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Original Article

IMPACT ON PROLINE CONTENT OF *JATROPHA CURCAS* IN FLY ASH AMENDED SOIL WITH RESPECT TO HEAVY METALS

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ABSTRACT

Objective: To reveal the property of *Jatropha curcas*, to retain itself under the heavy metal stress of high concentration of fly ash through the increase in proline content in plants.

Methods: A pot culture experiment was conducted to investigate growth performance, biochemical and physiological responses of the *Jatropha curcas* (n=15) in fly ash amended the soil. The present study was performed as an attempt to determine the growth performance of *Jatropha curcas* using various concentrations of fly ash and soil [100% soil (T_1), 25% fly ash+75% soil (T_2), 50% fly ash+50% soil (T_3) and 75% fly ash+25% soil (T_4) and 100% fly ash (T_5)]. The elemental composition (Zn, Ca, Mg, Pb, Cu, Fe, Mn, Ni and Cd) was studied by Atomic Absorption Spectrophotometer in base material at the beginning and at the end of the study. The three years response was reported and observed that the proline content in *Jatropha curcas* leaves increased as the fly ash concentration increased (as proline is a stress protein which is formed according to the defensive capability of plants).

Results: After three years of complete plant growth the elemental (heavy metals) uptake increased with respect to the availability. The overall proline content increased as 2.48 µg/ml, 3.97 µg/ml, 4.78 µg/ml, 5.25 µg/ml and 5.60 µg/ml in T_1, T_2, T_3, T_4 and T_5 respectively. After evaluating the correlation between heavy metal uptake and proline content, all the results showed positive significance at 0.05% and 0.01% significance level.

Conclusion: According to the results it has been proved that when heavy metal uptake by *Jatropha curcas* increases through fly ash, the proline content increases according to its capability to defence itself in stress conditions. This research motivated to waste utilization, sustainable development, and environment protection.

Keywords: Soil, Fly ash, *Jatropha curcas*, Proline, Growth performance

INTRODUCTION

Fly-ash is a solid waste consisting of completely burnt or unburnt particles of carbon resulting from the burning coal. Fly ash constitutes the large portion of the total quantity of residues produced in a coal-fired thermal power plant. The electrostatic precipitator separates dust particles from the flue gases. Now the bottom ash as non-combustible by-product obtained through combustion in a furnace. Fly ash is a good soil ameliorate [1, 2] and very useful for agriculture [3]. Fly ash addition to soil improves or changes various physical, chemical and biological properties of soil. It is also observed that tomato plant grown in fly ash mixture showed luxuriant growth with bigger leaves. Plant growth, yield, pigment content were enhanced in 40-80 % fly ash amended soils. With 100% fly ash, yield was considerably reduced. The most economical level of fly ash incorporation was 40%, which improved the yield and market value [4]. By fly ash application the seed yield of black gram increases with fly ash at the rate of 250g Kg^{-1} [5]. The increase in chlorophyll content and photosynthetic rate of *Jatropha curcas* has been observed with a low dose of fly ash (20%) with soil [6]. It was also reported that *Jatropha curcas* has medicinal properties [7], which also can enhance by using 25% fly ash with soil in the base material [8].

Proline is a α-amino acid (imino acid), one of the 20 DNA-encoded amino acids found in proteins. It is distinctive among the 20 protein-forming amino acids in that the α-amino group is secondary. The more common 'L' form has 'S' stereochemistry. In plants, proline is synthesized from glutamic acid through a path catalysed by pyrroline-5-carboxylate synthetase and pyrroline-5-carboxylate reductase. Its presence in various abiotic stresses (heat, cold, drought, moisture, and salinity) in important crop plants considered as a tolerance mechanism. It is recommended to act as compatible as well as a source of nitrogen during recovery from stress. Compatible products act an as chemical chaperone, which protects proteins during various abiotic stresses. Due to the presence of heavy metals in fly ash the stress protein (proline) produced in plants for the survival. This was reported in 2012 [9], that when the seedlings of *Jatropha curcas* under cadmium and lead and their combined stress. The plant biomass, gas exchange rate, and photosynthetic pigment contents decreased while leaf conductivity, the soluble proteins, and free proline content increased significantly. The present study involves the evaluation of the effect of fly ash on proline content in leaves in different concentrations.

