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Original article

EFFECT OF COLCHICINE ON THE GROWTH AND PROXIMATE COMPOSITION OF *Jatropha curcas* L.

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ABSTRACT

Jatropha curcas has numerous benefits, which necessitates efforts to increase yield and value. In this study, seeds of matured, healthy Jatropha were collected and induced with four concentrations (zero, 0.25 mM, 1.25 mM and 2.50 mM) of colchicine to evaluate their effects on the growth and proximate composition of the plant. The growth parameters considered were evaluated relative to different colchicine treatments. The proximate composition of the leaves, stems and roots were carried out twelve weeks after planting. Findings from the analysis revealed that treatments 1.25 mM have high mean values across all the parameters considered compared to the control. It was determined that there was no significant difference ($p > 0.05$) between the control (91.67 %) and 1.25 mM (91.67 %) treatments, which shows the highest germination percentage among all treatments. The 1.25 mM Colchicine treated seeds have high mean values in their plant height (28.44 cm). Also, leaf area (49.73 cm^2) and fresh weight (15.90 g) revealed promising results with the 1.25 mM treatment highly significantly from other treatments and control. More so, the colchicine treatments increased significantly ($p < 0.05$) at the number of leaves and dry weight compared to the control. There is no significant difference in the dry weight among all the colchicine treatments (2.45 g, 2.94 g and 2.59 g), but they are significantly different ($p < 0.05$) from the control (1.63 g). Results from the proximate analysis show no significant difference ($p > 0.05$) between the various colchicine treatments and the control. Colchicine at 1.25 mM treatment should be considered for further study, and chemical treatments at lower concentrations should be progressed.

Key Words: Mutagens, Colchicine, *Jatropha curcas*, Mutation, Crop improvement.

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INTRODUCTION

Jatropha curcas is a species of flowering plant in the family Euphorbiaceae. It is native to the tropical areas of America from Mexico to Argentina and has been widely dispersed in subtropical and tropical areas worldwide, becoming naturalized or invasive in many areas [1]. It is resistant to a high degree of aridity, allowing it to grow in deserts [1].

It is an uncultivated non-food wild-specie plant with great potential for bioenergy development in the country [2]. It is a perennial undomesticated plant species with a high, untapped potential for sustainable production of bioenergy in the tropics and subtropics. *Jatropha curcas* has been used traditionally for medicinal purposes. The plant possesses anti-inflammatory, anti-parasitic, wound healing, insecticidal, disinfectant, anti-mestatic, antitumor, co-agulant, pregnancy terminating activities and anti-diarrhoea [3].

Because of the easiness in propagation, rapid growth, drought tolerance, pest resistance, high oil content, adaptation to a wide range of environmental conditions, short gestation period, large genetic variation and optimum plant size, this makes *Jatropha curcas* a promising crop for biofuel [4].

Planting of unimproved materials not only leads to low returns on investments on *Jatropha curcas*. It is therefore important to carry out studies to figure out a suitable strategy for increasing the yield of *Jatropha curcas*. The challenge is to transform *J. curcas* into a competitive crop by applying successful plant breeding technologies [5]. Massive progress in *Jatropha curcas* breeding has been achieved during the past decade [5]. Nigeria still suffers enormous fuel and energy crises, manifesting in various forms, despite her position as Africa's largest crude oil exporter. Thus, the development of new energy sources such as biofuels from the agricultural sector has been viewed as a way of expanding domestic energy supply, preventing too much dependence on imported oil, and diversifying the economy, particularly in the face of falling oil prices [6].

Mutation breeding is the process of exposing seeds to chemicals or radiation to generate mutants with desirable traits to be bred with other cultivars [7]. Mutation breeding is a proven addition and a successful replacement of conventional breeding that provides a specific improvement in a variety without significantly affecting its acceptable phenotypes [8]. The main advantage of mutation breeding is the possibility of improving one or two quantitative characters without changing the rest of the genotypes [8]. Colchicine is an example of a chemical mutagen. It is an alkaloid obtained from autumn crocus (*Colchicum autumnale*), which belongs to the family Colchicaceae. Colchicine binds specifically to tubulins to prevent microtubules' polymerization and induce polyploidy cells [9, 10]. It may induce artificial polyploidy in plants, and various reports have been published to show artificial polyploidy in various plant species and families [9, 10]. These artificial polyploidial effects by colchicine may induce some changes in morphological, cytological, histological and even in gene expression level [11]. The ability of this chemical to induce polypoid in crop species depend on the chemical concentration, duration of exposure and species of crop plant being investigated [11]. Several high yielding variants of crop plants have been developed using colchicine. This study, therefore, describes the growth and proximate composition of the induced mutant population of Jatropha in Nigeria against different doses of colchicine.

