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# EVALUATION OF NATIVE AND COMMERCIAL STRAINS OF *TRICHODERMA* AND *AZOSPIRILLUM* IN VEGETATIVE PROPAGATION OF *JATROPHA CURCAS* L.

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**ABSTRACT**
The plant of jatropha is cultivated in all the world of form conventional, utilized artificial inputs. Because of this reality, it is necessary generate technology of production sustainable. The aim of this research was to evaluate combinations of natives strain and commercial strains of *Azospirillum* y *Trichoderma* in vegetative propagation for cutting in jatropha. The experiment was performed in April of 2013 in a greenhouse of crop field of the Universidad Tecnológica de Izúcar de Matamoros (UTIM). The experimental randomized complete block design was used with ten replications. The native strains of *Azospirillum* y *Trichoderma* was obtained from crops of the UTIM and the commercial strains from the products: Azofer® (*Azospirillum*) and Tricovel® (*Trichoderma*), also was evaluated the rooting chemical hormonal Rooting® (auxins and cytokinins). It turned out that commercial strains *Azospirillum* + *Trichoderma* generated more dry weight of root and leaf. The product chemical hormonal Rooting® generate more height of plant and number of leaves by branch. The low dose of combination of native strains of *Azospirillum* and Trichoderma, presented more dry weight of leaf and the high doses, generate more diameter of apical branch. In conclusion, an alternative sustainable for promote rooting and vigor of plant in the propagation by cuttings of jatropha, is use the combination of commercial strains *Azospirillum* and *Trichoderma*.

**KEY WORDS**: Plant, experimental design, rooting, dry weight.

## INTRODUCTION

*Jatropha* is a genus of the family Euphorbiaceae has 175 species, 45 of them are in Mexico, where 77% are endemic (Martínez *et al*., 2002). In Puebla they found 11 species distributed mainly in the Sierra Norte de Puebla and the Mixteca Poblana, all endemic to Mexico, except *Jatropha curcas*, this goes to Central and South America (Rodríguez *et al*., 2009). In Mexico, the plant *J. curcas* is distributed in 20 states, including the state of Puebla. It is located in tropical and semitropical climates, associated with the tropical deciduous forest, with over 600 mm rainfall, at altitudes from 0 to 1, 600 meters above sea level, in types of sandy soil and soil unfit for agriculture (Martínez, 2009). The *J. curcas* has a variety of uses among which are: the production of biodiesel, as living fences to control erosion, medicinal products, and cosmetic products such as soap (Oyuela *et al*., 2012); however only in Mexico are edible genotypes noted for their high nutritional value (Makkar *et al*., 1998).
Vegetative propagation is defined as the reproduction of a plant from a cell, tissue or plant organ. In theory, any part of a plant can give rise to another of the same characteristics depending on the conditions of growth (light, humidity, temperature, nutrients and health) (Rojas *et al*., 2004). Usually in the Soconusco region in Chiapas, Mexico, *J. curcas* is spread by twigs or cuttings, how is replaced or supplemented new areas in hedgerows (Dardon *et al*., 2015), on the other hand vegetative propagation of jatropha is useful when planting is used for planting seed production, which is important to select vigorous and good production plants (Oyuela *el al*., 2013).
Regarding the use of hormones in vegetative propagation, an experiment with cuttings of *J. curcas*, immersed in solution with auxins (indolebutyric acid and naphthaleneacetic acid at 10 and 100 mg $L^{-1}$), they found that indolebutyric acid generates greater survival of cuttings (91 to 93%), greater number of sprouts (2.5 to 2.7) and shoot length (1.4 to 1.8 cm) (Kochhar *et al*., 2008); in contrast three types of auxins (IAA, IBA and NAA) and thiamine were evaluated also evaluated season and found a good rooting, root length and number of roots with IBA and thiamine in spring, but in summer thiamine fall short the IBA in the variables evaluated (Dillon *et al*., 2010). In another study in *J. curcas* in vegetative propagation by cuttings, evaluated length cutting (15 and 25 cm), cutting position of the plat (apical, middle and basal) and two auxins, AIA and IBA (100, 200, 400 , 800 and 1000 ppm), obtained the best results with the treatment of apical cutting, 25cm in length and 100 ppm of AIA, as it generated more fresh biomass (73.21 g), dry biomass (34.06 g), rooting percentage (86.66 %), root length (30.66 cm) and number of roots (16.33) (Sahoo *et al*., 2014).
Based on the above it follows that the length of cutting, cutting position in the plant, season and use of hormones, are factors that influence vegetative propagation by

