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EVALUATION OF BIOFERTILIZERS AND HORMONAL ROOTING (NURSERY AND FIELD) IN *Jatropha curcas* L.

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ABSTRACT
The aim of this study is to evaluate the effect of commercial biofertlizers and chemistry rooting, in morphological variables. The plant of jatropha is cultivated in a conventional form using artificial inputs all over the world. That is why it is imperative to generate technology of production sustainable. The experiment in nursery is performed from July to October of 2013 and it is performed on field from May to December of 2013. Both of these experiments are carried on in the experimental crop field of the Universidad Tecnológica de Izúcar de Matamoros. The evaluated products are: Azofer® (*Azospirillum*), Micorrizafer® (mycorrhiza of gender *Glomus*), Tricovel® (*Trichoderma*) and chemical rooting Rooting® (auxins and cytokinins). A totally random experiment design with ten replications is performed in nursery. In the field, the experimental randomized complete block design was used with twelve replications. It turned out that the combination of *Azospirillum* + *Trichoderma* both doses generates more height of plant (23.3 cm), number of leaves (14.2), fresh weight of leaf and stem (30.5 g) and total fresh weight (36.2 g); in the field the chemical Rooting® was the more active to generate more number of lateralbranches (5.2), fruits matures for plant (45.8), and weight of seed for plant (68.4 g).

KEYWORDS: auxins, mycorrhiza, leaves.

INTRODUCTIÓN

The *Jatropha*, belongs to the Euphorbiaceae family that has 175 species. From this number, 45 of them are found in Mexico, where 77% are endemic (Martínez *et al*., 2002). In Puebla there are 11 species distributed mainly in the Sierra Norte from Puebla and the Mixteca Poblana. These species are all endemic to Mexico, with the exception of *Jatropha curcas*, which reaches Central and South America (Rodríguez *et al*., 2009).In Mexico, the *J. curcas* plant is distributed in 20 states, including the state of Puebla. It is located in tropical and semitropical climates, associated with the low deciduous forest, with rainfall exceeding 600 mm, at altitudes of 0 to 1, 600 meters above sea level, in types of sandy soil and unsuitable soils for agriculture (Martínez, 2009).Jatropha has a wide variety of uses. Some example are: the production of biodiesel, as living fences to control erosion, medicinal products, cosmetic products such as soap (Oyuela *et al*., 2012); however, only in Mexico are the edible genotypes that stand out for their high nutritional value (Makkar *et al*., 1998).

Puebla, Mexico is one of the states with the greatest potential (mainly in the Mixteca Poblana), as shown by studies conducted by the London-based Global Exchange for Social Investment (GEXSI) (Renner *et al*., 2008).Regarding biofertilizers, Kannan and Rajendran (2015), evaluated seaweed fertilizer, compost and biofertilizers (Mycorrhiza +*Azospirillum* + Fosfobacteria), alone and in mixture; found that the fertilizer combination of seaweed + (Mycorrhiza + *Azospirillum* + Fosfobacteria) + compost, generated greater total dry weight of plant, plant height, stem diameter, root length, number of leaves, root dry weight, leaf and stem.Regarding mycorrhizas Charoenpakdee *et al*. (2010), used jatropha as a natural attractant of rhizospheric mycorrhizae, with the objective of reproducing them for application in cereals and observed that mycorrhizae increased dry weight of root and stem of corn, rice, sorghum and weed of tears.Ravikumar *et al*. (2011), evaluated the inoculation in soil of several species of *Azospirillum*, later seeds of jatropha were planted, the results showed that *Azospirullum brasilense* increased stem length, root length, stem biomass, leaf and root.In a study conducted by Neyra *et al*. (2013), in chili culture, inoculated with 5 ml of a suspension of 108 spores/ml of *Trichodema viride*, found an increase in length of stem and root at ten days after inoculation and increased dry weight of root and stem at 20 days after inoculation.