MATERIALS AND METHODS

Collection of planting materials and mutagens

Seeds of *Jatropha curcas* were obtained from Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria, Kaduna State. One gramme (1g) of colchicine was purchased from BDH Chemical Industry, Poole, England and prepared at the Multi-User Laboratory, Ahmadu Bello University, Zaria. Topsoil was collected from uncultivated land within the Botanical Garden, Ahmadu Bello University, Zaria and transferred for sterilization and physio-chemical analysis as previously described by Bello *et.al* [12] at the General Laboratory Department of Soil Science, Institute for Agricultural Research, Zaria. These locations fall within Nigeria's tropical guinea savannah zone (Latitude 11^{o} $11^{'}$ N, Longitude 7^{o} $38^{'}$E and altitude of 674m above sea level).

Seed viability

The seed viability test was done before seed dressing using the germination test method described by AOSA [13]. Before the dressing, 200 seeds were used for the viability test. The seeds were spread on separate Petri dishes containing moist filter paper and covered with another moist paper. The experiment was monitored daily. Seven days after setup, the germination percentages were calculated as follows;

$$\% \text{ Germination} = n / N \times 100\ \%$$

Where; n is the Total number germinated, and N is the Total number of seeds planted

Treatment of seeds

The treatment of the seeds was carried out in the screen house located at the Department of Botany, Faculty of Life sciences, Ahmadu Bello University, Zaria. The seed treatment was conducted as previously described by Manzoor *et.al* [14]. Thirty-six (36) dry seeds of *Jatropha curcas* were soaked in four treatments of colchicine (0.25 mM, 1.25 mM and 2.50 mM) and distilled water (zero) for six hours.

Experimental design and sowing of seed

In this experiment the seeds were planted and observed for growth parameters at different growth stages. A total of twelve polythene bags were used, comprising of three polythene bags per treatment. The polythene bags filled with sterilized topsoil were arranged in a Complete Randomized Design (CRD) with three replications. The polythene bags were half-filled with soil, weighing 8.5 kg with intra-spacing and interspacing of 22 cm and 45 cm respectively. Four seeds were sown in each bag.

Evaluation of colchicine on treated seed

At twelve (12) weeks after planting, the morphological parameters such as; germination percentage (%), plant height (cm), number of leaves, leaf area (cm^2), fresh weight (g) and dry weight (g) were recorded. Also, the analysis on the proximate composition of the leaves, stem and roots of the cultivated plants were carried out at the Department of Agronomy, Faculty of Agriculture, using a standard procedure [15]. Nutrients analysed are; moisture content, ash content, crude fibre content, crude protein content, crude lipid content and nitrogen-free extracts (digestible carbohydrates).

Data analysis

The data obtained from the parameters were subjected to one-way analysis of variance (ANOVA) using SAS (2002) version 9.1 to determine the effect of different concentrations of the mutagen on *Jatropha curcas.* Duncan's Multiple Range Test (DMRT) was used to separate

the means obtained, where significant ($p \leq 0.05$).

RESULTS

The results on the physicochemical parameters are presented in table 1. The textural class of the soil used for the research is sandy – loam. For the soil parameters, the soil contained 0.2 % Nitrogen and 1.62 % organic carbon. The pH ratio 1:2:5 in water (H_2O) (dS/m) is 6.99. The pH ratio in 0.01m Calcium Chloride ($CaCl_2$) (dS/m) is 6.54. The electrical conductivity is 0.35, while the available phosphorous (ppm) is 4.0. At exchange bases, Calcium (Ca) is 8.64 cmol+/kg, Magnesium (Mg) is 2.36 cmol+/kg, Potassium (k) is 0.52 cmol+/kg, Sodium (Na) is 0.23 cmol+/kg, Hydrogen and Aluminium (H^+Al) is 0.20 cmol+/kg while the Cation exchange capacity (CEC) is 12.30 cmol+/kg. Also, for particle size distribution, clay is 6 %, silt is 22 %, while sand is 72 %.

