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Experimental Evaluation of 10 Ecotypes of Toxic and Non-Toxic *Jatropha curcas* as Raw Material to Produce Biodiesel in Morelos State, Mexico

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Abstract*—Jatropha curcas* is a perennial oleaginous plant that is currently considered an energy crop with high potential as an environmentally sustainable biofuel. During the last decades, research in biofuels has grown in tropical and subtropical regions in Latin America. However, as far we know, there are no reports on the growth and yield patterns of *Jatropha curcas* under the specific agro climatic scenarios of the State of Morelos, Mexico. This study presents the results of 52 months monitoring of 10 toxic and non-toxic ecotypes of *Jatropha curcas* (E1M, E2M, E3M, E4M, E5M, E6O, E7O, E8O, E9C, E10C) in an experimental plantation with minimum watering and fertilization resources. The main objective is to identify the ecotypes with the highest potential as biodiesel raw material in the select region, by developing experimental information. Specifically, we monitored biophysical and growth parameters, including plant survival and seed production (at the end of month 52), to study the performance of each ecotype and to establish differences among the variables of morphological growth, net seed oil content, and toxicity. To analyze the morphological growth, a statistical approach to the biophysical parameters was used; the net seed oil content -80 to 192 kg/ha- was estimated with the first harvest; and the toxicity was evaluated by examining the phorbol ester concentration (μg/L) in the oil extracted from the seeds. The comparison and selection of ecotypes was performed through a methodology developed based on the normalization of results. We identified four outstanding ecotypes (E1M, E2M, E3M, and E4M) that can be used to establish *Jatropha curcas* as energy crops in the state of Morelos for feasible agro-industrial production of biodiesel and other products related to the use of biomass.

***Keywords*—**Biodiesel production, *Jatropha curcas*, morphologic growth, toxic and non-toxic ecotypes, seed oil content.

I. Introduction

BIOFUELS are becoming increasingly important worldwide as the best option to minimize greenhouse gas emissions in the transport sector and as an alternative to reduce our reliance on fossil fuels [1]-[3]. At present, interest in liquid biofuels is rising, particularly in ethanol and biodiesel; in this scenario, the production of biofuels and the R&D of energy crops play an extremely important role.

Jatropha curcas has long been deemed a raw material with an exceptional potential to produce biodiesel [4]. This perennial oleaginous plant is a species of the Euphorbiaceae family native to America, with heights up to 5 m and an average life expectancy of 50 years [5]. Research has shown that there are toxic and non-toxic genotypes. Phorbol esters are considered the main components of toxic *Jatropha curcas*, making these seeds inedible for animals or humans. Non-toxic *Jatropha curcas* genotypes have seeds with the same or similar chemical composition to the toxic varieties, except for the fact that they lack phorbol esters. This implies that the non-toxic *Jatropha curcas* seeds may be used for feeding livestock [6].

A relevant feature of *Jatropha curcas* is its ability to grow in marginal soil with minimum resources for survival [7]–[9]. This makes this shrub an excellent option to cultivate as an energy crop. In addition, *Jatropha curcas* plantations can grow in terrains that are not suitable for agriculture; therefore, biofuel production using this crop would not compromise food security [10]–[12].

Currently, the *Jatropha curcas* crop has been used mainly for oil extraction. This oil is obtained from the seeds and transformed into biodiesel by chemical processes [13], [14]. A significant amount of non-edible oil, ranging from 27% to 40% [15], can be obtained from these seeds. In addition, the energy that can be extracted from the *Jatropha curcas* seeds oil is considered high, compared to palm or cottonseed oils, with a favorable energy balance in the transformation to biodiesel [16]–[18]. However, crops of *Jatropha curcas* can have alternative uses as they contain some by-products with industrial applications and they can be used as carbon sink and for degraded land recovery [19]–[21]. One potential value-adding by-product is the seed kernel residue obtained after the extraction of the oil (seed cake). This residue has a high protein content, a high calorie count and low fiber amounts that make it suitable for animal or human consumption [22]. In order to use toxic *Jatropha curcas* as animal feed, there must be a detoxification process to remove or inactivate the phorbol esters. In contrast, the non-toxic *Jatropha curcas* seeds may be used for feeding livestock without the need for detoxification processes [23]. Therefore, non-toxic *Jatropha curcas* ecotypes would be the best option to cultivate as an energy crop.

The potential of *Jatropha curcas* plantations to produce energy has been evaluated in several countries from Africa, Asia, South America and North America [24], [25]. However, the results reported in the literature about the plant growth and the seed productions as well as the physical, chemical, and toxicity properties are diverse. The growth performance and

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yield within varying agroclimatic scenarios are uncertain [26]–[29], which makes plantation yield forecasts for other sites challenging. Furthermore, some crops have been inadequately surveyed, considering only one year performance after being planted [30], [31].