A research carried out in the INIFAP C.E. Valle de Apatzingán, Mexico, evaluated chemical fertilization (based on nitrogen and phosphorus) and biological fertilization based on mycorrhizae of the genus *Glomus* and *A. brasilense*, found similar results in both types of fertilization, on the number of branches, number of inflorescences and seed production, so they suggest biological fertilization, at least for that region (Teniente *et al*., 2011).

Several studies have shown excellent results when applying the combination *Azospirillum* + *Trichoderma*: in tomato (El-Katatny, 2010), in pomegranate (Patil *et al*., 2004), in corn and wheat (Fadl-Allah *et al*., 2012) and in sugar cane (Serna *et al*., 2011).Based on the above, the objective of this research was to evaluate commercial biofertilizers based on mycorrhiza (*Glomus*), *Azospirillum* and *Trichoderma*, and Rooting® hormonal chemical rooter in *Jatropha curcas*, in nursery and open field.

MATERIALS & METHODS

The research was conducted in the experimental field of the Technological University of Izúcar de Matamoros, located in the south of the state of Puebla and geographically at the coordinates 18º 36 'north latitude, and 98º 28' west longitude, at an altitude close to 1300 masl (INEGI, 2009).For the experiments, seeds of a jatropha genotype collected in Huaquechula, Puebla were used, with a harvest time of eight months and homogeneously selected (on average 19 mm in length). The experiment in nursery, was carried out in July-October 2012, the substrates used were: 50% compost (composed of 60% corn stubble, 15% grass, 20% manure and 5% agricultural land) and 50% agricultural land, collected in the UTIM field. Previuos to the experiment, 120 seeds of jatropha were germinated in a nursery of $1m^2$, for 20 days (the plants germinated with an average height of 8 cm), then the most vigorous plants were transplanted into black polyethylene bags (10 x 23 cm)), after 8 days the biofertilizers and chemical rooting were applied. The field experiment was carried out from June 2015 to January 2016, the treatments were applied at the beginning of June 2015 (third year of production), with a planting frame 2 x 2 m, irrigation was also applied in the dry season of the temporality.The commercial products applied in the nursery and field were: Azofer® (bacteria of the genus *Azospirillum*), Tricovel® (fungi of the genus *Trichoderma*) and Micorrizafer® (mycorrhizas of the genus *Glomus*) and rooting hormone Rooting® (based on auxins, cytokinins and phosphorus). In the field, two chemical fertilizations were applied: at the beginning of June and mid-November with 50 g/tree of Triple 16® (16% N, 16% P and 16% K). The experimental nursery unit was one plant per polyethylene bag, generating seven treatments with 10 repetitions per treatment (Table 1). The nursery experiment was established in a 7x10m chapel greenhouse, using a completely randomized experimental design, and a 50% shadow mesh was also placed to reduce affectation by transplant and frequency of irrigation. In the field, the experimental unit consisted of four plants with three replications and a randomized complete block design was used.The variables evaluated in the nursery were plant height, number of leaves, root length, fresh weight of root, fresh weight of leaf and stem and total fresh weight (root, leaf and stem) (they were registered with an Ohaus® balance of 2610g ±0.1g capacity). In the field, the evaluated variables were: stem diameter, number of lateral branches, number of branches with floral clusters, number of branches without floral clusters, number of mature fruits per plant and weight of seed per plant.

Statistical package SAS (Statistical Analysis System for Windows 9) was used for the statistical analysis. An analysis of variance was performed to establish the significance of variables, and Tukey's mean comparison tests were performed ($P \leq 0.05$).

