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Euphorbia antisyphilitica : A potential petro-crop for degraded calcareous soils and saline water irrigation in dry regions of India

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ABSTRACT

Euphorbia antisyphilitica commonly known as Candelilla Wax plant is a succulent laticiferous potential hydrocarbon yielding petro-crop. It yields 8-10 % biomass utilized as bio-fuel. It can be grown successfully on degraded sandy and calcareous soils in arid and semi-arid regions. The crop, when irrigated with saline water, produced ~ 23 t ha^{-1} dry biomass in 2 years. Results of present investigation proved it to be a low nutrient demand crop as it required only 16 and 40 kg ha^{-1} of phosphorus and nitrogen, respectively for optimum biomass production. It is also low-water requiring and produced 17.5 and 15.25 t ha^{-1} dry biomass with saline water (12 dS m^{-1}) irrigation at Diw: CPE ratio of 0.1 and 0.2, respectively as compared to 10.9 t ha^{-1} under rain-fed condition. For large scale cultivation it can be grown successfully on marginal calcareous and sandy soils of dry regions and one or two life saving irrigations with saline water will give optimum biomass.

Keywords: Bio-fuel, calcareous soil, *Euphorbia antisyphilitica*, Candelilla, petro-crop

Introduction

Comparing the forecasted increase in energy demand and available crude mineral oil resources, it is evident that the future energy requirement cannot be met by fossil fuels alone. Without suitable alternatives to crude mineral oil, the global economy will suffer a drastic collapse because of exploding oil prices as a consequence of continuously increasing energy demand expected to arise for maintaining the sustainable future growth of society and economy (Sake *et al.*, 2013). To meet the challenge of the increasing demand of energy, alternative sources of energy and new technology for exploiting the available biomass sources, the biologists need special focus. The bio-fuels have been categorized based on their method and principle application namely as first, second, third and fourth generation bio-fuels, *etc.* (Sorrel *et al*., 2009). The first generation bio-fuels are prepared from biomass consisting of either sugar from sugarcane, sugar beet; starch from corn/maize, cassava; second generation bio-fuels are vegetable oils from soybean, rapeseed; third generation include non-edible oils from *Jatropha, Pongamia*, Castor, animal fats, etc.; and the fourth generation bio-fuels are derived from ligno-cellulosic biomass such as stalks of wheat, corn, switch grass and wood like poplar using advanced technological process. Food and bio-fuels are reliant on same resources for productions *i.e.* land, water and energy. Most of the arable fertile lands cannot be diverted from food producing crops to bio-fuel crops; hence the degraded marginal lands need to be brought under cultivation of bio-fuel crops. Therefore, selecting suitable species from non-food sources, having ability to grow on marginal and degraded lands and sustain production with low quality and quantity water usage may help in mitigating this conflict to a greater extent (Escamilla-Trevino *et al.*, 2010).

The importance of hydrocarbon producing plants, also popularly known as "petro-crops" can provide alternative energy source have come into focus in recent years (Kumar, 1994, 1995; Johari and Kumar, 2013; Sake *et al.*, 2013). Many of these (e.g. *Euphorbia* species-*lathyris, tirucalli, antisyphilitica, caducifolia, neriifolia*; *Pedilanthus tithymalidas*, *Calotropis procera* and *C. gigantea*) can be grown successfully on waste lands or marginal lands of semi-arid and arid regions with no water requirement or meagre requirement of low quality water and without much agricultural inputs and management (Sake *et al.*, 2013). *Euphorbia antisyphilitica* Zucc., commonly known as Candelilla wax plant, is a species of spurge native to the Trans-Pecos of Texas and Southern New Mexico in the United States as well as Chihuahua, Coahuila, Hidalgo and Queretaro in Mexico (http://en.wikipedia.org/wiki/Euphorbia_antisyphilitica). The white sap of this plant was historically used in Mexico to treat sexually-transmitted diseases. Commercial harvesting of Candelilla wax began at the start of 20th century, with demand greatly increasing during World War I & II. The succulent plant has milky latex in the vacuoles of specialized secretory cells called laticifers. It possesses unique features with regard to chemical composition that makes it possible bio-resource for production of bio-fuel. Recently, the residue has been found as a new source of ellagic acid (Ascacio-Valdes *et al.*, 2010) adding the extra value to the plant.