MATERIALS AND METHODS

The fly ash from Rajghat thermal power station, New Delhi, India, was brought to the experimental site. The soil was collected from a garden near Badshahpur, Sohna road, Gurgaon, Haryana. The fly ash and garden soil were mixed. Experiments were conducted under natural conditions in cement pots. For the growth of *Jatropha curcas* fly ash and soil are mixed in five different concentrations. The five different treatments and each treatment had sample size 15 (15-15 pots of each treatment) prepared. These are as follows:

1. Treatment 1 ($T1/T_1$): 100% soil (control)

2. Treatment 2 ($T2/T_2$): 25% fly ash+75% soil

3. Treatment 3 ($T3/T_3$): 50% fly ash+50% soil

4. Treatment 4 ($T4/T_4$): 75% fly ash+25% soil

5. Treatment 5 ($T5/T_5$): 100% fly ash

The seeds of *Jatropha curcas* were collected from "National Oilseeds and Vegetable Oils Development Board, Sector-18, Gurgaon". The seeds were sowed in the month of July. The germination of seeds started in the month of August. For three consecutive years, the plant growth performance was evaluated from May to October ($M1/M_1$ to $M6/M_6$). Initially before the sowing of *Jatropha curcas* seeds in pots, the elemental composition was evaluated by AAS (Atomic Absorption Spectrophotometer). These elements are as follows: Zn, Ca, Mg, Pb, Cu, Fe, Mn, Ni and Cd. The proline content

was evaluated in the continuous process from May to October (M_1 to M_6) in three years of plant growth performance by L. S. Bates *et al.* [10]. The method is as follows:

Proline estimation is based on the formation of a brick red coloured proline-ninhydrin complex in acidic medium. This complex is soluble in toluene and thus, can be separated from the liquid phase. This gives the positive response that there is no interference with other amino acids, which also form a blue coloured complex with ninhydrin. The toluene brick-red coloured complex absorbs at 520 nm.

Reagents

1. Glacial acetic acid (Analytical grade)

2. Sulphosalicylic acid (3%): Three gram of sulphosalicylic acid was dissolved in 100 ml of distilled water.

3. Orthophosphoric acid (6 N): Required volume of orthophosphoric acid (38.1 ml) was taken, and volume was made to 100 ml, using distilled water to get 6 N orthophosphoric acid.

4. Acid ninhydrin: Ninhydrin (1.25 g) was dissolved in a blend of 30 ml of glacial acetic acid and 20 ml of 6 N orthophosphoric acid.

Procedure

1. 0.5 g plant tissue was taken and homogenized in 5 ml of 3% sulfosalicylic acid using mortar and pestle.

2. The homogenate was filtered through 'Whatman No. 1' filter paper and collected filtrate was used for the estimation of proline content.

3. 2 ml of extract was taken in the test tube, and 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added.

4. The reaction mixture was heated in a boiling water bath at 100 ^oC for 1hour. The brick red colour developed.

5. After cooling the reaction mixtures, 4 ml of toluene was added and then transferred to a separating funnel.

6. After thorough mixing, the chromospheres containing toluene was separated, and its absorbance read at 520 nm in spectrophotometer next to toluene blank.

7. A standard curve of proline was plotted by taking 2 to 10 µg/ml concentrations.

8. The unknown proline concentration determined from a standard curve.

After proline content determination in treatment levels, the interaction in between treatments, interaction in between months and interaction in between months and treatments were determined through CRD (Complete Randomize Design) by "OPSTAT software of HAU", Hisar, Haryana. The correlation in between the elemental (heavy metals) uptake and plant growth was determined, for the significance level of results at 0.05% and 0.01% [11].

