J Plant Nutr19 : 247–264.

Fernández F, Dell'Amico JM, Alarcón JJ, Nicolás, E, Pedrero, F. 2010. Mejora del rendimiento del cultivo del arroz mediante la aplicación de micorrizas aisladas en condiciones salinas. Agricultura Diciembre 916-926.

Fernández F, Juarez J. 2012. Proceimiento de obtención de un agente micorrizógeno. Patente Europea. No: ES20110030566 20110411.

Fernández F, Rivera R, Herrera R, Furrazola E. 2005. Efecto de la inoculación dehongos micorrízicos arbusculares y diferentes relaciones suelo: humus de lombriz sobre el crecimiento del cafeto *(Coffeaarabica*. L) var. Catuaí bajo la etapa de vivero en Cuba. Revista Chapingo. Serie Horticultura. Revista Chapingo: Serie

Horticultura. Vol. 11, no. 1 (enero-junio 2005), pp. 175-184. 0186-3231.

Gadkar V, David-Schwartz R, Kunik T, Kapulnik Y 2001. Arbuscular mycorrhizal fungal colonization. Factors in- volved in host recognition. Plant Physiol. 127:1493–1499.

George E, Römheld, Marschner H 1994. Contribution of mycorrhizal fungi to micronutrient uptake by plants. In: Manthey JA, Crowley DE, Luster DG (eds.)

Biochemistry of metal micronutrient in the rhizosphere. CRC, Boca Raton, Fla, pp. 93-109.

Giovannetti M, Mosse B. 1980. An evaluation of technique for measurement vesicular arbuscular mycorrhizal infection in roots.New Phytologist 84: 489-500.

Hamel, C and Plenchette, C. 2007. Mycorrhizae in Crop Production (eds.). Chantal

Hamel and Christian Plenchette. Haworth Press, Binghamton, NY Hard Cover ISBN: 978-1-56022-306-1 (CL).

Herrera RA, Hamel C, Fernandez F, Ferrer, RL Furazola E. 2011. Soil-strain compatibility: the key to effective use of arbuscular mycorrhizal inoculants? Mycorrhiza 21: 183-193.

Ortas I, Akpinar C. 2006. Response of Kidney bean to arbuscular mycorrhizal inoculation and mycorrhizal dependency in P and Zn deficient soils. Acta Agriculture Scandinavica, Section B- Plant Soil Sci. 56: 101-109.

Phillips DM, Hayman, D.S.(1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55: 158-161.

Raju PS, Clark RB, Ellis JR, Maranville JW 1990. Effects of species of VA-mycorrhizal fungi on growth and mineral uptake of sorghum at different temperatures. Plant & Soil 121: 165-170.

Rivera R.A, Fernández, F. 2005. Inoculation and management of mycorrhizal fungi within tropical agroecosystems. Chapter 33. In: Biological approaches to sustainable soil systems. Norman Uphoff. Cornell University, New York. 479-488.

Ruiz-Lozano JM. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectivesfor molecular studies. Mycorrhiza 13: 309–317.

Sánchez-Blanco MJ, Ferrández T, Morales A, Morte A, Alarcón JJ. 2004. Variations in water status, gas exchange and growth in *Rosmarinus officinalis* plant infected with *Glomus deserticola* under drought conditions. J Plant Physiol. 161: 675-682.

Sánchez-Vicente, J. 2011. Efecto del riego con aguas regeneradas sobre el crecimiento y estado nutricional de plantas de lechuga "iceberg" micorrizadas con el hongo *Glomus iranicum var. tenuihypharum*. Tesis de Master. Facultad de Biología. Murcia.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis, 2nd edn.Academic Press, London.

Terry, E.; Núñez, M.; Pino, M.A.; Medina, N. (2002). Efectividad de la combinación biofertilizantes-análogo de brasinoesteroides en la nutrición del tomate (Lycopersicum esculentum Mill). Cultivos Tropicales 22 (2): 56-59, 2002.

Treeby MT. 1992. The role of mycorrhizal fungi and non-mycorrhizal microorganisms in iron nutrition of citrus. Soil Biol Biochem. 24: 857–864.

Wu QS, Zou YN, Xia RX, Wang MY. 2007. Five Glomus species affect water relationsof Citrus tangerine during drought stress. Bot Stud 48:147–154.

Xian-Can Zhu, Feng-Binn Song, Hong-Wen Xu. 2010. Arbuscular mycorrhizae low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil. 331: 129-137.

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