Though seeing the importance, people advocate strongly for cultivation of bio-fuel crops (Bhatia *et al.*, 1993; Kumar, 2001) but the interest in the area of production of bio-fuels hydrocarbon yielding plants has declined, because of the fact that growing these crops merely to obtain bio-fuel by tapping, extraction and by hydro-cracking of bio-crude cannot be cost-effective (Sake *et al.*, 2013). However, if the spent residue acquired after the extraction of bio-crude from petro-crops could be effectively utilized to get value added fuels and chemicals then petro-farming could become a viable alternative for production of bio-fuels from petro-crops. As stated above, many species of *Euphorbia* do not compete with conventional food crops and can be cultivated successfully on degraded marginal lands with very little or no requirement of water as is the case in arid and semi-arid regions. Most of the regions do not have sufficient good quality irrigation water and posses only saline groundwater aquifers. Therefore, in present investigation, both in field and pot-house experiments have been conducted to work out the possibility of utilizing saline water for cultivation of *Euphorbia antisyphilitica*, one of the most potential petro-crops (Kumar, 1990, 1994, 1995, 2001; Johari and Kumar, 2013).

Material and methods

Site climate and soil description

The field study was carried out at Bir Forest, Hisar ($29^0$10'N and $75^0$44'E with altitude of 215.2 m above MSL) in Haryana state in north-western part of India. The climate of the experimental site is semi-arid monsoon type with an annual rainfall of 499 ± 165 mm and open pan evaporation 1888 ± 243 mm (average of 21 years from 1991-2011). Most of the rain (70-80 %) occurs during July to September. The mean daily maximum and minimum temperatures recorded during same period were 31.3 ± 0.8 and 16.1± 0.8 ^{0}C, respectively. The annual rainfall during two years of study period was 340 and 493 mm. The soil characteristics of the experimental site are given in table 1. The soil is sandy loam highly calcareous (Typic Haplustalf) with mean $CaCO_3$ in profile ranged from 6.2 to 9.4 %. The water used for irrigation had electrical conductivity (EC) of 12 dS m^{-1} and SAR ~20. The following experiments were conducted in field:

Experimental details

Experiment 1: Effect of nitrogen and phosphorus on biomass production

This field experiment consisted of 12 treatment combinations comprising of 4 levels of nitrogen (0, 40, 60 and 80 kg ha^{-1}) and 3 levels of phosphorus (0, 16 and 32 kg P_2O_5 ha^{-1}) arranged in complete block factorial RBD design with 4 replications. Full dose of P and half dose of nitrogen were applied basal and remaining half dose of nitrogen was applied after 12 months of crop growth. The rooted slips of *Euphorbia* were planted at 50 x 50 cm plant to plant and row to row distance in 4m x 4m size plots, thus accommodating 64 plants in each treatment plot.

Experiment 2: Effect of saline water irrigation schedules on biomass production

This experiment consisted of 5 irrigation schedules based upon ratio of irrigation water applied (7cm) and cumulative pan evaporation, i.e. Diw: CPE of 0.1, 0.2, 0.4, 0.6 and 0.8, respectively. One treatment was also kept without supplemental irrigation *i.e.* rain-fed. The experiment was laid out in randomised complete block design with four replications.

Two pot-house experiments were carried out under control conditions. In one experiment 16 pots (40 cm height and 40 cm diameter) were filled with 20 kg of each of 5 soil mixtures. Five soil mixtures were prepared using air dried calcareous soil brought from the experimental field site and mixed with sand in soil: sand ratio of 0:4, 1:3, 2:2, 3:1 and 4:0. One rooted *Euphorbia* sapling was planted in each pot and such 20 pots were irrigated with water of each of the four salinity levels i.e. 0.4 (tap water), 4, 8 and 16 dS m^{-1}. Initially 8 litre designated salinity water was applied to bring the soil moisture to field capacity followed by two litre applied at weekly interval. The

Table 1. Initial physico-chemical properties of the soil

Soil depth (cm)	Clay (%)	Silt (%)	Sand (%)	pH (1:2)	ECe (dS m^{-1})	$CaCO_3$ (%)
0-15	18.2	19.4	62.4	8.1	2.0	6.2
15-30	18.0	22.2	59.8	8.3	1.8	8.2
30-60	17.6	21.8	60.6	8.0	2.1	9.4
60-90	17.2	21.2	61.6	7.9	2.6	8.6
90-120	17.4	22.4	60.2	7.8	2.9	8.1

treatment pots were arranged in complete block randomised design (RBD) with four replications of each treatment combination.

