GROWTH-PROMOTION EFFECT OF *TRICHODERMA* SP. ON HORTICULTURAL SEEDLINGS DEPENDS ON INOCULUM AND SUBSTRATE TYPE

EFECTUL DE STIMULARE A CREŞTERII AL *TRICHODERMA SP.* ASUPRA RĂSADURILOR HORTICOLE ÎN FUNCTIE DE INOCUL ŞI TIPUL DE SUBSTRAT

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Abstract. *Fungi in the genus Trichoderma have been reported to have biostimulant and biofertilizer qualities in many types of horticultural crops, but the effects are highly variable. Thus, practical use of Trichoderma sp. requires feasible formulated products and suitable substrates. The aim of this study was to evaluate the survival and the growth-promotion effect of a Trichoderma sp. rice formulation compared with the incorporation of this fungi as a nonformulated conidia suspension, both at three different dosis (7 treatments in total), on tomato, pepper and cucumber seedlings grown in two substrates: 1) rich in organic matter (OM) and 2) mineral substrate without OM. Three replicates of each treatment were performed, and the trials were carried out twice. The results showed beneficial effects on seedling growth in the OM-rich substrate when Trichoderma sp. was applied by the formulated form (mainly at maximum concentration), but the effects were opposite when the mineral substrate without OM was used. The effects were closely linked to the survival and growth of the fungus, and therefore to the level of inoculum in the substrate, which was greater on application of the formulated one as opposed to the nonformulated one. These results provide evidence of the complexity inherent in the use of microorganisms in agriculture, while also confirming that the activity of the biofertilizers based on Trichoderma sp. depends on the type of inoculum and its concentration, as well as the properties of the medium in which the fungi develop.*
Keywords: *biostimulant, biofertilizer, in vitro, cucumber, pepper, tomato*

Rezumat*. Ciupercile genului Trichoderma sunt considerate ca având calități de biostimulator şi biofertilizant, însă cu efecte variabile. Astfel folosirea Trichoderma sp pentru a fi fezabilă necesită utilizarea diverselor substraturi sustenabile. Scopul acestui studiu a fost acela de a stabili influența diverselor substraturi şi tipuri de inocul de Trichoderma sp. A fost studiată dezvoltarea pe inocul de orez în comparație cu o soluție de conidii în suspensie, ambele în diferite doze (7 tratamente în total) administrate pe culturi de răsaduri de tomate,*

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ardei şi castraveți. Substratul pe care au crescut răsadurile a fost de două tipuri 1) bogat în materie organică (OM) şi 2) substrat mineral fără materie organică (OM). Au fost stabilite trei variate cu două repetiții fiecare. Rezultatele au arătat efectele benefice asupra creşterii răsadurilor în cazul folosirii variantei Trichoderma sp. pe substrat îmbogățit cu materie organică cu inocul pe orez (mai ales la folosirea concentrației maxime), însă efectul a fost opus când a fost utilizat substratul fără materie organică. Rezultatele au fost strâns legate de de supraviețuirea şi creşterea ciupercii, de concentrația ciupercii din substrat care a fost mai ridicată la variata cu orez față de cea cu soluții de conidii. Aceste rezultate oferă dovada complexității inerente a utilizării microorganismelor în agricultură, în timp ce, confirmând că activitatea ca biofertilizator bazat pe Trichoderma sp. depinde de tipul de inocul şi concentrația sa, precum şi proprietățile mediului în care ciupercile se dezvoltă.