cuttings, however there are other important factors in the propagation by cuttings in *J. curcas* as: cutting diameter (Kumar *et al*., 2010 y Parthiban, *et al*., 2014), moisture, fertilization and shading (Peer, 2010).

The information generated from research on vegetative propagation of *J. curcas* is related to the application of auxins mainly, however there is no information regarding the use of native and commercial strains of beneficial microorganisms in vegetative propagation of *J. curcas* hence the importance of generating research in this direction. Based on the above, the objective of this research was to evaluate native and commercial strains of *Trichoderma* and *Azospirillum* as promoters of rooting and plant vigor.

## MATERIALS & METHODS

The research was conducted in the experimental field of the Universidad Tecnológica de Izúcar de Matamoros, located south of the state of Puebla and geographically in the coordinates 18º 36' north latitude and 98º 28' west longitude at an altitude close to 1300 m (INEGI, 2009).

The experiment was conducted in April-July 2015. They were used cuttings of 35 cm in length and 3 to 3.5 cm in diameter (these dimensions based on optimal results obtained by Kathiravan *et al*., 2007 and Enciso and Castle, 2010), which were cut from a genotype of *J. curcas*, collected in the municipality of Huaquechula, Puebla, later were placed in black bags of 10 x 23 cm, which were filled with a mixture of substrates with 50% compost and 50% sand (substrate volume of 1.56 lt) to promote rooting commercial biological products were used: Azofer® (*Azospirillum*), Tricovel® (*Trichoderma*) and native strains of *Trichoderma* and *Azospirillum*; also it used a rooting hormonal chemical called Rooting® (auxins, vitamins, cytokinins and assimilable phosphorus). The strain *Azospirillum* sp. was isolated in culture medium PY, supplemented with 20 µg $mL^{-1}$ nalidixic acid and 10 µg $mL^{-1}$ of tetracycline (Caballero *et al*., 1992), taking cuts roots of 2 cm length of *J. curcas*, washed and surface sterilized with 70% ethanol for 5 minutes, rinsed in sterile distilled water and transferred to the culture medium above mentioned, remaining incubation interval of 10 days to 30 °C. Culture media were screened positive growth and the methodology described by Ilyas *et al*., 2012 was followed for identification of *Azospirillum*. The ability to grow on different carbon sources according to the methodology proposed by Barbosa (2006) was also determined. Regarding the native strain of *Trichoderma* rhizosphere soil of the experimental field of the Technological University of Matamoros Izucar was used, of the sample obtained it was used a subsample of 10 g, which was dissolved in 90 ml of sterile water and stirred for 20 was minutes. From the resulting solution 100 µl were taken and inoculated into medium semi-selective E (Papavizas y Lumsden, 1982). Petri dishes were placed in incubation at 28 °C for 5 days, later were removed from the incubator and allowed to light and at room temperature for 3 days. Subsequently temporary assemblies were held in Lactophenol blue and observed under a microscope to identify the presence of fungi of the genus *Trichoderma* as described by Barnet and Hunter (1987) and Bisset (1991a, 1991b). The obtained strain was grown on Potato Dextrose Agar medium (PDA) at 30 °C for 72 hours in darkness and then start to light for 48 hours to induce sporulation (Casas *et al*., 2004). Subsequently, the strain was grown on substrate sterilized rice at 121°C and 15 atmosphere pressure for 60 minutes, after 20 days the spores were obtained to prepare an aqueous suspension containing $1x10^8$ spores/ml following the methodology described by Guigon and González (2004), which was used to inoculate cuttings of jatropha. For native strains *Azospirillum* and *Trichoderma* were used at a concentration of 5x108 CFU/ml solution.