TABLE 1. Treatments generated from the research of biofertilizers and hormonal rooting in *Jatropha curcas* in nursery and field

Treatments in nursery	Treatments in the field
T1 - Control	T1 – 7 g/pta. Micorrizafer®
T2 - 0.3 g/pta. Azofer® + 0.5 g/pta. Micorrizafer®	T2 – 5 g/pta. Azofer®
T3 - 0.6 g/pta. Azofer® + 1 g/pta. Micorrizafer®	T3 – 5 g/pta. Tricovel®
T4 - 0.3 g/pta Azofer® + 0.5 g/pta. Tricovel®	T4 – 5 g /pta. Azofer® + 7 g/pta. Micorrizafer®
T5 - 0.6 g/pta. Azofer® + 1 g/pta. Tricovel®	T5 – 5 g/pta. Azofer® + 5 g/pta. Tricovel®
T6 - 0.5 ml/pta. Rooting®	T6 - 5 ml/pta. Rooting®
T7 - 1 ml/pta. Rooting®	T7 – Control

RESULTS & DISCUSSION

Experiment in nursery

The plant height variable showed significant statistical differences (Table 2), the highest value (23 cm) was recorded by the mixture 0.3g/pta. Azofer® +0.5g/pta. Tricovel®. In this sense, dried tomato seeds inoculated with encapsulates of *A. brasilense* + *T. harzianum* significantly increased the seedling growth (El-Katatny, 2010), while with the inoculation of *A. brasilense* + *T. harzianum* free or encapsulated significantly increased all growth parameters of wheat and corn (Fadl-Allah *et al*., 2012) or, the application of *T. harzianum* (30 gm^{-2}) mixed with the organic matter (cow dung, 24 $kg.m^{-2}$) and efficient microorganisms (15 $mL.m^{-2}$) increased the height of the onion (*Allium cepa*) of 13.15 cm of control at 15.02 and 18.93 cm, respectively (González *et al*., 2015).Significant differences were not registered in the number of leaves, however, the treatment obtained 0.6 g/pta. Azofer® + 1 gr/pta. Tricovel® with 14.2 leaves (Table 2), this suggests the feasibility of using said mixture to reduce the use of chemical and hormonal products, due to the biological fixation of nitrogen and the secretion of hormones such as auxins, gibberellins and cytokinins (Mehboob *et al*., 2009). On the other hand, Serna *et al*. (2011), found higher number of leaves of sugarcane with *A. brasilense* (2.5 x 107 CFU/ml) than with *Trichoderma lignorum*. But the application of *T.harzianum* alone or in combination with bovine manure and efficient microorganisms increased the number of onion leaves from 3.10 to 13.46, respectively (González *et al*., 2015).Significant differences were shown in the root length variable, the treatment 0.3 gr/pta. Azofer® +0.5 gr/pta. Tricovel®) was the most active (28.9cm) (Table 2), followed by Rooting® hormonal rooting at 0.5 ml/pta. (26.6 cm). In this sense the rooters Radix 1500® (indole butyric acid at 1500 ppm) and Rootone F® (1-naphthalene acetamide 0.057% + 2- methyl-1-naphthalene acetic acid 0.033% +2-methyl-1-naphthalene acetamide 0.013% + acid 3- indolebutyric 0.057%, thiram 4%) applied on stakes of *K. blossfeldiana* sown in the mixture of substrates: cachaza + pet-moss + tezontle (1:1:1) recorded the largest root length of 144 and 1.1 cm, respectively, while salicylic acid in 10^{-5} M reactive grade applied in the mixture of substrates: cachaza + tezontle (1:1) generated a root length of 1.37 cm of this plant (Villanueva *et al*., 1998). Or, the application of *T. harzianum* alone or combined with cow manure and efficient microorganisms increased the root length of the 7.90cm onion of the control to 8.46 and 9.81cm, respectively (González *et al*., 2015).Significant differences were not shown in the fresh root weight, however a similar effect is observed in the length of the root, because the hormonal treatment with Rooting® a (1ml/pta.) And the mixture 0.6 g/pta. Azofer® + 1 gr/pta. Tricovel®, obtained 5.7 g/pta. and 5.6 g/pta., respectively (Table 2), suggesting the feasibility of using biological fertilization with these microorganisms to promote root growth. Several studies argue that *Azospirillum* produces growth regulators, mainly auxins (indoleacetic acid, AIA), which promotes root growth (Jain and Patriquin, 1985, Van de Broek *et al*., 1999, Carcaño *et al*., 2006), similarly *Trichoderma* produces AIA, promoting plant growth and lateral roots (Pieterse *et al*., 2009) because it induces large structural changes at the cellular level of plants, as in cucumber (*Cucumis sativus*) when fungi colonize the roots (Yedidia *et al*., 1999).Significant statistical differences were shown in fresh weight of leaf and stem. It should be noted that the treatments 0.3 gr/pta. Azofer® + 0.5gr/pta. Tricovel® and 0.6 g/pta. Azofer® + 1 gr/pta. Tricovel®, recorded the highest values (28 g/pta. and 30.5 g/pta.) (Table 2), which shows the feasibility of using this mixture at both doses, for the production of plant in nursery. In contrast, the application of *T. harzianum* alone or in combination with bovine manure and efficient micro-organisms increased the fresh weight and dry weight of the onion leaf area of 2.96-1.61g of the control at 3.15-1.85g and 3.95-2.90g, respectively (González *et al*., 2015), while maize plants incubated for 30 days with *A. brasilense* DSM1690 increased their fresh weight and dry leaf and stem weight (Nezarat and Gholami, 2009). On the other hand, the application of free *T. harzianum* (1×109 CFU) or immobilized with chitosan hydrogel (1×109 CFU +6g Hydrogel) did not stimulate the production of tomato biomass *Solanum lycopersicum* in nursery (Francisco *et al*., 2012). Neither plants of the melon incubated 42 days with *T. harzianum* (106 conidia g^{-1}) increased the fresh weight and dry weight of the plant (Martínez *et al*., 2009).Regarding total fresh weight (root, leaf and stem), the treatments: 0.6 g/pta. Azofer® + 1gr/pta. Tricovel® and 0.3 gr/pta. Azofer® + 0.5 gr /pta. Tricovel®, recorded the highest values (36.2 g/pta. and 32.8 g/pta.) (Table 2). In contrast, Kannan and Rajendran (2015) found that when applying seaweed fertilizer + biofertilizers (Mycorrhiza + *Azospirillum* + Phosphobacterium) + compost, they generated greater total dry weight of plant (40% more than the control). On the other hand tomato seeds (*Lycopersicon esculentum*) incubated with three strains of *A. brasilense* (Sp7, Sp7-S and Sp245) increased their fresh weight and total dry weight (Mangmang *et al*., 2015).