Cuvinte cheie: *biostimulator, biofertilizator, in vitro, castraveți, ardei, tomate*

INTRODUCERE

Fungi in the genus *Trichoderma* show qualities that allow them their natural presence in all kind of soils (Papavizas, 1985; Widden and Scattolin, 1988; Jackson *et al*., 1991). Likewise, their development is favoured by the presence of roots, to which they colonise quickly, and establish chemical stimulants that modify how the different genes behave in the plants (Harman *et al*., 2012). In this way, species belonging to *Trichoderma* genus are considered as endophytic simbionts with diversity of properties beneficial for plants (Benítez *et al*., 2004; Harman *et al*., 2012). *Trichoderma* spp. has been studied during decades as a biological control agent (BCA) (Weindling, 1934; Mukherjee *et al*., 2013), and specialised literature mainly highlights the existence of several species used for biocontrol of soil phytopathogen organisms, the aerial and post-haverst parts through different mechanisms, to which biostimulant and biofertilizer qualities are shown to generate a growth promoting effect, on seedlings as well as adult plants, and even a production increase (Chang *et al*., 1986; Windham *et al*., 1986; Harman *et al*., 1989, 2004, 2012; Lo *et al*., 1996; Björkman *et al*., 1998; Altomare *et al*., 1999; Harman, 2000, 2011; Yedidia *et al*., 2001; Benítez *et al*., 2004; Savazzini *et al*., 2009; Mastouri *et al*., 2010; Shoresh *et al*., 2010). In this respect, the effects are very variable, and depend mainly on the strain used, the considered crop and its growth conditions, as well as on the rate and type of inoculum, and the substrate used (Lindsey y Baker, 1967; Ahmad y Baker, 1987; Kleifeld y Chet, 1992; Ousley *et al*., 1994; Martínez-Medina *et al*., 2009; Bashan *et al*., 2014; Rajput *et al*., 2014 a, b). In this way, one of the main limitations in the application of BCA such as *Trichoderma* spp., is the development of commercial preparations formulated properly (Fravel, 2005; Bashan *et al*., 2014; Rajput *et al*., 2014a). The development of new effective formulations for the inoculation of microorganisms is a very slow process that has been widely ignored, only another interest shown has been in the evaluation of patent formulations, and such formulations are useless to a large extent, apart from the manufactures' exhibition to the public (Tello *et al*., 2011). While many studies describe plant inoculation with microorganisms, only a few of them study in depth the formulations and the application methods that are hidden between materials and methods. Therefore, the

advantages and/or disadvantages of each formulation are seldom investigated in depth (that is, amounts, conditions, relation of mixtures), nor the improvements in the production process (Bashan *et al*., 2014).

Usually, trials with *Trichoderma* spp. under controlled conditions use solid as well as liquid inocula with concentrations included in the order of 10^4 and 10^9, and referred to propagules (mainly conidia) and also to Colony-Forming Units per g or mL respectively (Sivan *et al*., 1984; Ousley *et al*., 1994; Bernal-Vicente *et al*., 2009; Martínez-Medina *et al*., 2009), while in greenhouse and land the recommended concentration must not be lower than 10^8 (Cooney *et al*., 1997). With reference to the substrate used, one of the main obstacles to use *Trichoderma* efficiently is the maintenance of a high inoculum density after its application (Kleifeld and Chet, 1992; Martínez-Medina *et al*., 2008). For this reason, considering the saprophytic nature of fungi belonging to *Trichoderma* genus, a substrate with high content of organic matter could favour the growth, reproduction and maintenance of the fungus (Rajput *et al*., 2014b). Consequently, the application of the fungus through solid inoculum obtained from its reproduction on organic materials such as cereals (that is, rice, barley, wheat, oats, sorghum), could be favourable because these materials provide food for fungi, which allow them to stay in the source substrate keeping a higher level of inoculum, even under unfavourable conditions for their establishment (Martínez-Medina *et al*., 2009). However, when using liquid inoculum is more important to have favourable conditions and a substrate rich in organic matter (OM) to avoid possible losses (Bernal-Vicente *et al*., 2009; Franco *et al*., 2014).