The experiment was set in a greenhouse with overhead ventilation hood 7x10 m, a mesh shadow of 50% was placed to reduce watering frequency; six treatments and a control, which were applied at 40 days after setting up the experiment (when they emerged the first roots) were generated; 8 days of treatments applied, a chemical fertilization Triple 16 (16 N, 16 P and 16 K) was performed at a dose of 2g/plant. It was used a completely randomized design with 10 replications, the experimental unit was defined by a black polyethylene bag, with a cutting of jatropha (Table 1).

The variables evaluated were: dry weight of root, leaf and branch, branch length, root length, number of branches per plant, number of leaves per branch, plant height, diameter of apical branch, and length of basal leaf (of apical branch). Samples for drying a drying oven (Scorpion Scientific) were used. The samples were placed in paper bags (10 x 30 cm) for eight days at a temperature of 80 °C. The dry weight of the sample was recorded with an Ohaus® balance of 2610g ±0.1 g capacity. SAS (Statistical Analysis System for Windows 9) statistical package was used for statistical analysis. An analysis of variance and mean comparison tests by Tukey ($P \leq 0.05$) was performed.

**TABLE 1.** Treatments evaluated in vegetative propagation by cuttings of *Jatropha curcas*

| Number | Treatament |
|---|---|
| 1 | SNA (0.5 ml/pta.) + SNT (0.5 ml/pta.) |
| 2 | SNA (1.0 ml/pta.) + SNT (1.0 ml/pta.) |
| 3 | SCA (0.3 g/pta.) + SCT (0.5 g/pta.) |
| 4 | SCA (0.6 g/pta.) + SCT (1 g/pta.) |
| 5 | ROOTING® (0.5 ml/pta.) |
| 6 | CONTROL |

SCA = Commercial strain *Azospirillum*, SCT = Commercial strain *Trichoderma*, SNA = Native strain *Azospirillum* and SNT = Native strain *Trichoderma*.

## RESULTS AND DISCUSSION

The root dry weight variable, showed statistically significant differences, treatment 3 (low dose of *Azospirillum* and commercial *Trichoderma*) showed the highest dry weight (1.42 g), followed by the low dose of native strain of *Azospirillum* and *Trichoderma* (1.08 g), showing its potential as a rooting sustainable to enhance anchoring and nutrition of the plant to be planted in the field, it was expected to generate greater chemical rooting Rooting® root weight, although it should be noted that this product is applied to plants with roots already established and possibly when applied had small number of roots, or poorly developed (Table 2). Several studies argue that *Azospirillum* produces growth regulators, particularly auxins (indoleacetic acid), which promotes root growth (Jain y Patriquin, 1985; Van de Broek *et al*., 1999; Carcaño *et al*., 2006), similarly *Trichoderma* produces AIA, promoting plant growth and lateral roots, because it induces major structural changes at the level plant cell (Pieterse *et al*., 2009).

The dry weight of branch showed no statistically significant differences and highlights treatment 2 (low dose of *Azospirillum* and *Trichoderma* native strain) with 6.18 g, followed by the low dose of native strain of *Azospirillum* and *Trichoderma* (5.96 g), these results confirm the potential of native strains for the production of jatropha plants, so it would be important to evaluate them in nursery and field so that in the later convert them into commercial biofertilizers adapted to the region, and even evaluate other crops; in this regard Bécquer *et al.* (2015), in a study in wheat, with rhizosphere and *Trichoderma* wheat bacteria, they found that the combination of *Sinhorizobium meliloti* + *A. zeae* + *Trichoderma*, applied five days after planting, generated higher dry weight biomass (0.58 g/ plant), 5 grams more than the control.