In second pot experiment, plants were raised in pots filled with same calcareous soil after mixing different doses of N, P and K fertilizers. The treatments consisted of 3 nitrogen (0, 20, 40, mg kg^{-1} soil), 3 phosphorus levels (0, 7.5 and 15 mg P_2O_5 kg^{-1} soil) and 3 potassium (0, 20 and 40 mg kg^{-1} soil) levels. The total 27 combinations each with 4 replications were arranged in RBD design. The crop in pots was irrigated with tap water as per the schedule followed in earlier pot experiment. Both the experiments were carried out for 9 months (February – October).

Sampling and analytical procedures

After preparing the field for planting, the soil samples were collected from different places in the field to make the representative sample. These were air dried, ground and passed through a 2 mm sieve. Extract of saturated soil paste prepared in distilled water and obtained using the vacuum pump was analyzed for electrical conductivity (ECe) as described by Richards (1954) and pHs using digital pH meter. The mechanical analysis of initial soil samples was done as per the International Pipette method (Piper, 1966). Soil samples were also collected after 2 years of experimentation from each of the treatment plots and analyzed for ECe and pHs to ascertain the changes with imposition of respective treatments. The samples of irrigation water were analyzed for EC, pH and composition of different cations and anions described by Richards (1954). The Na^+ and K^+ were determined with the help of flame photometer while the Ca^{2+} + Mg^{2+} as per standard procedure described in Jackson (1967). As most of the saline nature ground waters contain chlorides and sulphates of sodium, magnesium and calcium, therefore, the saline water used in pots was prepared dissolving salts of NaCl, $MgSO_4$ and $CaCl_2$ in distilled/tap water to make the water of desired salinity ranging from 4 to 16 dS m^{-1}. Care was taken that sodium adsorption ratio (SAR) was maintained < 10 while preparing the water of desired salinity levels. The treatment with tap water (EC 0.4 dS m^{-1}) was kept as control. In both pot experiments, the plants were harvested in first week of November at 9 months of growth and fresh biomass was recorded after taking the height of the plants. Chlorophyll content in plants subjected to different treatments in second experiment was measured as per Arnon (1949).

Statistical analysis

The significance of differences among means of different treatments and their interactions in respective field and pot experiments were judged using least significant difference (LSD) computed at 5% level of Tukey's adjustment after ANOVA test in CRD and factorial RBD designs using MStat-C program.

Results and discussion

Euphorbia antisyphilitica, a succulent plant found growing naturally on marginal soils in arid and semi-arid conditions in Texas and southern New Mexico, shows natural adaptability to prevailing water deficit and resistance to plant and insect attacks (Sake *et al.*, 2013). Cultivation of this plant offers more productive utilization for bio-fuel production and carbon sequestration of otherwise lying fallow marginal and degraded lands (Kalita and Saikia, 2003). To find out the water requirement, one field experiment was conducted to optimize the irrigation schedules of utilizing available saline (of ECiw 12 dS m^{-1}) groundwater regimes ranging from rain-fed (control) to irrigations at Diw:CPE ratio 0.1, 0.2, 0.4, 0.6 and 0.8. Maximum biomass production was recorded when crop received irrigations at Diw: CPE ratio 0.2 plus rains followed by irrigations at Diw: CPE ratio of 0.4. The increase in dry biomass with irrigations scheduled at Diw: CPE ratio of 0.1 and 0.2 was 60 and 40%, respectively as compared to rain-fed. But with further wetter regime irrigation schedules there was drastic reduction in biomass production of *Euphorbia* (Table 2), showing that annually only 4-5 irrigations are sufficient for this crop to produce the optimum biomass. There was also increase in soil salinity build up with application of more number of irrigations (Figure 1) suggesting that though this is extremely low water requiring crop but only moderately tolerant to salts. Four or five life saving irrigations of even saline water increases the biomass to the considerable amount. It is unlike other low water requiring crops such as psyllium (*Plantago ovata*) which could provide higher yield with increase in number of irrigations in 4 months crop period (Tomar *et al.*, 2010) and gradual increase in biomass recorded with increased frequency of saline irrigation in lemon grass (Dagar *et al.*, 2013). For increasing productivity of *E. antisyphilitica* Johari and Kumar (1992) studied the impact of fertilizers in pots and found that supplementation of nutrients

Table 2. Effect of depth of irrigation (Diw/CPE ratio) of saline water (ECe 12 dS m^{-1}) on biomass production of *Euphorbia antisyphilitica*

Depth of Irrigation (Diw/CPE)	Fresh biomass (t ha^{-1})	Dry biomass (t ha^{-1})
Rainfed	68.13	10.92
0.8	30.25	4.85
0.6	40.63	6.55
0.4	55.75	8.96
0.2	104.52	18.29
0.1	94.38	17.40
LSD (p = 0.05)	11.82	2.01