Therefore, as agriculture cannot support inoculants with high production costs or expensive support materials, it is important to make effective formulations that are also economically profitable. In this way, given that *Trichoderma* spp. has the capacity of being cultivated on different substrates, and rice grain is an economic substrate and one of the most used in mass production under In Vitro conditions (Hjeljord and Tronsmo, 1998), it is interesting to evaluate the potentiality of adding *Trichoderma* sp. through solid rice formulation. As far as we know, there is not any specialised literature that considers this type of inoculum for the evaluation of possible beneficial effects on plants and/or seedlings. Its use would help, among other aspects, to reduce preparation labours of commercial formulations. For this reason, the purpose of this work was to evaluate the effects on tomato, pepper and cucumber seedlings growth after the addition of *Trichoderma* sp. through different inocula (liquid and solid) in two different substrates, one mineral without OM and the other rich in OM and commonly used in commercial nurseries.

MATERIAL AND METHOD

Vegetable Material

Assessments were carried out for the three most significant horticultural species in the study area crops: tomato (*Solanum lycopersicum* L. cv. Río Grande; Ramiro Arnedo S.A.), pepper (*Capsicum annuum* cv. Sonar; Clause Spain S.A.) and cucumber (*Cucumis sativus* cv. Marketmore 76; Ramiro Arnedo S.A.).

***Trichoderma* sp. Origin and Inocula preparation**

Trichoderma sp. was isolated from the agricultural soils of Almería and sown in Petri dishes which contained PDA medium (Potato-Dextrose-Agar) and incubated during 7 days at 28ºC. After the incubation period, fifteen mL of a preservative solution (KCl at 10%) were added to each Petri dish and was scraped with a lancet on the colony to detach the spores. The resulting solution was deposited in 250 mL flasks to which 3 drops of Tween® 80 (biological detergent) were added to decrease the surface tension and make easier the release of spores or conidia. In this way, a fungus suspension which contained 10^8 conidia·mL^{-1} was obtained and used for the preparation of the inocula used in the trials. Then, the fungus was multiplied in commercial round grain rice, given that in previous evaluations, differences were not shown in the production of spores when using other types of rice (Table 1). To that end, aluminium trays (146x121x40 mm) which contained 50 g. of rice and 25 mL of distilled water were autoclaved (120ºC/21 minutes) and then inoculated with 10 mL of fungus suspension. After a 21 day period of incubation in darkness at 23-25 ºC until arriving at the stationary phase, a concentration of the order 10^9 conidia·g^{-1} was reached.

Table 1

Production of *Trichoderma* sp. conidia according to the different types of rice used as solid substrate for the fungus growth and multiplication. The results show the average ± typical deviation. Values obtained from the three counting carried out with Neubauer chamber of the conidia present in each of the three repetitions of each type of rice.

Type of rice	Production of conidia (x10^9 conidia·g^{-1})
Long	4,7±0,8a[x]
Round	4,1±0,8ab
Brown	3,6±0,6b
p-value	*

[x] *Values in the same column with different letters shows significant differences through Tukey's test (HSD) honestly significant difference test. * significance at p≤0,05.*

Trials description

Each horticultural species was sown separately at a rate of three tomato seeds, one of pepper, and one of cucumber, in 175 mL volume pots (experimental unit). Seeds were previously disinfected through immersion in a commercial dissolution of sodium hypochlorite at 20% during 15 minutes, and then they were rinsed and soaked during 48 hours in a wet chamber before sowing. Trials were carried out during 30 days in a controlled environmental chamber at 12.000 lux, photoperiod of 14 h light/day and maximum and minimum temperatures of 24,9±0,6 and 21,2±0,8 ºC respectively. Plants were fertilised on demand with a common fertilising solution (NPK 19-19-19) at a rate of 1g·L^{-1}.

Sowing was made in two different substrates: 1) vermiculite, mineral substrate without organic matter (OM) and 2) substrate rich in OM (60% blonde peat, 15% black peat, 15% coconut fiber, 10% Perlite) which is commonly used in commercial nurseries for seedling growth. To each substrate *Trichoderma* sp. was added at different concentration using 2 types of inoculum: 1) solid: *Trichoderma* sp. rice formulation and 2) liquid: nonformulated conidia suspension. The treatments with solid inoculum consisted of adding rice colonised by fungus at a rate by weight of 1%, 5% and 10% to the substrates. Treatments with liquid inoculum were prepared from a conidia suspension obtained from the same rice colonised by fungus and adjusted from successive dilutions at 10^8, 10^7 and 10^6 conidia·mL^{-1}. For each type of substrate, the control treatment consisted of sowing the horticultural species without adding

Trichoderma sp. In this way, seven treatments were considered in the trials (Table 2). Three repetitions of each treatment were carried out for each horticultural species and substrate. Trials with each substrate were repeated twice over time.