The analysis of the results of the present study indicated that the application of *Azospirillum* mixed with mycorrhizae (*Glomus*) in both doses, did not increase any of the variables evaluated. On the other hand, the application of 0.3 gr/pta. Azofer® + 0.5 gr/pta. Tricovel®, increased the height of the plant and the length of the root, while this same mixture at its high dose, increased the number of leaves, the diameter of the stem, the fresh weight of the root, or, in both doses (low and high) increased the fresh weight of the leaves and stem and the fresh weight of the root, leaf and stem. Also the rooting Rooting® (0.5 ml/pta.), increased the length of the root, while at its highest dose (1 ml/pta.), increased the fresh weight of the root, or, with both doses increased the diameter of the stem.

TABLE 2. Effect of biofertilizers and hormonal rooting in morphological variables of jatropha in the nursery

Treatment	HG	NL	SD	RL	RFW	FWLS	TFW
T1	21.4 ab	12.2 a	0.95 ab	23.1 b	4.5 a	24.7 bc	29.2 bc
T2	19.4 b	13.4 a	0.98 ab	24.4 ab	5.1 a	25.4 abc	30.5 abc
T3	21.5 ab	12.4 a	0.9 b	23.1 b	4.7 a	21.5 c	26.2 c
T4	23.3 a	13.2 a	0.95 ab	28.9 a	4.8 a	28.0 ab	32.8 ab
T5	22.2 ab	14.2 a	1.05 a	26.0 ab	5.6 a	30.5 a	36.2 a
T6	22.1 ab	12.2 a	1.05 a	26.6 ab	5.3 a	26.4 abc	31.7 abc
T7	21.6 ab	12.2 a	1.0 ab	25.8 ab	5.7 a	26.2 abc	31.9 abc
LSD	3.78	2.60	0.13	4.68	1.42	5.13	5.99