In Mexico, several large-scale commercial *Jatropha curcas* crops have been planted. Nonetheless, the yield of these plantations has been lower than expected due to a lack of information for selecting the best seeds, denoting data scarcity about their growth and development under different agroclimatic scenarios [32], [33]. One of the cornerstones for optimizing the production of any plantation is to understand the growth pattern; therefore, it is necessary to monitoring the plantation of the selected *Jatropha curcas* ecotype long enough to obtain reliable yield predictions.

In this work, an experimental plantation including 10 ecotypes of *Jatropha curcas* from the Mexican states of Michoacán, Morelos and Oaxaca was established to collect field measurements of selected biophysical parameters (plant height, stem base diameter, canopy area), of plants survival and of seed production to understand the crop growth and development of the selected ecotypes under a minimum resource scenario. The data collected on the experimental crop for the *Jatropha curcas* over a period of 52 months were processed using a statistical approach to identify those ecotypes with the largest morphologic growth. The collected data on survival and seed production were sorted out to identify those with the best net oil seed content yield. Additionally, toxicity testing was performed to classify the toxic and non-toxic ecotypes.

The results obtained from each variable (morphologic growth, net oil seed content, and toxicity) were normalized to compare and select the ecotypes with the highest potential to propose an eventual *Jatropha curcas* biodiesel production in the state of Morelos.

II. Material and Methods

A. Study Site

The experimental plantation covers around 1.3 ha (3.2 acres) located in the Miacatlan Municipality of the state of Morelos in Mexico (latitude 18° 47' 41.3''N, longitude 99° 21' 06.0'' W at 1000 MASL). The climate at the site is warm and humid with summer rains, and the annual average temperature is 24ºC. The rainy season runs from May to October with precipitation ranging from 800 mm to 1200 mm. Soil texture is clayey and slightly alkaline (pH = 7.4). The natural vegetation of the site includes acacia and other annual herbaceous plants and before establishing the plantation, the land was used to grow sugar cane.

The experimental plantation was initiated in March 2009 and it contained 10 toxic and non-toxic *Jatropha curcas* ecotypes distributed across 10 experimental micro-plots. A total number of 569 *Jatropha curcas* plants were planted and meaningful plant samples were included to represent each ecotype.

The field methodology involved a selection of seeds from the Mexican states of Michoacán, Morelos and Oaxaca to obtain germplasm of *Jatropha curcas* by generative propagation (direct seeding). Seed germination was carried out under controlled laboratory conditions and the obtained seedlings remained in the nursery for three months. Subsequently, the seedlings were transplanted into the experimental plot with pits of 0.4 x 0.4 x 0.4 m and spacing of 2 m x 2.8 m, keeping the spacing between plants constant. This resulted in a crop density of 1250 plants per hectare.

Each ecotype was identified with an alphanumeric ID code to recognize its location in the experimental plot. We used the codes E1M, E2M, E3M, E4M, E5M, E6O, E7O, E8O, E9C and E10C (M stands for Morelos, O for Oaxaca and C for Michoacán).

The *Jatropha curcas* experimental plantation was set up in a place with minimum resources to be described below. Fertilizers were used only once during the transplantation process by adding compost mix to each pit. Plantation watering was through natural rainfall. Ancillary irrigation was used for three months after the transplanting; 20 liters of water per plant were poured fortnightly. No insecticides or fungicides were used for pest and disease control.

B. Evaluation of Ecotypes

The comparison and selection of *Jatropha curcas* ecotypes were carried out following the methodology shown in Fig. 1. Three variables were obtained from field survey, specifically: Morphological growth, net seed oil content and toxicity. These variables were normalized from zero to 1 in order to compare all the ecotypes.

C. Morphological Growth

After the plantation set up, a permanent monitoring of the growth parameters of the *Jatropha curcas* ecotypes was conducted. The measurements of basic biophysical parameters included plant height or vertical distance from the ground level to the highest green plant, stem base diameter (i.e., the cross-section of the stem at the apex height), and maximum and minimum diameter coverage. With these parameters, the canopy area was calculated assuming an ellipsoidal shape as the best approximation to the shape of the plants.

During the first year, data were collected on a monthly basis for three months (months 2-5: May-July, 2009); there was a waiting period of two months and data were collected again in month 7 and month 9 (September and November 2009). Subsequent measurements were taken on an annual basis from July 2010 to July 2013. Overall, data were collected at the experimental plantation for a period of 52 months. All data were recorded systematically in a database [34] for subsequent processing.