Table 2

Treatments with *Trichoderma* sp. carried out in the trials for the evaluation of the growth-promoting effect on tomato, pepper and cucumber seedlings. Treatments consist of adding *Trichoderma* sp. to the substrate (vermiculite and commercial nursery substrate) through solid and liquid inoculum at different concentrations.

Treatment	Type of inoculum	Concentration	Dosis[z]
T1	Control	--	--
T2	Solid	1% p/p[x]	0,8 g
T3	Solid	5% p/p	4,0 g
T4	Solid	10% p/p	8,0 g
T5	Liquid	10^8 conidia·mL^{-1} [y]	1,75 mL
T6	Liquid	10^7 conidia·mL^{-1}	1,75 mL
T7	Liquid	10^6 conidia·mL^{-1}	1,75 mL

[x] *% by weight of solid inoculum (10^9 conidia·g^{-1}) in the substrate*
[y] *through counting in Neubauer chamber*
[z] *corresponds with the amount of inoculum added, according treatment, in each experimental unit constituted by 175 mL container with 80 g of substrate.*

Microbiological Analysis of Substrates

The successive-dilutions method was used to know the microbiological composition of substrates when trials ended (Tello *et al*., 1991). The culture medium used was acidified malt extract agar. Ten replications of each sample for each of the treatments carried out and dilution were made. Incubation was carried out in the laboratory at room temperature during 2-5 days. After the indicated time, the counting of total Colony-Forming Units (CFU) present in each repetition was carried out.

Evaluated parameters in seedlings

Several variables (plant height, fresh and dry weight, leaf length and width, among others) were measured in previous trials (Marin-Guirao *et al.,* unpublished data). Finally, plant height and dry weight were taken as being the most representative and constant variable (Guerrero *et al*., 2014)

To obtain the dry weight, the washed plants were dried in a muffle oven at 72°C for 48 h until constant weight as measured in a Mettler Toledo PB 303-S analytical scale with a precision of 0.001 g.

Statistical Analysis

The analysis carried out for the comparisons between treatments consisted of simple analysis of variance ANOVA. The method used for the comparison of the average was the procedure of Tukey's test (HSD) honestly significant difference test at 95% of confidence. The results are shown for each horticultural species as the average of the two trials carried out over time and depending on the substrate used. The statistical package used was Statgraphic Plus 5.1 (Manugistic Incorporate, Rockville, MD, USA) for Windows.

RESULTS AND DISCUSSIONS

The results are classified by sections according to the aspects and parameters considered in the materials and methods.

Level of inoculum of *Trichoderma* sp. in the substrates

When trials ended with the mineral substrate made of vermiculite and the substrate rich in OM of the nursery, and in the case of all the horticultural species evaluated, the treatments through solid inoculum showed a inoculum level higher than the treatments with liquid inoculum (Table 3).

Table 3

Level of inoculum of *Trichoderma* sp. present in the nursery and vermiculite substrate 30 days after inoculations. Results according to the treatments carried out with solid and liquid inocula at different concentrations, as well as, of the horticultural species grown during the trial