RESULTS AND DISCUSSION

According to the results, the concentration of elements was decreased in pots of all treatment levels as per the availability. According to the results (table 1 and table 2), the percent absorption of Zn in T_5 (48.28% of 1.28 ppm), in T_4 (36.29% of 0.89 ppm), in T_3 (15.26% of 0.48 ppm), in T_2 (14.41% of 0.34 ppm) and in T_1 (6.67% of 0.24 ppm). The percent absorption of Ca in T_5 (42.38% of 1364 ppm), in T_4 (51.31% of 1302 ppm), in T_3 (20.00% of 1296 ppm), in T_2 (18.02% of 1204 ppm) and in T_1 (10.34 % of 406 ppm). The percent absorption of Mg in T_5 (62.89% of 127.2 ppm), in T_4 (64.40% of 122.2 ppm), in T_3 (51.38% of 110.2 ppm), in T_2 (49.29% of 98.4 ppm) and in T_1 (29.92% of 26.4 ppm). The percent absorption of Pb in T_5 (30% of 0.02 ppm), in T_4 (25.71% of 0.21 ppm), in T_3 (21.65% of 0.40 ppm), in T_2 (19.14% of 0.58 ppm) and in T_1 (12.92% of 0.72 ppm). The percent absorption of Cu in T_5 (64.80% of 1.96 ppm), in T_4 (62.66% of 1.58 ppm), in T_3 (18.78% of 1.14 ppm), in T_2 (16% of 0.50 ppm) and in T_1 (7.89% of 0.38 ppm). The percent absorption in Fe in T_5 (75.36% of 20.86 ppm), in T_4 (59.23% of 16.80 ppm), in T_3 (40% of 12.4 ppm), in T_2 (38.62% of 7.12 ppm) and in T_1 (21.10% of 4.36 ppm). The percent absorption in Mn in T_5 (60.59% of 3.40 ppm), in T_4 (61.47% of 2.18 ppm), in T_3 (51.67% of 1.80 ppm), in T_2 (36.04% of 1.44 ppm) and in T_1 (17% of 1.60 ppm). The percent absorption in Ni in T_5 (60.25% of 400 ppb), in T_4 (55.71% of 280 ppb), in T_3 (18.67% of 195 ppb), in T_2 (15% of 50 ppb) and in T_1 (11.57% of 28 ppb). The percent absorption of Cd in T_5 (37.93% of 319 ppb), in T_4 (45.65% of 322 ppb), in T_3 (44.48% of 326 ppb), in T_2 (32% of 343 ppb) and in T_1 (30.75% of 370 ppb).

The plant growth results were studied by CRD (Complete randomize design) to evaluate. The year wise proline content was also determined. According to results of CRD, it is evident from the data in table 3 the time interval and different base material had a significant effect on proline content in *Jatropha curcas* leaves. The highest proline content (3.29 µg/ml) was recorded from September which was statistically at par with October (3.18 µg/ml), and minimum proline content (2.67 µg/ml) was observed from May, which was statistically similar to proline content, recorded from M_2 (2.71 µg/ml). Plants were grown in 100 % fly ash (T_5) contain highest proline content followed by T4 (3.40 µg/ml), whereas, lowest proline content (1.88 µg/ml) was obtained from T_1 (100% soil). The interactive effects of month interval and different base material were also significantly influenced the proline content in Jatropha leaves. Thus, present data showed that the plants grown in T_5M_6 combination recorded maximum proline content (4.23 µg/ml) and found superior in the treatment combinations except T_5M_5 (3.81 µg/ml), while minimum proline content was recorded from T_1M_1 (1.68 µg/ml) and T_1M_2 (1.68 µg/ml) which was statistically at par with interaction of T_1M_4 (1.77 µg/ml) (fig. 1). According to year wise results the average proline content was increased in all treatment as the growth of plant occurred but the maximum proline content was present in T_5 treatment 5.60 µg/ml with standard deviation of 0.69 (table 4 and fig. 2) because due the heavy metal stress condition the stress protein or proline also enhanced and the plant was survived, but its plant growth suppressed as compared to other treatments.