The dry weight of leaf variable did not show statistically significant differences, however, it highlights the treatment 3 (low dose of *Azospirillum* and commercial *Trichoderma*) with the highest value of dry leaf weight (10.92 g) and low dose of native strain *Azospirillum* and *Trichoderma* (10.72 g), it should be noted that dry root weight also highlighted treatment 3, so it follows that the greater root development, further development of the stem and thus of blade above the highest capacity they have roots to absorb nutrients and feed the plant, in contrast Ontiveros et al. (2005), to conduct a study with different soil drying rates found in beans and faster drying root slower growth and less development of the aerial part; on the other hand Gonzalez *et al.* (2015) performed the application of *T. harzianum* (30 gm-2) mixed with organic matter (cattle manure, 24 kg $m^{-2}$) and effective microorganisms (15 mL. $m^{-2}$) increased the dry weight of leaf *Allium cepa*, 1.29 g with respect to control. Root length, showed statistically significant differences, include treatments 4 and 3 (high and low dose of *Azospirillum* and *Trichoderma* commercial strain) with 13.85 cm and 13.25 cm, respectively, from the above it follows that the commercial strains of *Azospirillum* and *Trichoderma* they have a great capacity to generate auxins, which promotes root growth in a sustainable manner. In this sense, tomato seedlings inoculated with encapsulated dry beads of *A. brasilense* + *T. harzianum* significantly increased root length 3 cm, compared with the control, however when applied wet beads encapsulated with *A. brasilense* exceeded by 4.5 cm to the combination *A. brasilense* + *T. harzianum* (El-Katatny, 2010); it should be noted that some strains *Azospirillum* produce B vitamins that promote rooting capacity and affect microbial populations (Rodelas *et al*., 1993); on the other hand *Trichoderma* has several mechanisms to promote plant growth, among which: increased solubilization of soil nutrients, increased root growth, number of root hairs and favoring deeper rooting (Harman, 2006). The variable number of branches, did not show statistically significant differences, however treatments 1 and 2, low and high doses of *Azospirillum* and *Trichoderma* native strain, generated greater number of branches (both treatments 3.5 branches), it should be noted that these treatments also generated higher dry weight of leaf, only surpassed by treatment 3 (low dose *Azospirillum* and *Trichoderma* commercial strain), in contrast Kannan and Kannan (2013), in an experiment with bioinoculants, farmyard manure and chemical fertilizers (NPK) in field found that mixtures containing *Azospirillum* + *Trichoderma* + Arbuscular mycorrhizal, generated greater number of branches per plant in *J. curcas* (27 to 36 branches); on the other hand Kochhar *et al.* (2008) they performed vegetative propagation by cuttings in *J. curcas* with auxins (indolebutyric acid and naphthaleneacetic acid at 10 and 100 mg $L^{-1}$), it turned out that the indolbutyric acid generates larger number of buds (2.5 to 2.7), however in this research the hormonal product Rooting® generated an average of three branches per plant.

**TABLE 2.** Comparison of means of variables: root dry weight, branch and leaf, root length and number of branches in *Jatropha curcas*

| TREATMENTS | RDW | BDW | DWL | RL | NB |
|---|---|---|---|---|---|
| SNA + SNT (LD) | 1.08 b | 5.96 a | 10.72 a | 9.92 c | 3.5 a |
| SNA + SNT (HD) | 1.07 b | 6.18 a | 10.43 a | 10.20 c | 3.5 a |
| SCA + SCT (LD) | 1.42 a | 5.52 a | 10.92 a | 13.25 ab | 3.1 a |
| SCA + SCT (HD) | 1.13 b | 5.1 a | 9.28 a | 13.85 a | 3.3 a |
| ROOTING® | 1.07 b | 5.87 a | 10.27 a | 11.08 bc | 3.0 a |
| CONTROL | 0.87 b | 5.15 a | 9.52 a | 8.90 c | 3.4 a |
| LSD | 0.274 | 1.75 | 2.98 | 2.75 | 1.08 |
| CV (%) | 14.12 | 17.66 | 16.68 | 13.99 | 24.87 |

RDW = Root dry weight (g), BDW = Branch dry weight (g), DWL = Dry weight of leaf (g), RL = Root length (cm), NB = Number of branches. SCA = Commercial strain *Azospirillum*, SCT = Commercial strain *Trichoderma*, SNA = Native strain *Azospirillum*, SNT = Native strain *Trichoderma.* LD = Low dose, HD = High dose. LSD = Least significant difference. CV = Coefficient of variation. Means with the same letter are not significantly different according to Tukey test ($P \leq 0.05$).