	CFU *Trichoderma* sp.·g^{-1}					
	Tomato		Pepper		Cucumber	
Treatment	Substrate	Vermiculite	Substrate	Vermiculite	Substrate	Vermiculite
T1:Control	7,4·10^4 c	--	3,0·10^4 d	--	2,1·10^4 d^y	--
T2:Solid 1% p/p^x	4,5·10^6 a	2,2·10^6 c	5,6·10^6 a	5,2·10^6 c	4,4·10^6 a	2,2·10^6 c
T3:Solid 5% p/p	6,0·10^6 a	4,7·10^7 b	8,3·10^6 a	2,8·10^7 b	6,0·10^6 a	8,9·10^7 b
T4:Solid 10% p/p	9,0·10^6 a	1,8·10^8 a	9,6·10^6 a	1,7·10^8 a	7,3·10^6 a	3,0 10^8 a
T5:Liquid 10^8 conidia/ml	4,8·10^5 b	3,1 10^4 d	5,4·10^5 b	1,0 10^5 d	2,4·10^5 b	1,2 10^5 d
T6:Liquid 10^7 conidia/ml	4,8·10^4 c	2,3 10^4 ed	6,6·10^4 c	3,6 10^4 e	5,4·10^4 c	2,7 10^4 e
T7:Liquid 10^6 conidia/ml	4,7·10^4 c	1,2 10^4 e	4,7·10^4 c	1,3 10^4 f	3,7·10^4 c	1,6 10^4 f
p-value	***	***	***	***	***	***

^{x} % by weight of Solid inoculum (10^9 conidia/g) in the substrate

*^{y} Values in the same column with different letters shows significant differences through Tukey's test (HSD) honestly significant difference test.). *. **. *** significance at p≤0,05. 0,01. o 0,001 respectively. Statistical analysis carried out with the transformation log CFU·g^{-1}.*

So that, in the vermiculite, the difference in the solid treatments at 1, 5 and 10% was one, two and three orders of magnitude respectively, compared with the liquid treatment at the maximum concentration (10^8 conidia·mL^{-1}), while when treatments were carried out in the substrate rich in OM of the nursery, the difference was a magnitude order in the three cases. In both substrates, these differences are increased by one order of magnitude compared with the other treatments through liquid inoculum (10^7 and 10^6 conidia·mL^{-1}). These last ones showed in both substrates a level of inoculum of the order 10^4, level similar to that obtained in the control treatment when considering the substrate rich in OM. It is important to highlight that this substrate contained *Trichoderma* and inoculations permitted increasing inoculum density 2 points of power. It would seem that this level would be the maximum acceptable level for OM used in the substrate. Likewise, it is noteworthy that the maximum level of inoculum in the mineral substrate (10^8 CFU·g^{-1}), which was obtained in the solid treatment by 10% weight, exceeded two orders of magnitude the maximum inoculum density obtained in the substrate rich in OM when applying the same treatment.

Table 4

Height and dry weight of tomato, pepper and cucumber seedlings according to the treatments with *Trichoderma* sp. applied to 2 different substrates (Verm: Vermiculite and Subs: nursery substrate) through solid and liquid inoculum at different concentrations. The results correspond with the average of the two trials carried out in each substrate

	Height (cm)						Dry weight (g)					
	Tomato		Pepper		Cucumber		Tomato		Pepper		Cucumber	
Treatment	**Subs.**	**Verm.**	**Subs.**	**Verm.**	**Subs.**	**Verm.**	**Subs.**	**Verm.**	**Subs.**	**Verm.**	**Subs.**	**Verm.**
T1:Control	7,32 b	4,83 a	6,22 b	3,30 a	7,25 ba	5,40 a	0,257 b	0,055 a	0,089 b	0,017 a	0,508 b	0,113 a
T2:Solid 1% p/p[x]	8,02 ba	3,05 b	6,50 ba	2,10 b	6,78 ba	4,13 ba	0,444 ba	0,028 b	0,100 b	0,012 a	0,562 ba	0,073 b
T3:Solid 5% p/p	8,81 a	2,43 b	7,07 ba	2,17 ba	7,43 ba	4,22 ba	0,596 ba	0,023 cb	0,196 ba	0,008 ba	0,701 ba	0,069 cb
T4:Solid 10% p/p	8,80 a	2,28 b	7,82 a	1,50 b	7,88 a	3,25 b	0,762 a	0,018 c	0,260 a	0,003 b	0,849 a	0,036 c
T5:Liquid 10^8 conidia/ml	6,83 b	4,31 a	6,15 b	2,87 ba	6,32 ba	4,93 a	0,245 b	0,052 a	0,116 b	0,018 a	0,462 b	0,112 a
T6:Liquid 10^7 conidia/ml	7,12 b	4,25 a	6,13 b	3,18 a	6,27 ba	4,80 a	0,364 ba	0,054 a	0,145 ba	0,016 a	0,559 ba	0,108 a
T7:Liquid 10^6 conidia/ml	7,06 b	4,33 a	5,90 b	2,67 ba	5,43 b	5,12 a	0,323 b	0,055 a	0,106 b	0,014 a	0,438 b	0,102 ba
p-value	***	***	**	***	**	***	***	***	**	***	**	***