The database comprising all the parameters measured in the plantation during the 52 months period was analyzed using a statistical approach. Some statistical tests were applied in order to find patterns, identify similarities or differences among the several *Jatropha curcas* ecotypes selected for this study. The methodology involved (i) a comprehensive review of all data collected in the field to avoid data entry mistakes;

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(ii) plotting of histograms to see whether data were normally distributed; (iii) analysis of outliers with the software UDASYS [35] to identify discordant outliers, and (iv) application of ANOVA and Tukey HSD significance tests to compare core trend parameters against the set of normal data. The tests for outliers and the significance tests were all applied at 95% confidence level (0.05 significance level).

The morphological growth variable was computed from the average measure of the biophysical parameters of plant height, stem diameter and canopy area, which were normalized from zero to 1, where 1 was the highest value in each setting. The values obtained from this normalization for each parameter were added up for each one of the ecotypes and the obtained result was equally normalized, from zero to 1.

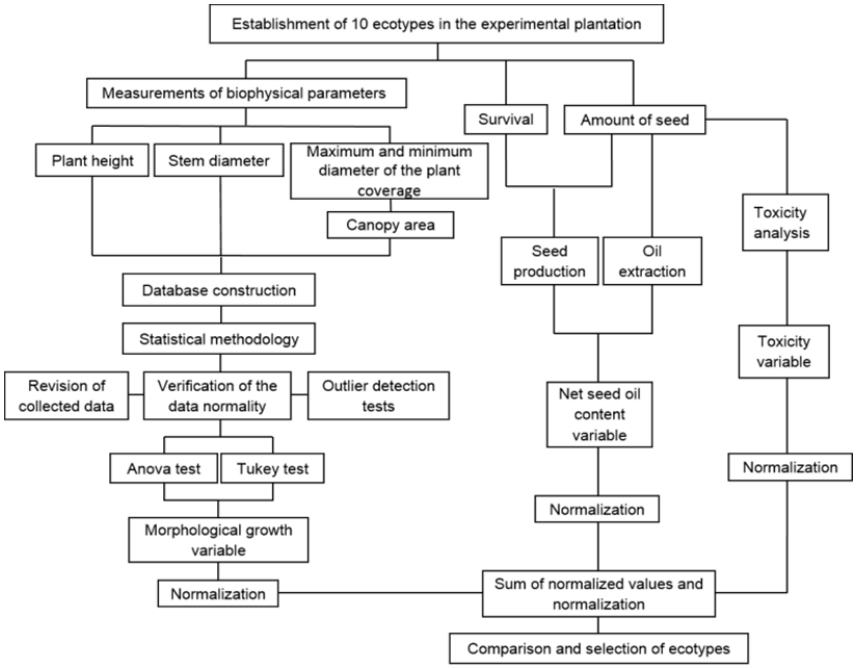


Fig. 1 Methodology evaluation of 10 ecotypes of *Jatropha curcas* in the experimental plantation

D. Net Seed Oil Content

The net seed oil content was obtained from the evidence of the surviving plants, by estimating the amount of seed produced by ecotype. The plant survival rate under the site environmental conditions was calculated by counting the live and dead plants at the end of months 4, 28, 40 and 52. A specific number of seeds were obtained with the first harvest of *Jatropha curcas* in the year 2013. With these seeds, a first calculation of the oil extraction percentage was carried out for each of the ecotypes. The oils were extracted from the seeds using a cold mechanical process, by means of a laboratory hydraulic press that was specially designed for this study. The oil extraction rates (in percentage) were obtained by triplicate for each ecotype, with 200 g and 300 g of seeds per sample, calculating the relationship between the weight of collected oil and the weight of seeds.

For each ecotype, survival and the weight of seeds were used to estimate seed production per hectare. After that, the seed production and oil extraction rates were multiplied to obtain the net seed oil content. The results obtained were normalized from zero to 1, where 1 was the highest value.

E. Toxicity

The toxicity of the *Jatropha curcas* in the experimental plantation was assessed by quantifying phorbol esters in oil. HPLC analysis was carried out (LC/MS instrument 1100, Agilent) based on the modified method of Haas (2002). For each ecotype of *Jatropha curcas,* 1.5 g of oil was weighted on an electronic balance (+/- 0.0001 g) and diluted with 5 mL of ether. Samples were treated with a solid phase extraction device. The silica column was activated and conditioned with ether. The samples were poured and washed with 5 mL of ether and 3.5 mL of acetone to eliminate the column interferences or contaminants. Finally, the phorbol esters elution was performed with 5 mL of methanol and 5 mL of ethanol. Methanol and ethanol were evaporated and the sample was diluted with 2 mL of acetonitrile for the HPLC analysis. The quantitative analysis was performed following the same method, but with some modifications and the addition of 40 µL of phorbol 12-myristate 13-acetate (SIGMA) of known concentration (0.005 mg/µL) as the reference standard.