Table 1: Elemental compositions of all elements in all treatment levels (at the beginning of study)

S. No.	Zn (ppm)	Ca (ppm)	Mg (ppm)	Pb (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Ni (ppb)	Cd (ppb)
T_1	0.24	406	26.4	0.72	0.38	4.36	1.60	28	370
T_2	0.34	1204	98.4	0.58	0.50	7.12	1.44	50	343
T_3	0.48	1296	110.2	0.40	1.14	12.4	1.80	195	326
T_4	0.89	1302	122.2	0.21	1.58	16.80	2.18	280	322
T_5	1.28	1364	127.2	0.02	1.96	20.86	3.40	400	319

T_1:100% soil (control), T_2:25% fly ash+75% soil, T_3:50% fly ash+50% soil, T_4:75% fly ash+25% soil, T_5:100% fly ash; M_1 to M_6: May to October. Ppm = parts per million and ppb = parts per billion.

Table 2: Percent absorption of elements by plants at all treatment levels (at completion of the study)

S. No.	Zn	Ca	Mg	Pb	Cu	Fe	Mn	Ni	Cd
T_1	6.67	10.34	29.92	12.92	7.89	21.10	17.00	11.57	30.75
T_2	14.41	18.02	49.29	19.14	16.00	38.62	36.04	15.00	32.00
T_3	15.26	20.00	51.38	21.65	18.78	40.00	51.67	18.67	44.48
T_4	36.29	51.31	64.40	25.71	62.66	59.23	61.47	55.71	45.65
T_5	48.28	42.38	62.89	30.00	64.80	75.36	60.59	60.25	37.93

T_1:100% soil (control), T_2:25% fly ash+75% soil, T_3:50% fly ash+50% soil, T_4:75% fly ash+25% soil, T_5:100% fly ash.

Table 3: Interaction of time interval (month) and different base material on proline content (µg/ml) in *Jatropha curcas*

Months interval	Treatments/Base material					Mean
	T_1	T_2	T_3	T_4	T_5	
M_1	1.68	2.50	2.83	3.06	3.29	2.67
M_2	1.68	2.51	2.87	3.09	3.39	2.71
M_3	2.02	2.68	2.97	3.22	3.55	2.89
M_4	1.77	2.77	3.23	3.52	3.93	3.04
M_5	2.06	2.72	3.30	3.70	4.10	3.18
M_6	2.10	2.87	3.43	3.81	4.23	3.29
Mean	1.88	2.68	3.11	3.40	3.75	
CD at 5%	Month = 0.12, Treatment = 0.11, Month × Treatment = 0.26					

T_1:100% soil (control), T_2:25% fly ash+75% soil, T_3:50% fly ash+50% soil, T_4:75% fly ash+25% soil, T_5:100% fly ash; M_1 to M_6:May to October. µg/ml = microgram per millilitre.

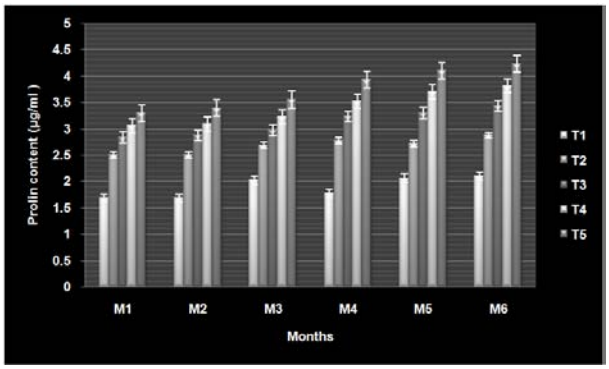

Fig. 1: Proline content (µg/ml) in all treatments month wise according to CRD in *Jatropha curcas*. T1:100% soil (control), T2:25% fly ash+75% soil, T3:50% fly ash+50% soil, T4:75% fly ash+25% soil, T5:100% fly ash; M1 to M6: May to October. Data are the means of all replicate measurements (n=15). µg/ml = microgram per milliliter