Table 1: Soil parameters and their values

Soil Parameters	Values
pH ratio 1:2:5 in H_2O (dsm)	6.99
pH ratio in 0.01m $CaCl_2$ (dsm)	6.54
Electrical Conductivity	0.35
Organic Carbon (%)	1.62
Total Nitrogen (%)	0.21
Available Phosphorous (ppm)	4.01
Exchange bases (Cmol/kg)	Values
Ca	8.64
Mg	2.36
K	0.52
Na	0.23
H+Al	0.20
CEC	12.30
Particle size distribution (%)	Values
Clay	6.00
Silt	22.00
Sand	72.00
Textural class	Sandy-loam

The effect of different concentration of colchicine in the germination date, agro-morphological parameters and proximate composition of leaves, stems and roots of *Jatropha curcas* are shown in tables 2, 3, 4, 5 and 6 respectively. Colchicine treated seeds started to emerge four (4) days after planting, while the control (untreated seeds) were delayed for ten (10) days as shown in table 2.

Table 2: The different treatments and their number of days to emergence

TREATMENT	Number of days to emergence
COLCHICINE 0.25mM	4
COLCHICINE 1.25mM	4
COLCHICINE 2.50mM	4
CONTROL	14

For the percentage germination, colchicine induced seeds show a significantly high rate than non-induced seeds (control). The 1.25 mM colchicine treatment and the control (non-treated seeds) have the highest values (91.67 %), followed by 2.50 mM colchicine treatment (83.32 %), and 0.25 mM colchicine treatment (75.01 %). A similar trend was observed for the plant height, where 1.25 mM colchicine treated seeds have the highest mean value (28.44 cm), which is significantly ($p > 0.05$) different from other colchicine concentrations [0.25 mM (20.28 cm) and 2.50 mM (21.44 cm)] and the control (20.78 cm). Also, for the number of leaves, all the colchicine treated seeds, 0.25 mM (8.00), 1.25 mM (8.75) and 2.50 mM (8.13), are not significantly different from each other but are highly significant ($p > 0.05$) from the control (6.75). Moreover, for the leave area, the mean with the highest value is the 1.25 mM colchicine treatment (49.73 cm^2), and it is significantly different from the 0.25 mM colchicine treatment (45.76 cm^2), the 2.50 mM colchicine treatment (39.60 cm^2) and the control (40.64 cm^2). Whereas, for fresh weight and dry weight, the colchicine treatments are highly significant from the control.

Table 3: Effect of Colchicine on some agro morphological traits of *Jatropha curcas*

TREATMENTS mM	P/GERM (%)	P/HGHT (cm)	N/LVS	L/AREA (cm^2)	F/WHGT (g)	D/WHGT (g)
0.00	91.67^a	20.78^b	6.75^b	40.64^c	10.86^b	1.63^b
0.25	75.01^c	20.28^b	8.00^a	45.76^b	11.68^b	2.45^a
1.25	91.67^a	28.44^a	8.75^a	49.73^a	15.90^a	2.94^a
2.50	83.32^b	21.44^b	8.13^a	39.60^c	12.78^b	2.59^a

NB: Means with the same letter(s) within each column are not significantly different ($p > 0.05$). P/GERM= Percentage germination, P/HGHT= Plant height, N/LVS= Number of leaves, L/AREA= Leaf area, F/WHGHT= Fresh weight and D/WHGT= Dry weight

Table 4: Effect of Colchicine on the proximate composition of *Jatropha curcas* Leaves

TREATMENTS (mM / %)	MC (%)	LC (%)	PC (%)	AC (%)	FC (%)	CC (%)
CONTROL	4.09[a]	0.37[a]	22.30[a]	10.11[ab]	1.89[b]	61.12[d]
0.25	4.12[a]	0.36[a]	18.21[b]	10.12[ab]	1.93[ab]	65.28[b]
1.25	4.09[a]	0.32[b]	15.42[c]	10.13±[ab]	1.92[ab]	68.13[a]
2.50	4.12[a]	0.33[b]	15.41[c]	10.09±[b]	1.93[ab]	68.15[a]

NB: Means with the same letter(s) within each column are not significantly different ($p > 0.05$). MC = Moisture content, LC = Lipid content, PC = Protein content, AC = Ash content, FC = Fiber content and CC = Carbohydrate content

There is no significant difference in the colchicine treated and non-treated plants for the proximate composition of the leave parameters (moisture content, ash content and fibre contents). While for lipid content, the control and the 0.25 mM colchicine treated plants are not significantly different. Moreover, the control is highly significant at protein content from the colchicine treated plants, unlike carbohydrate content, where the colchicine treated plants are highly significant from the control.

The proximate composition of the stem shows that moisture content, lipid content, protein content, ash content and fibre content of the colchicine treated plants and the non-colchicine treated plants (control) are not significantly different from each other. While at carbohydrate content, the control is highly significant from the colchicine treated plants.