The samples were collected from the reverse phase column Agilent Eclipse XDB C18 (5 µm-150 x 4.6 mm). The separation conditions were: Flow rate of 1 mL/min, mobile phase 75/25 acetonitrile-water, and isocratic mode. The phorbol ester concentration in seeds was only evaluated for the harvest of 2013.

The results for toxicity were normalized so that 1 is for non-toxic ecotypes and 0 for toxic ecotypes, since the former crop would not entail risks of food poisoning for animals eating

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Jatropha curcas plant leaves and because the seed cake of non-toxic ecotypes can be used to produce cattle feed.

Finally, the normalized values of morphological growth, net seed oil content and toxicity were added up for each one of the ecotypes and subsequently the results of this addition were again normalized from zero to 1. The ecotypes with a final value greater than 0.6 were selected as those with the highest potential to be used for biodiesel production in the state of Morelos.

III. Results and Discussion

A. Plant Morphologic Growth

The average growth trend of *Jatropha curcas* ecotypes for each biophysical parameter is shown in Fig. 2. All ecotypes showed similar trends except for E9C, which consistently exhibited the highest values in plant height, stem diameter and canopy area in contrast with E8O, which always showed the lowest mean values. The average increase of the measures and the standard deviation in plant height, stem diameter and canopy area for all 10 *Jatropha curcas* ecotypes during their phases of growth are shown in Table I. The average growth showed a rapid growth stage in plants during the set-up year (Table I, row 1Y) and a slow development phase in subsequent years (Table I, row AJ), where the average annual growth was significantly lower compared to the first year.

During the rapid growth stage, the lower average increase of the plant height and stem diameter happened in the period from December to March (Table I, row DM). The canopy area was not recorded in this period due to a dormancy period of all ecotypes, this was a vegetative phase when the plants lost all their leaves.

At the end of the rapid growth stage, the average annual growth in the plant height ranged from 95.1 cm to 165.0 cm. The annual average of the stem diameter and canopy area increased from 3.74 cm to 7.09 cm, and from 5,458.0 cm^2 to 28,663.6 cm^2, respectively. The ecotypes E8O and E9C showed the lowest and highest annual average increase for all parameters during this stage (Table I, row 1Y).

After the initial growth period of *Jatropha curcas*, the average annual increase decreased substantially across all ecotypes (see Table I, row AJ). Regarding plant height measurements, the average annual increase ranged from 7.2 cm up to 30.4 cm, E4M and E9C had the lowest and highest values, respectively. The average annual increase for stem diameter fluctuated between 0.83 cm to 3.22 cm, and E10C and E9C ecotypes showed the lowest and highest measurements, respectively. The canopy area ranged between 894 cm^2 to 9,309.6 cm^2; ecotypes E8O and E9C showed the highest and lowest average annual increase, respectively. In Fig. 3, the final average growth per ecotype (after 52 months) and its corresponding standard deviation are displayed for plant height (Fig. 3 (a)), stem diameter (Fig. 3 (b)) and canopy area (Fig. 3 (c)). The final average growth interval of the plant height was 145.3 ± 25.0 cm to 264.0 ± 53.8 cm, that of the stem diameter was 8.4 ± 1.0 cm to 15.6 ± 2.9 cm, and that of the canopy area was 8075.9 ± 2355.1 cm^2 to 49054.5 ± 20507.4 cm^2. Most of the ecotypes show small standard deviation values for these parameters; however, the ecotypes with the highest growth in the biophysical parameters also showed the largest variability. Nevertheless, the mean values are still representative of the average growth of the plant height, stem diameter, and canopy area.

To detect if there were statistically significant differences of growth biophysical parameters, we applied ANOVA and Tukey tests at 95% confidence level. The ANOVA test showed two groups of ecotypes: (i) with no statistically significant differences E1M, E2M, E3M, E4M, E5M, and E8O and (ii) with statistically significant differences E6O, E7O, E9C, and E10C. For the same growth of biophysical parameters, the application of Tukey test classified ecotypes in (A) higher growth; (AB) high growth; (B) medium growth; (BC) low growth; and (C) lower growth (see Fig. 3). After the application of these statistical tests, we were able to differentiate those ecotypes displaying the highest growth for each parameter.