HG = Plant height (cm), NL = Number of leaves, SD = Stem diameter (cm), RL = Root length (cm), RFW = Root fresh weight (g), FWLS = Fresh weight of leaf and stem (g), TFW = Total fresh weight (g). LSD = Least significant difference. Means with the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

Field experiment

Statistical significance in diameter of stem was not shown (Table 3), however the treatments: 7 g/pta. of Micorrizafer® and 5g / pta. of Azofer®, presented high values (10 and 10.1 cm), these results agree with those obtained by Díaz *et al*. (2013) when applying 40 g of mycorrhiza *G. intraradices* increased stem diameter above 10% with respect to the control in *J. curcas*. It should be noted that the sampling was carried out 26 months after planting, which is consistent with the sampling done in this experiment, so that biological fertilization is a sustainable alternative for the production of jatropha.The number of lateral branches did not show significance, however the treatments: 5g/pta. of Azofer® and 7 g/pta. of Micorrizafer®, presented high values (31.5 and 30.8 branches). On the other hand, Kannan and Kannan (2013), in an experiment carried out with bio-inoculants, farm manure and chemical fertilization (NPK), recorded that the mixtures containing *Azospirillum* +*Trichoderma* + Arbuscular mycorrhiza, generated greater number of branches per plant of *J. curcas* (from 27 to 36 branches).

Statistical significance was not presented in the variable number of branches with inflorescences, (Table 3), however the treatment 5 g/pta. of Azofer® +5 g/pta. of Tricovel® and 5 g/pta. of Azofer®, generated high values (22.2 and 22.1 branches with inflorescence); these results contrast with those obtained by Teniente *et al*. (2011), when using *Azospirillum brasilense* and Micorriza of the genus *Glomus* obtaining 19 flower clusters per plant of *J. curcas*, nevertheless it is necessary to evaluate the mixture of *Azospirillum* + the mycorrhiza (*Glomus*) and perform the sampling when the jatrropha plant is in full inflorescence to determine the increase of floral clusters. On the other hand (Janarthanam, 2013), argues that the application of the multifunctional microbial formulation Suma Grow-F2 (14 bacteria and 7 strains of *Trichoderma*, 1 x10^{10}/ pot) or mixed with chemical fertilization (NPK 20-20-20) , increased the number of ears of corn, the number of pods of sorghum and the number of peanuts, as well as increases the total production of each of these crops.Statistical significance was observed in the number of branches without inflorescence, being the treatments 5 g/pta. of Azofer® and 5g/pta. of Tricovel®, those that presented high values (10.0 and 9.2); It should be noted that treatment 2 also generated a greater number of branches with inflorescence.

Significant differences were shown in the variable ripe fruits per plant,(Table 3), the treatment 5 ml/pta. of Rooting® and 5 g/pta. of Azofer® + 5 g/pta. of Tricovel®, presented high values (326.3 and 322.0 fruits per plant). The weight of seed per plant records significant statistical differences, the treatment 5 ml/pta. of Rooting® and 5 g/pta. of Azofer® + 5 g/ pta. of Tricovel®, presented higher yield (492.6 and 469.0 g/pta.); in contrast Díaz *et al*. (2013) to apply 40g of mycorrhizae *G. intraradices* alone or mixed with 1 kg of cane compost increases the number of fruits and weight of seed per plant higher than 20% with respect to the control in *J. curcas*. Com all this, the biological fertilization is a sustainable alternative for the production of jatropha, nevertheless in this experiment the mycorrhizae had no significant effect on the yield. On the other hand Sathianachiyar and Devaraj (2013), applied biofertilizers (*Trichoderma viride* + *Azospirillum* + Fosfobacteria) and found an increase in the content of oil and seed harvested.