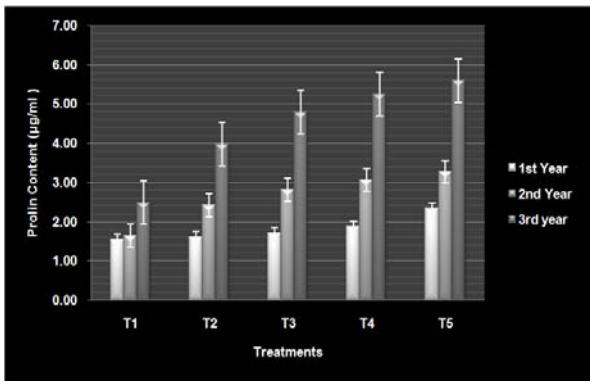

Fig. 2: Year-wise average proline content (µg/ml) in all treatments of *Jatropha curcas*, T1:100% soil (control), T2:25% fly ash+75% soil, T3:50% fly ash+50% soil, T4:75% fly ash+25% soil, T5:100% fly ash. Data are the means of all replicate measurements (n=15)

Table 4: Year-wise average proline content (µg/ml) in all treatments of *Jatropha curcas* where n=10

Proline content (µg/ml)					
	T_1	T_2	T_3	T_4	T_5
1st year	1.54	1.61	1.70	1.88	2.33
	±0.080	±0.042	±0.028	±0.136	±0.25
2nd year	1.63	2.42	2.82	3.07	3.27
	±0.05	±0.30	±0.46	±0.41	±0.32
3rd year	2.48	3.97	4.78	5.25	5.60
	±0.53	±0.15	±0.31	±0.47	±0.69

T_1:100% soil (control), T_2:25% fly ash+75% soil, T_3:50% fly ash+50% soil, T_4:75% fly ash+25% soil, T_5:100% fly ash. Data are the means with standard deviation of all replicate measurements (n=15). µg/ml = microgram per milliliter

Table 5: Average proline content (µg/ml) in all treatments in all three years

Sample	Proline (µg/ml)
T_1	1.88
T_2	2.68
T_3	3.11
T_4	3.40
T_5	3.75

T_1:100% soil (control), T_2:25% fly ash+75% soil, T_3:50% fly ash+50% soil, T_4:75% fly ash+25% soil, T_5:100% fly ash. Data are the means of all replicate measurements (n=10). µg/ml = microgram per millilitre.

After correlation (table 6) of the average proline concentration (table 5) in all treatments with the absorbed percentage of elements, at 0.01% significant level the positive correlation was shown by Mg, Pb, Fe and Mn and at 0.05% significance level the positive correlation was Zn, Ca, Cu and Ni. With Cd positive correlation also studied. The three-year response was reported in which the proline content increased as the fly ash concentration increased. As proline is a stress protein which formed according to the defensive capability of plants, not only because of heavy metals but also in drought stress conditions the proline content increases [12].

Table 6: Correlation results between proline content (µg/ml) and percent element absorption in all treatments (n=5, df= 4)

Plant growth parameter	Zn (ppm)	Ca (ppm)	Mg (ppm)	Pb (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Ni (ppb)	Cd (ppb)
Proline (µg/ml)	0.891*	0.838*	0.965**	0.988**	0.859*	0.947**	0.979**	0.844*	0.695

* P<0.05 (r =0.811) ** P<0.01 (r = 0.917) (r = Correlation coefficient and µg/ml = microgram per millilitre)

CONCLUSION

According to the results, it was proved that when heavy metal uptake by *Jatropha curcas* increases through fly ash, the proline content increases according to its capability to defence itself in stress conditions. This research motivated for waste utilization, sustainable development, and environment protection. This research also gives a way to phytoremediation of fly ash by non-edible plants.

CONFLICT OF INTERESTS

Declared none

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