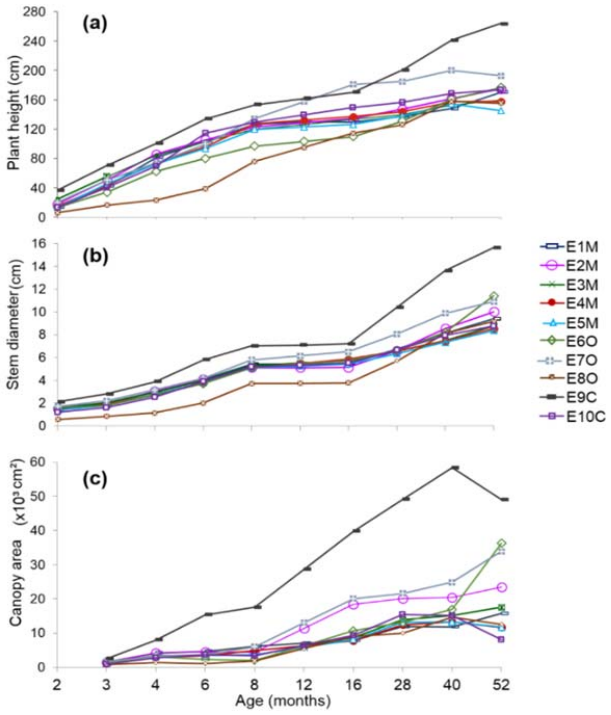


Fig. 2 Growth evaluation of 10 ecotypes of *Jatropha curcas* (C= Michoacán, M= Morelos and O= Oaxaca) by means of biophysical parameters at different plant ages in the plantation. (a) Plant height; (b) Stem diameter; (c) Canopy area

In sum, the improvement of average parameters in *Jatropha curcas* ecotypes during the monitoring of the plantations, in ascending order, was: For height plant growth: E5M, E8O, E4M, E3M, E1M, E10C, E2M, E6O, E7O, E9C; for stem diameter E5M, E4M, E10C, E3M, E8O, E1M, E2M, E7O, E6O, E9C; and for the canopy area E10C, E4M, E5M, E8O, E1M, E3M, E2M, E7O, E6O, E9C.

TABLE I
MORPHOLOGICAL GROWTH OF THE 10 *JATROPHA CURCAS* ECOTYPES AT THE EXPERIMENTAL PLANTATION (P≤0.05)

Average Increase / row		Ecotypes														
		E1M			E2M			E3M			E4M			E5M		
Plant height (cm)	MJ	27.2	±	10.0	32.3	±	7.0	30.2	±	9.0	28.1	±	13.3	28.8	±	10.8
	SN	20.0	±	10.9	20.2	±	9.7	17.4	±	7.8	30.3	±	11.7	26.0	±	13.6
	DM	6.6	±	5.0	10.7	±	5.1	8.7	±	5.6	7.5	±	5.1	10.0	±	3.9
	1Y	130.2	±	15.1	128.7	±	14.6	126.5	±	16.6	132.0	±	15.8	123.0	±	17.6
	AJ	14.3	±	8.3	12.7	±	6.8	7.2	±	6.3	5.7	±	3.1	11.6	±	8.6
Stem diameter (cm)	MJ	1.0	±	0.3	1.1	±	0.3	1.0	±	0.2	0.5	±	0.3	0.9	±	0.2
	SN	1.3	±	0.4	1.0	±	0.3	1.4	±	0.3	1.3	±	0.3	1.1	±	0.3
	DM	0.3	±	0.1	0.5	±	0.2	0.2	±	0.1	0.3	±	0.2	0.2	±	0.1
	1Y	5.4	±	0.6	5.1	±	0.9	5.5	±	0.6	5.5	±	0.8	5.3	±	0.4
	AJ	1.3	±	0.5	1.5	±	0.7	1.1	±	0.4	1.3	±	0.5	1.4	±	0.5
Canopy area (cm^2)	MJ	3031.2	±	1600.7	2667.4	±	1390.7	1822.7	±	820.9	1986.4	±	1474.4	2246.4	±	1336.4
	SN	1243.1	±	984.0	161.5	±	111.4	380.0	±	103.3	1111.4	±	1075.7	594.9	±	238.2
	DM		D			D			D			D			D	
	1Y	6962.3	±	2391.6	11383.7	±	4210.3	6114.7	±	3291.9	6321.8	±	2982.2	5854.9	±	2986.9
	AJ	4031.3	±	2290.6	1748.0	±	455.7	5138.3	±	1493.2	4672.2	±	1215.9	5020.3	±	962.7
Average Increase / row		Ecotypes														
		E6O			E7O			E8O			E9C			E10C		
Plant height (cm)	MJ	28.4	±	10.1	24.9	±	8.6	8.1	±	2.8	33.9	±	5.0	31.0	±	7.0
	SN	16.4	±	10.5	34.5	±	13.5	35.0	±	18.5	32.8	±	11.5	42.9	±	12.3
	DM	14.7	±	6.2	25.3	±	11.5	23.4	±	29.1	22.4	±	7.0	9.6	±	5.5
	1Y	103.2	±	19.5	157.5	±	24.9	95.1	±	25.5	161.8	±	14.5	139.4	±	19.7
	AJ	25.5	±	13.0	8.3	±	4.7	11.6	±	9.1	30.4	±	15.0	7.0	±	6.5
Stem diameter (cm)	MJ	1.0	±	0.2	0.9	±	0.1	0.7	±	0.3	1.0	±	0.2	0.8	±	0.2
	SN	1.4	±	0.3	1.6	±	0.4	1.7	±	0.5	2.0	±	0.5	0.1	±	0.3
	DM	0.2	±	0.1	1.0	±	0.3	0.7	±	0.3	0.8	±	0.3	0.6	±	0.2
	1Y	5.4	±	0.6	6.2	±	1.2	3.7	±	1.1	7.1	±	1.0	5.3	±	0.8
	AJ	1.1	±	0.4	1.6	±	0.7	1.9	±	0.6	3.2	±	0.9	0.8	±	0.4
Canopy area (cm^2)	MJ	2460.4	±	1166.4	1538.2	±	702.3	583.0	±	175.7	5575.6	±	1912.9	2183.9	±	953.6
	SN	289.3	±	164.8	2421.5	±	1045.5	881.3	±	495.2	7303.0	±	3000.0	956.4	±	651.0
	DM		D			D			D			D			D	
	1Y	6331.5	±	3503.7	13024.2	±	5483.0	5458.5	±	2266.0	28663.6	±	8001.8	6366.1	±	3710.2
	AJ	2474.0	±	1012.6	1484.0	±	944.5	894.0	±	174.5	9309.6	±	7274.3	6130.0	±	1400.8