Whereas, for the proximate composition of the roots, the moisture content, lipid content and ash content of the colchicine treated and non-treated plants (control) are not significantly different. While the protein content and fibre content of the colchicine treated plants are significantly different from the control plants. Also, the carbohydrate content of the control plant is significant from the colchicine treated plants.

Table 5: Effect of Colchicine on the proximate composition of *Jatropha curcas* Stem

TREATMENTS (mM / %)	MC (%)	LC (%)	PC (%)	AC (%)	FC (%)	CC (%)
CONTROL	4.63[a]	0.26[a]	9.82[a]	15.53[b]	3.94[a]	66.01[a]
0.25	4.58[a]	0.26[a]	9.85[a]	15.64[ab]	3.95[a]	65.76[c]
1.25	4.54[a]	0.27[a]	9.81[a]	15.78[a]	3.91[a]	65.74[c]
2.50	4.51[a]	0.24[a]	9.81[a]	15.77[a]	3.92[a]	65.82[b]

NB: Means with the same letter(s) within each column are not significantly different ($p > 0.05$). MC = Moisture content, LC = Lipid content, PC = Protein content, AC = Ash content, FC = Fiber content and CC = Carbohydrate content

Table 6: Effect of Colchicine on the proximate composition of *Jatropha curcas* Root

TREATMENTS (MM / %)	MC (%)	LC (%)	PC (%)	AC (%)	FC (%)	CC (%)
CONTROL	4.39^{ab}	0.18^{b}	13.99^{d}	11.13^{a}	6.41^{b}	63.86^{a}
0.25	4.30	0.30^{a}	16.70^{c}	11.12^{a}	6.53^{a}	60.78^{c}
1.25	4.36^{ab}	0.17^{b}	16.83^{b}	11.09^{a}	6.49^{a}	61.02^{b}
2.50	4.41^{a}	0.19^{b}	21.10^{a}	11.12^{a}	6.50^{a}	56.77^{d}

NB: Means with the same letter(s) within each column are not significantly different ($p > 0.05$). MC = Moisture content, LC = Lipid content, PC = Protein content, AC = Ash content, FC = Fiber content and CC = Carbohydrate content

DISCUSSION

The application of different concentrations of colchicine to the seeds of *Jatropha curcas* was found to influence the growth of *Jatropha curcas* plant significantly. This observation could be a result of the mutagenic effect of colchicine on agronomic traits of plants. The percentage germination of *Jatropha curcas* at a certain concentration (1.25 mM) does not significantly affect colchicine treatments. Although, there was no clear trend on the effect of colchicine on percentage germination of the plant. This observation could mean that the performance of colchicine on the seeds of *Jatropha curcas* depends on the dose/concentration of the mutagen. Also, the colchicine induced seeds germinated earlier than non-colchicine induced seeds. Growth parameters (plant height, number of leaves and leaf area) are important morphological traits which contribute to increased biomass.

Statistical test on data collected from this research revealed significant differences in plant height, number of leaves, leaf area, fresh weight and dry weight of colchicine treated plants and non-colchicine treated plants that are in accordance with the work of Adelanwa *et al.* [16]. Among the various colchicine concentrations used for *Jatropha curcas* in this present research, 1.25 mM concentration showed the highest mean values (performance) in all the agronomic traits considered [17].

As reported by Victor *et al*. [18] colchicine is said to be beneficial for improving nutrient quality of plants when induced in the seeds. It was observed from this study that the proximate composition of the leaves, stem and roots of *Jatropha curcas* colchicine induced plants are majorly not significantly different from the non-colchicine induced plants (control). Unlike, the leaves' carbohydrate content,

protein content of the roots and fibre content of the roots where significantly increased in the colchicine induced plants compared to the control. There was an exception to the roots' carbohydrate content where there is significant decrease in the colchicine induced plants [18]. The result from the proximate analysis indicated that the results of other parameters considered were concentration-dependent [19]. There is a need for screening the corresponding concentrations for an optimal result on the desired traits to be considered. The present finding revealed that concentrations at 0.25 mM and 1.25 mM could be utilized for improvement of traits in plant.

CONCLUSION

It is evident from this study, that colchicine especially at 1.25 mM concentration, is essential for the fast growth of *Jatropha curcas*. It can also be concluded that the application of colchicine to enhance the growth of *Jatropha curcas* does not negatively affect the proximate composition of its leaves, stem and roots. This indicates that *Jatropha curcas* induced colchicine could still be used for other purposes aside from biofuel, including traditional medicines. Studies on Colchicine concentrations between 0.25 mM to 1.25 mM should be further conducted to the fruiting stage to ascertain the best concentration that could enhance the growth of *Jatropha curcas* for the use of biofuel.

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