The variable plant height showed no statistically significant differences, however, treatment 5 (chemical rooting Rooting®), generated greater plant height (73.04 cm) and treatment 2 (high dose of *Azospirillum* and *Trichoderma* native strain) with 71.3 cm (Table 3); regarding the use of hormones, a study in tomato assessed a commercial product based on 0.12% of indolebutyric acid and 0.004% forchlorfenuron, found that the dose of 3 mL. $L^{-1}$ generated greater plant height (6.75 cm) at 47 days after planting (Cuesta y Mondaca, 2014); in contrast tomato seedlings inoculated with encapsulated dry beads *A. brasilense* + *T. harzianum* significantly increased seedling height (4 cm, compared to the control), however when applied wet beads was encapsulated *A. brasilense* exceeded by 4 cm in height to the combination *A. brasilense* + *T. harzianum* (El-Katatny, 2010); on the other hand, the application of *T. harzianum* (30 gm-2) mixed with organic matter (cattle manure, 24 kg $m^{-2}$) and effective microorganisms (15 mL. $m^{-2}$) increased the height of the onion (*Allium strain*) of 13.15 cm to 15.02 and control 18.93 cm, respectively (González *et al*., 2015).

The variable diameter of apical branch, showed no statistically significant differences, however, treatment 3 (low dose of *Azospirillum* and *Trichoderma* commercial strain) presented the highest value in diameter (1.2 cm), followed by rooting Rooting ® (1.08 cm). In this regard, tomato seedlings inoculated with encapsulated dry beads *A. brasilense* + *T. harzianum* significantly increased stem diameter (1.2 cm, compared to the control), however when applied wet beads encapsulated *A. brasilense*, exceeded by 1.4 cm stem diameter to the combination *A. brasilense* + *T. harzianum* (El-Katatny, 2010); in contrast to a study in vegetative propagation by cuttings *Kalanchoe* commercial rootings auxin-based powder (Radix 1500® and Rotone F®) and salicylic acid $10^{-5}$M, in combination with substrates (peat-moss, cachaza and tezontle ); It turned out that treatment with Radix 1500®, generated greater stem diameter (0.71 cm) compared to the control (0.46 cm) (Villanueva *et al*., 1998).

The variable number of leaves showed no significant statistical difference, however, treatment 5 (rooting hormonal chemical Rooting®), had the highest number of leaves (13 leaves), also highlighted the treatment 3 (12.31 leaves) and 4 (12.29 leaves) of commercial strains of *Trichoderma* and *Azospirillum*. In a study in vegetative propagation by cuttings *Kalanchoe*, commercial rootings auxin-based powder (Radix 1500® and Rotone F®) and salicylic acid $10^{-5}$M, in combination with substrates (peat-moss, cachaza and tezontle ); it turned out that the largest number of leaves was obtained by the mixture of the three substrates with salicylic acid (19.25) and the lowest result for the tezontle with salicylic acid (Villanueva *et al*., 1998), this suggests the possibility of using salicylic acid to see the effect on rooting *J. curcas* and simultaneously evaluate the effect of substrate mixture. On the other hand Serna *et al.* (2011) found more 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 cattle manure and effective microorganisms increase the number of leaves of onion from 3.1 to 13.46 respectively (González *et al*., 2015); It should be noted that the number of leaves is related to the root length and the number of roots, since the greater length and number of roots, more leaves (Kumar *et al*., 2011).