* This is the sum of the normalized values of the plant height, stem diameter and canopy area of each ecotype
** Normalization according to normalized values of sum column
*** Normalization according to net seed oil content
**** Normalization according to toxicity analysis
***** This is the sum of the normalized values of the fifth, sixth and seventh column

The normalization of morphological growth variables was also estimated. The normalization of the biophysical parameters is shown in Table II (columns 2-4). The sum of these values (column 5) was normalized to obtain the morphological growth variable, and finally, the normalization of the morphological growth variable is calculated in column 6. The normalized values of the morphological growth in ascending order is as follows: EM5, E4M, E10C, E8O, E3M, E1M, E2M, E7O, E6O, and E9C.

B. Net Seed Oil Content

To estimate the net seed oil content, seed production and oil extraction rates were calculated. Seed production is the product of plant survival times the amount of seed collected in the experimental plot at the end of the study.

Plant survival was monitored throughout the study. After the fourth month, we recorded plant mortality percentages of around 10% among all ecotypes, indicating a rapid acclimatizing to the experimental site conditions. Plant mortality increased over time and the highest plant loss occurred 40 months after the establishment of the plantation. It was confirmed that in most cases, *Jatropha curcas* plant mortality was due to a stem borer pest (*Diatraea saccharalis*).

By the end of the study (month 52), E9C kept a survival rate of 100%, while ecotypes E6O and E8O showed the lowest survival rates with 64% and 65% mortality, respectively. The survival rate percentage of ecotypes by the end of the study is shown in Fig. 4, in ascending order: E6O (64%), E8O (65%), E4M (73%), E5M (75%), E10C (82%), E1M (84%), E2M-E7O (89%), E3M (92%) and E9C (100%).

In the "x" axis of Fig. 4, the production of seeds per hectare is presented according to the quantity of collected seeds by ecotype over the last year of the study. The ecotypes E8O, E4M and E3M yielded the highest amount of seed per hectare with 140.09 kg/ha, 88.80 kg/ha and 82.39 kg/ha, respectively, whereas E9C showed the lowest amount of seed with 1.73

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kg/ha. The ascending order of the amount of seed in kg/ha is E9C (1.73), E10C (32.64), E7O (34.87), E2M (54.46), E1M (61.13), E5M (62.87), E6O (69.37), E3M (82.39), E4M (88.80) and E8O (140.09).

According to these results of plant survival and amount of seed, the seed production was calculated and is reported in Fig. 5. The ascending order of the seed production of the 10 ecotypes in kg/ha was: E9C (1.73), E10C (26.76), E7O (31.05), E6O (44.40), E5M (47.15), E2M (48.47), E1M (51.35), E4M (64.82), E3M (75.80), and E8O (91.05).