*Values in the same column with different letters shows significant differences through Tukey's test (HSD) honestly significant difference test. *. **. *** significance at p≤0,05. 0,01. o 0,001 respectively.).*

Plant height

In general, the length of the aerial part of the seedlings of the three crops studied was higher when using nursery substrate compared with the substrate made of vermiculite (Table 4). The use of solid inoculum on the mineral substrate made of vermiculite had a negative effect on the seedlings of the three horticultural species, obtaining lower height plants at the end of the trials, compared with those grown when the inoculum used was liquid and those grown with the controlled treatment, there being no difference between them. However, the same treatments with solid inoculum applied in the nursery substrate which is rich in organic matter caused an increase of seedling height compared with those obtained from the treatments with liquid inoculum and control treatment. These differences are shown mainly for solid treatment at 10% by weight.

Dry weight

In general, the dry weight of seedlings of the three crops studied was higher when using nursery substrate compared with the substrate made of vermiculite (Table 4).

The use of solid inoculum on mineral substrate made of vermiculite did not have a beneficial effect on the seedlings of the three horticultural species. In this way, the aerial dry weight obtained from the treatments with solid inoculum was always inferior, although in some cases was similar to those obtained in the treatments with liquid inoculum and with the control treatment, which in few cases differed between them. However, the seedlings grown from the treatments with solid inoculum at the highest concentration and applied in the nursery substrate rich in organic matter, in general, showed higher dry weight of the aerial part compared with those obtained from the treatments with liquid inoculum and the control treatment.

With the addition of solid formulations, although a decrease approximately of one order of magnitude was registered in the case of commercial substrate of the nursery rich in OM, this decrease was not detected in the mineral substrate, the levels of inoculum of *Trichoderma* sp. were kept close to the initial levels after 30 days. However, in both substrates, in the treatments with liquid formulations, the initial level of inoculum was reduced two or three orders of magnitude, compared with the treatments with the solid formulations and the values of the initial liquid inocula, respectively. Considering the liquid formulations added to the commercial substrate of the nursery, only the addition at the maximum concentration (10^8 conidia·mL^{-1}) increased one order of magnitude (up to 10^5 CFU·g^{-1}) the initial values of the substrate used, which as they came from a specific nursery of the province of Almería, showed initial values of 10^4 CFU·g^{-1}, a sample from a voluntary inoculation by the same nursery and not from a contaminated one. This low survival rate suggested the *Trichoderma* sp. sensitivity to environmental conditions, although an optimum environment was provided when it was applied. The decrease of *Trichoderma* sp. population which occurred mainly in the treatments with liquid inocula, could be

attributed reasonably, and, in general, to a lack of protection against different factors, including, temperature, humidity and competitors, among others (Jones and Burges, 1998). This last aspect should be considered with interest, because the microbiological analysis carried out, showed the presence of other fungi (*Absidia* sp. and *Penicillium* spp.) and bacteria, and a great amount of actinomycetes that showed a strong antagonistic power compared with the fungus object of study (data not shown). Furthermore, although the nutritive capacity of substrates together with root exudates did not allow the establishment of fungus as it was introduced, the host capacity of substrate increased with the application of solid inoculum made of rice, this could be due the fact that rice supplies food to fungus, which permits it remaining as the source substrate keeping a higher level of inoculum, even under unfavourable conditions for its establishment (Martínez-Medina *et al*.,2009).