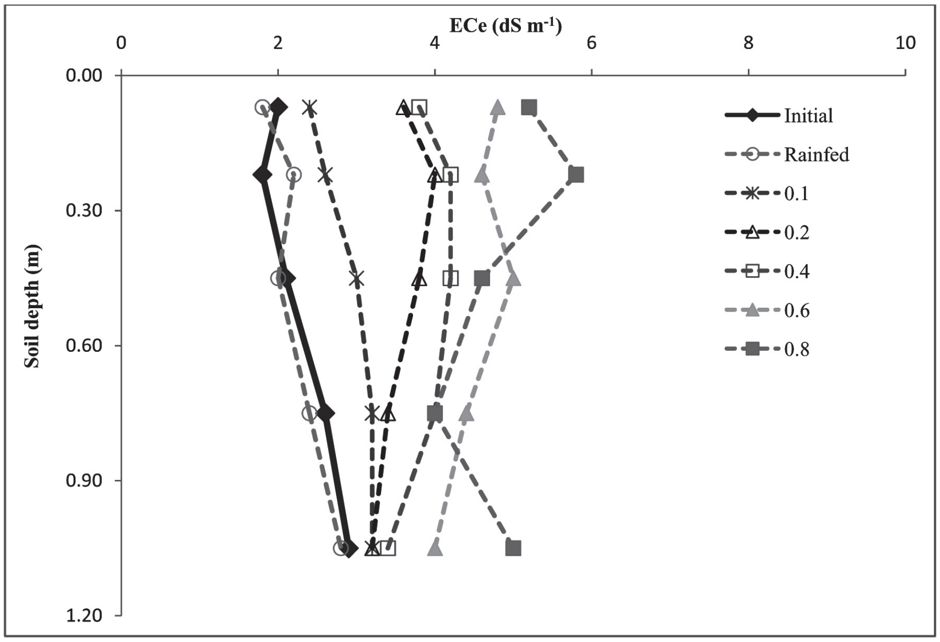

Fig. 1. Development of salinity in soil profile when *Euphorbia antisyphilitica* was irrigated with different Diw: CPE ratio

promoted the growth and productivity including the bio-crude contents which are 8-10 % of dry biomass. In present study, there was gradual increase in biomass with increase in phosphorus and nitrogen doses (Table 3) but there was no significant difference in biomass when 32 kg ha^{-1} phosphorus was applied showing that only 16 kg ha^{-1} phosphorus was sufficient. Similarly, no significant difference in biomass was recorded at 60 and 80 kg ha^{-1} nitrogen implying that nitrogen at the rate of 60 kg ha^{-1} is sufficient. Similar trend in biomass production was true when the study was carried out in pots (Table 4). But in pot-studies the impact of potassium was clearly visible. Earlier, Kaicker *et al*. (1975) in poppy and Johari and Kumar (1992) in *Euphorbia* reported increased biomass and latex yield with application of N whereas with application of K, could produce higher bio-crude than to N and P application. Chlorophyll content and biomass production increased significantly when N, P, & K fertilizers were applied (Table 5). Similar observations were recorded on cholorophyll content in *Euphorbia* by Johari and Kumar (1992).

In most of the arid and semi-arid areas the degraded soils are calcareous in nature. *Euphorbia antisyphilitica* has been found to be quite successful particularly in sandy soils. In one pot study Johari and Kumar (2013) observed highest biomass in red soils which are predominant in arid zone of peninsular India. This was followed when red soil was mixed with sand and gravel and also when sand alone was taken. In present pot study using water of different salinity the maximum biomass was obtained when sand and calcareous soil were mixed in 3:1 ratio followed by sand : calcareous 2:2 ratio and 1: 3 ratio when irrigated with water of ECiw 4 dS m^{-1}. The crop

Table 3. Effect of nitrogen and phosphorus on above ground biomass production (t ha^{-1}) of *Euphorbia antisyphilitica* after two years of growth

Nitrogen	Phosphorus					
	Fresh biomass			Dry biomass		
	P_0	P_{16}	P_{32}	P_0	P_{16}	P_{32}
N_0	95.2	102.3	116.5	16.2	17.4	19.8
N_{40}	118.2	126.7	128.0	20.2	21.8	22.3
N_{60}	123.2	128.4	129.5	21.2	22.2	22.4
N_{80}	124.4	132.5	133.5	21.3	22.8	23.3
Mean	115.3	122.5	122.5	19.7	21.1	22.0

LSD ($p = 0.05$)	Fresh	Dry
N	2.4	0.4
P	2.1	0.3
N x P	4.2	0.7

Table 4. Effect of N, P and K fertilizers on plant height and fresh biomass of *Euphorbia antisyphilitica*