The oil extraction rates obtained by mechanical extraction are reported in Fig. 5. This parameter varies amongst ecotypes from 13% to 24%: E7O-E10C (13%), E9C-E2M (18%), E8O (20%), E4M (21%), E1M-E5M-E6O (22%) and E3M (24%).

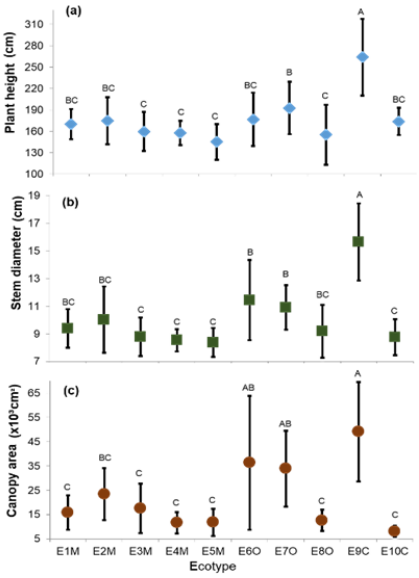


Fig. 3 Variation in biophysical parameters (mean±SD) at the end of the study (52nd month) for 10 ecotypes of *Jatropha curcas*: (a) Plant height; (b) Stem diameter; (c) Canopy area. Values according to Tukey test A = higher growth, AB = high growth, B = medium growth, BC = low growth, C= lower growth. Same letters mean they are not significantly different ($p \leq 0.05$)

TABLE II
NORMALIZATION OF THE VARIABLES MORPHOLOGICAL GROWTH, TOXICITY AND NET OIL CONTENT OF 10 *JATROPHA CURCAS* ECOTYPES

Ecotypes	Plant height	Stem diameter	Canopy area	Sum *	Morphological growth**	Net seed oil content ***	Toxicity ****	Total*****	Normalization value
E3M	0.3	0.3	0.6	1.2	0.4	1	1	2.4	1
E1M	0.4	0.6	0.4	1.4	0.6	0.7	1	2.3	0.9
E2M	0.7	0.7	0.7	2.0	0.7	0.3	1	2	0.8
E4M	0.2	0.1	0.1	0.4	0.1	0.8	1	1.9	0.7
E5M	0	0	0.2	0.2	0	0.6	1	1.6	0.4
E8O	0.1	0.4	0.3	0.9	0.3	1	0	1.3	0.2
E10C	0.6	0.2	0	0.8	0.2	0.1	1	1.3	0.2
E6O	0.8	0.9	0.9	2.5	0.9	0.4	0	1.3	0.2
E7O	0.9	0.8	0.8	2.4	0.8	0.2	0	1	0
E9C	1	1	1	3.0	1	0	0	1	0

* This is the sum of the normalized values of the plant height, stem diameter and canopy area of each ecotype
** Normalization according to normalized values of sum column
*** Normalization according to net seed oil content
**** Normalization according to toxicity analysis
***** This is the sum of the normalized values of the fifth, sixth and seventh column

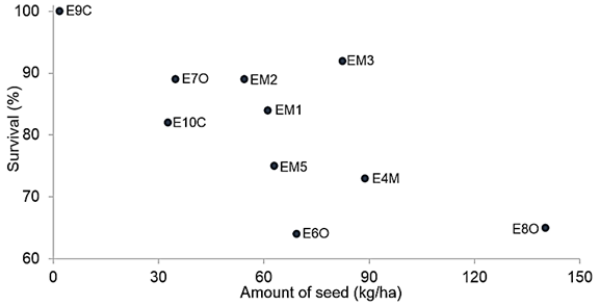


Fig. 4 Seed collected and Survival percentage at the experimental site by the end of the 52nd month. The product of the multiplication of these parameters is the seed production

On the basis of the results of the seed production and oil extraction rates, the net seed oil content was calculated for all ecotypes in kg of oil/ha: E9C (0.3), E10C (3.5), E7O (4), E2M (8.7), E6O (9.80), E5M (10.4), E1M (11.3), E4M (13.6), E3M-E8O (18.2). In Table II, the 7th column shows the normalization values of the net seed oil content variable.

C. Toxicity Analysis

The toxicity analysis results are described in Fig. 6. For the ecotypes E1M, E2M, E3M, E4M, E5M (with no statistically significant differences in growth biophysical according to ANOVA test) and E10C, no phorbol esters were detected; consequently, these are non-toxic ecotypes. The remaining four ecotypes are toxic, since concentrations of phorbol esters were detected in anomalous amounts of 1495.2 mg/l for E6O,

888.8 mg/l for E7O, 1262.56 mg/l for E8O and 599.3 mg/l for E9C. The normalization of the toxicity variable is shown in Table II (see Section II.*E*).