Regarding variable length basal leaf, it showed statistically significant differences, obtaining the highest value treatment 2 (high dose native and *Trichoderma* strain *Azospirillum*), followed by treatment 6 (Control). In this regard Ravikumar *et al*. (2011) evaluated the inoculation in soil for several species of *Azospirillum* subsequently jatropha seeds were planted, the results showed that *Azospirullum lipoferum* generated greater leaf area (28.57% more than the control), in contrast Aguado (2012), ensures that *Azospirillum* increase the absorption capacity of water and nutrients by plants by stimulating its growth through radical hormone production. On the other hand, the substrate is an important seed propagation and vegetative factor about research conducted in barley where evaluated compost based sawdust, manure of horses and sheep, green grass (in different proportions), as a control substrate formulated with 70% *Sphagnum* peat + 30% volcanic sand, was that most of blade length of barley, was achieved when composter the mix of horses and sheep manure with shaving (4 cm more than the control) (Contardi y Errasti, 2012), based on the above in this research we decided to use the compost as substrate.

**TABLE 3.** Comparison of means of variables: plant height, branch apical diameter, number of leaves per branch, length of basal leaf and branch length in *Jatropha curcas*

| TREATMENTS | HG | DAB | NLB | LBL | LB |
|---|---|---|---|---|---|
| **SNA + SNT (LD)** | 70.78 **a** | 1.06 **a** | 11.75 **a** | 17.42 **ab** | 29.19 **a** |
| **SNA + SNT (HD)** | 71.30 **a** | 1.20 **a** | 11.58 **a** | 20.03 **a** | 28.48 **a** |
| **SCA + SCT (LD)** | 67.65 **a** | 1.02 **a** | 12.31 **a** | 18.18 **ab** | 29.02 **a** |
| **SCA + SCT (HD)** | 70.92 **a** | 0.98 **a** | 12.29 **a** | 15.60 **b** | 28.95 **a** |
| **ROOTING®** | 73.04 **a** | 1.08 **a** | 13.00 **a** | 17.76 **ab** | 32.84 **a** |
| **CONTROL** | 69.90 **a** | 0.99 **a** | 11.81 **a** | 18.77 **ab** | 28.58 **a** |
| **LSD** | 7.79 | 0.16 | 2.78 | 4.29 | 7.27 |
| **CV (%)** | 8.38 | 11.72 | 17.36 | 18.06 | 18.65 |

HG = Plant height (cm), DAB = Diameter of apical branch (cm), NLB = Number of leaves per branch, LBL = Length of basal leaf (cm), LB = Length of branch (cm). SCA = Commercial strain *Azospirillum*, SCT = Commercial strain *Trichoderma*, SNA = Native strain *Azospirillum*, SNT = Native strain *Trichoderma*. LD = Low dose, HD = High doses. LSD = Least significant difference. CV = Coefficient of variation. Means with the same letter are not significantly different according to Tukey test ($P \leq 0.05$).

The variable length branch showed no statistically significant differences, however treatment 5 (hormonal chemical rooting Rooting®) showed the highest value of branch length (32.84 cm), followed by treatments 1 (29.19

cm) low dose of native strain of *Azospirillum* and *Trichoderma* and 3 (29.02 cm) low dose of commercial strains of *Azospirillum* and *Trichoderma*, in contrast with a similar study rooting cuttings in *J. curcas*, evaluated three types of auxins (IAA, IBA and NAA) and thiamine, also evaluated season, found that in the spring he IBA generated greater branch length (40.68 cm), however thiamine slightly exceeded in length branch of the IBA (41.78 cm), while in summer, sprouts had lower growth, however thiamine generated longest branch (1.92 cm) (Dillon *et al*., 2010); similarly, in *J. curcas* other research with auxins (indolebutyric acid and 10 naftanalacético acid and 100 mg $L^{-1}$), found that the indolbutyric acid generated greater length of sprouts (1.4 to 1.8cm) (Kochhar *et al*., 2008).

## CONCLUSIONS

The low dose of *Trichoderma* and *Azospirillum* (commercial strains), showed higher dry weight of root and leaf, so they are viable to improve anchoring root. The hormonal chemical Rooting®, showed higher plant height and number of leaves per branch. The low dose of native strain of *Trichoderma* and *Azospirillum*, showed higher dry weight of leaf and high dose, had higher apical diameter branch.

## 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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