Phosphorus	Potassium	Nitrogen								
		N_0			N_{20}			N_{40}		
		Height (cm)	Biomass (kg/plant) AG	BG	Height (cm)	Biomass (kg/plant) AG	BG	Height (cm)	Biomass (kg/plant) AG	BG
P_0	K_0	63.8	2.41	0.62	75.0	4.28	1.26	78.5	4.68	1.46
	K_{20}	64.5	3.07	0.74	84.0	4.36	1.32	86.5	4.80	1.58
	K_{40}	72.0	3.41	0.92	90.2	4.40	1.41	92.4	4.92	1.62
P_{16}	K_0	69.8	3.26	0.86	90.5	4.38	1.40	93.0	4.72	1.48
	K_{20}	76.8	3.36	0.90	91.2	4.42	1.45	93.8	4.97	1.65
	K_{40}	79.8	3.52	0.93	92.0	4.51	1.52	94.2	5.06	1.70
P_{32}	K_0	82.8	3.45	0.95	90.0	4.45	1.48	95.4	4.86	1.62
	K_{20}	83.0	3.81	1.08	91.5	4.56	1.50	96.0	5.15	1.73
	K_{40}	83.5	3.95	1.23	93.2	5.05	1.53	97.8	5.46	1.82

LSD(p = 0.05)	Height	Biomass
N	0.84	0.08
P	0.67	0.04
K	0.37	0.05
N x P	1.16	0.06
N x K	NS	0.08
P x K	0.65	0.08
N x P x K	1.12	0.14

AG-aboveground biomass, BG-belowground biomass
Subscript values represent amount applied as kg ha^{-1} of nitrogen (N), phosphorus (P) and potassium (K)

Table 5. Effect of N, P and K fertilizers on chlorophyll contents of *Euphorbia antisyphilitica*

Phosphorus	Potassium	Nitrogen								
		N_0			N_{20}			N_{40}		
		a	b	Total	a	b	Total	a	b	Total
		Chlorophyll (mg g^{-1}) fresh weight								
P_0	K_0	0.410	0.215	0.625	0.558	0.264	0.822	0.620	0.276	0.896
	K_{20}	0.556	0.294	0.850	0.610	0.303	0.913	0.660	0.282	0.942
	K_{40}	0.604	0.346	0.950	0.632	0.312	0.944	0.675	0.294	0.969
P_{20}	K_0	0.486	0.235	0.721	0.578	0.272	0.850	0.640	0.280	0.920
	K_{20}	0.525	0.305	0.830	0.634	0.345	0.979	0.784	0.362	1.146
	K_{40}	0.587	0.365	0.952	0.625	0.358	0.983	0.664	0.328	0.992
P_{40}	K_0	0.450	0.265	0.715	0.542	0.276	0.818	0.670	0.294	0.964
	K_{20}	0.480	0.225	0.705	0.584	0.282	0.866	0.680	0.360	1.040
	K_{40}	0.515	0.245	0.760	0.610	0.312	0.922	0.690	0.315	1.005

LSD (p=0.05)	a	b	Total
N	0.014	0.001	0.012
P	0.007	0.001	0.007
K	0.005	0.001	0.005
N x P	0.013	0.002	0.0013
N x K	0.009	0.002	0.008
P x K	0.009	0.002	0.008
N x P x K	0.016	0.003	0.014

biomass production with ECiw 4 dS m^{-1} water irrigation was even higher than when irrigated with tap water (ECiw ~ 0.4 dS m^{-1}) showing that the plant also requires some salt for its optimum growth. The pattern remained the same in different sand: soil relationships when irrigated with saline water but the biomass decreased when salinity of irrigation water increased (Table 6).

Table 6. Performance (biomass kg/plant) of *Euphorbia antisyphilitica* when grown in different proportion of sand: calcareous soil and irrigated with water of different salinity (After 9 months of growth March-November)

Sand: soil ratio	ECiw (dS m^{-1}) of irrigation water				
	Tap water	4	8	12	16
4:0	1.66	1.72	1.48	1.42	1.28
3:1	2.22	2.32	1.83	1.65	1.50
2:2	1.87	2.00	1.76	1.58	1.37
1:3	1.77	1.96	1.64	1.44	1.31
0:4	1.74	1.82	1.55	1.38	1.28

LSD (p = 0.05)	
Between sand: soil mixture (A)	0.18
Between ECiw of irrigation water (B)	1.17
Interaction (A) x (B)	NS

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