On the other hand, and related with what has been previously stated, the results obtained in this work showed that the effectiveness of the application of *Trichoderma* sp. on seedlings of the three species evaluated is directly related with the type of inoculum chosen and its concentration, as well as with the substrate used for the establishment and development of fungus and of the considered host, and therefore, with the survival and establishment of the fungus (Ousley, 1994; Martínez-Medina *et al*., 2009; Bashan *et al*., 2014). The results showed beneficial effects on seedling growth when *Trichoderma* sp. was applied through solid inoculated rice (mainly mixed at a rate of 10% by weight with the substrate) on the commercial nursery substrate rich in OM. These results are consistent with those obtained in other studies, in which the addition of *Trichoderma* sp. using other solid formulations, such as mushroom compost or the combination of oats with bentonite and vermiculite, showed better results in the parameters evaluated in the plants, as well as higher inoculum level in the substrate, compared with those obtained when fungus was applied from liquid formulations (Kleifeld and Chet, 1992; Lo and Lin, 2002; Martínez-Medina *et al*., 2008, 2009; Bernal-Vicente *et al*., 2009; Akoijam *et al*., 2014). The substrate used seems decisive because the development of the seedlings evaluated was always higher when they grew in the nursery substrate rich in OM. According to Rajput *et al*. (2014b) this could be due to nutritionally rich substrates have a longer useful life of microorganism compared with nutritionally poor substrates, however, in this study, a higher inoculum density is shown in the mineral substrate when treatments with solid inoculum are applied on it. On the other hand, it is probably that root exudates vary depending on the substrate where plants grow and that variation has an effect on the physiology of microorganisms that promotes growth in plants, and as a consequence, a possible production of molecules with function of phytohormones, or even capacity of dissolving some beneficial minerals for the plant (Ezziyyani *et al*., 2004). Furthermore, it must be highlighted that the same treatments with solid inoculum that showed beneficial effects on seedlings when using commercial nursery substrate rich in OM, appeared totally contrary, and even in some cases

they caused their death when were applied in the mineral substrate made of vermiculite. In this sense, there are studies in which after inoculating *T. viride* and *T. harzianum* a suppression of seed germination and of growth of wheat, cucumber, tomato and pepper seedlings has been observed (Menzies, 1993; Vujanovic *et al*., 2000), and even the death of forest seedlings caused by *T. viride* (Li Destri Nicosia *et al*., 2015) has been recently registered. However, as far as we know, no references were made to what happened in our trials with vermiculite. In this respect, there is literature that considers vermiculite as a substrate unsuitable for the evaluation of the growth-promoting effect that *Trichoderma* genus has on plants (Kleifeld and Chet, 1992). A possible reason could be the existence of a great competition between fungus and plant, due to the shortage of nutrients in the mineral substrates. In this sense, Martínez-Medina *et al*., (2009) attributed the decrease of nitrogen content in seedlings obtained from treatments with *Trichoderma harzianum*, to the possible competition between the plant and the fungus for the element. This suggests the possibility that under certain conditions of food shortage, *Trichoderma* sp. could show pathogenicity compared with seedlings under the studied conditions.

CONCLUSIONS

In conclusion, effectiveness of the application of *Trichoderma* sp. is directly related with its formulation, which had a clear influence on fungus survival, as well as on the substrate used for the development of fungus and seedlings. In this way, the use of rice to prepare the inoculum of *Trichoderma* sp. seems to be promising, as well as its application in a substrate rich in OM for seedlings' growth in nursery. The use of this type of inoculum could have great repercussion in different scopes: 1) at industry level it would help to reduce the labour and preparation costs of commercial formulations and 2) at nursery level it would reduce the growth period of seedlings. Future research must be focused on the improvement of formulation and its preservation in time, as on the possible effectiveness under greenhouse conditions.

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