TABLE 3. Effect of biofertilizers and hormonal rooting on morphological and performance variables of jatropha in the field

Treatment	SD	NLB	NBI	NBWI	NMFP	WSP
T1	10.0 a	30.8 a	22.0 a	8.8 ab	308.0 abc	435.6 ab
T2	10.1 a	31.5 a	22.1 a	10.0 a	309.2 abc	463.8 ab
T3	9.7 a	27.6 a	18.8 a	9.2 ab	262.5 bc	385.4 b
T4	9.4 a	27.0 a	19.4 a	7.8 ab	271.8 abc	411.3 ab
T5	9.8 a	28.4 a	22.2 a	5.9 b	322.0 ab	469.0 a
T6	9.9 a	27.8 a	21.8 a	5.9 b	326.3 a	492.6 a
T7	9.7 a	27.1 a	19.7 a	7.3 ab	253.0 c	383.2 b
LSD	1.2	6.7	4.8	3.3	80.8	83.6

SD = Stem diameter (cm), NLB = Number of lateral branches, NBWI =Number of branches with inflorescences, NBWI = Number branches without inflorescences, NMFP = Number of mature fruits per plant and WSP = Weight of seed per plant (g). LSD = Least significant difference. Means with the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

The analysis of results shows that the treatments: 5 g/pta. of Azofer® and 7 g/pta. of Micorrizafer®, generated greater diameter of stem, number of lateral branches and number of branches without inflorescence, so it can be assured that said biofertilizers promote vegetative growth, thus with the yield of fruit and seed treatment, also generated high values in number of branches with inflorescence. In this sense Moens (1998), ensures that *A. brasilense*, fixes molecular nitrogen in microaerophilic conditions, which promotes vegetative growth. On the other hand Merrywheather and Fitter (1996) and Alkaraki and Clark (1998), argue that the mycorrhizal symbiosis markedly increases the absorption of nutrients such as nitrogen, calcium, potassium, zinc, magnesium and especially phosphorus. In this sense, Ranjan *et al*. (2013), when characterizing strains of mycorrhizae associated with the rhizosphere of *J. curcas*, recorded 15 strains of mycorrhizae of the genus *Glomus*, which indicates the compatibility of applying this strain of mycorrhiza in jatropha. Regarding performance, the Rooting® chemical hormonal rooting agent (0.5 ml/pta.) was the most active when increasing the number of fruits per plant and the weight of the seeds per plant, while the mixture 5 g/pta. of

Azofer® + 5 g/pta. of Tricovel®, remained in second place in the performance variables, so it is a viable and sustainable economic option, for it is necessary to evaluate this mixture with organic fertilizers to enhance the yield in *J. curcas* and reduce the use of fertilizers chemical In this regard, several studies in different crops have shown good results when applying the combination *Azospirillum* + *Trichoderma*: in tomato (El-Katatny, 2010), in pomegranate (Patil *et al*., (2004) in corn and wheat (Fadl-Allah *et al*. al., 2012) and in sugarcane (Serna *et al*., 2011).

CONCLUSION

In the nursery, the mixture of Azofer® + Tricovel® in both doses generated higher plant height, number of leaves, fresh weight of leaf and stem and total fresh weight of jatropha, followed by Rooting® chemical hormonal rooting.

In the field, in contrast, Rooting® rooting was the most active because it generated the highest number of fruits per plant and seed weight per plant, followed by the Azofer® + Tricovel® mixture. The Azofer® biofertilizer generated greater stem diameter, number of branches and number of branches without inflorescence.

RECOMMENDATION

It is important to evaluate organic fertilization (bat guano or effluent vermicompost) in combination with biofertilizers (*Trichoderma* and *Azospirillum*) to generate organic plant.

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