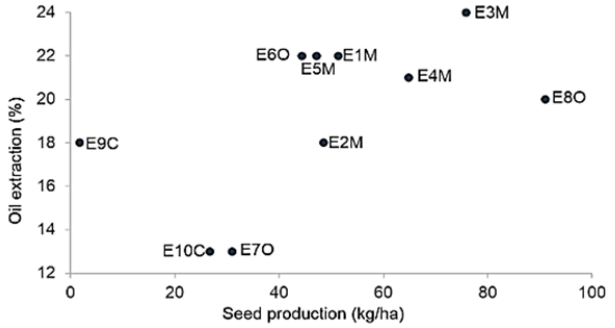


Fig. 5 Net seed oil Content calculated as the relation between seed production and oil extraction percentage

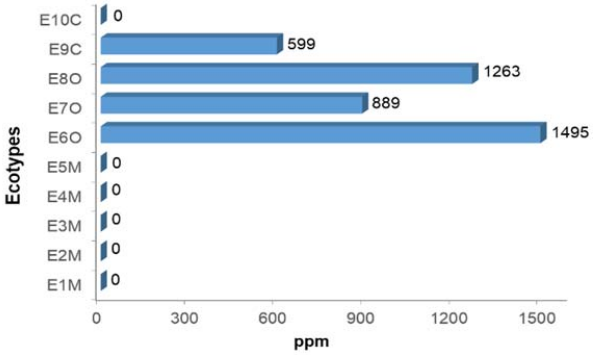


Fig. 6 Concentration of phorbol esters in the *Jatropha curcas* oil according to toxicity analysis

D. Ecotype Comparison and Selection

The sum of the normalized values of the morphological growth, net seed oil content and toxicity variables are detailed in Table II (9th column), and the normalization of these values is presented in the last column of the same table. The non-toxic ecotypes are those with the highest normalized values.

Among the non-toxic ecotypes E1M, E2M, E3M, and E4M with normalized values greater than 0.6 (Fig. 7), we found different features. On one hand, we found the ecotype E1M had high values of normalization in morphological growth, toxicity and net seed oil content. On the other hand, the ecotype E2M showed the highest normalized value in the morphological growth but a lower normalized value in the net seed oil content. Finally, the ecotypes E3M and E4M showed a high normalized value in the net seed oil content but a low morphological growth.

The differences among ecotypes suggest different end uses. For example, the most appropriate utilization for ecotype E2M could be as a carbon sink, through its use as shrub plant for reforestation, since it has a high storage capacity in terms of biomass. In contrast, E1M, E3M, and E4M are the most promising ecotypes for oil extraction. In this study, the ecotypes E1M, E3M, and E4M were identified as the raw material with the highest potential to be used for biodiesel production in the state of Morelos; ecotype E1M could also be used as a carbon sink, since it showed high morphological growth. In the case of E4M, which displayed a low growth index in the biophysical parameters, it could be beneficial to increase the plant density per hectare and facilitate the seed collection, taking advantage of this low index. Another application in order to take advantage of these non-toxic ecotypes is the use of the seed cake (the oil extraction waste) for animal feed production.

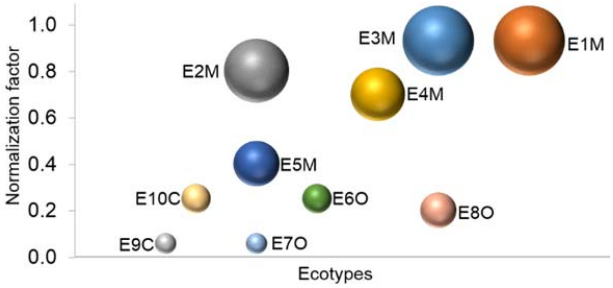


Fig. 7 Comparison of the normalization value of 10 ecotypes of *Jatropha curcas*

These results highlight the importance of studying agro-climatic scenarios in each region to more accurately forecast plantation yields and consider alternative sustainable uses for the *Jatropha curcas* crop.

IV. Conclusions

Based on the results obtained after monitoring an experimental plantation in Morelos state (Mexico) with 10 toxic and non-toxic ecotypes of *Jatropha curcas* during 52 months, we define the most relevant variables to select the best ecotypes to establish energy crops. The results also include new data useful for future studies under agro climatic scenarios like those found in the State of Morelos, México.

The ecotypes E1M, E2M, E3M, and E4M were the most outstanding varieties since they showed the highest seed productivity and oil content, as well as the maximum morphological growth. It is important to highlight that these ecotypes are non-toxic and native species of Morelos state. According to our results, these four ecotypes of *Jatropha curcas* are the best candidates to explore agro-production scenarios for biodiesel production and other use of biomass, in a sustainable way in Morelos state.

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