MUTAGENIC EFFECTS OF SODIUM AZIDE ON MORPHOLOGICAL PARAMETERS OF LAGOS SPINACH (*Celosia argentea* L.)

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Abstract

Despite the tremendous benefits of Lagos spinach (Celosia argentea L.) as food and in medicine, its diversity and uses are under threat in Nigeria, due to genetic erosion and low yield. Therefore, this study was aimed at inducing mutation in the seeds of the plant for the improvement of its morphological and yield parameters using sodium azide. Two hundred and fifty (250) seeds each were randomly selected and treated with five levels of sodium azide (Control, 2.00mM, 4.00mM, 6.00mM, and 8.00mM). The seeds were raised in the laboratory for germination study and experimental Garden. In the garden, the seeds were planted in experimental pots arranged in a completely randomized block design (CRBD) with each replicated five times for morphological and yield parameters evaluation. With the exception of treatment 2mM, increased concentration of Sodium azide resulted in reduction in seed germination and survival rate. Increased biological injury (Lethality) was observed as an apparent reduction in germination and root length with increasing concentration. The dichotomous branching mutant observed in the treated seeds resulted in increase number of leaves in 4mM and 6mM. The highest percentage branching (50%) correspond to the highest mean number of leaf (103.33) recorded in 6mM with distinct separation of the treatment by Euclidean pair group dendrogram based on morphological features. The LD_{50} for mortality was obtained at 4.74mM. Therefore, 6mM sodium azide was the most effective concentration for inducing desirable mutations at the highest frequency in C. argentea. Further study would be carried out on this plant to see if these useful characters could be transferred to the next generation.

Keywords: *Celosia argentea*, Dichotomous branching, morphology, Sodium azide

INTRODUCTION

Celosia argentea commonly known as Lagos spinach is an edible species of the Genus *Celosia* of the Amaranthaceae Family (Sudha and Mathanghi, 2012). It is a native plant in subtropical and temperate zones of Africa, South America and South East Asia; cultivated annually in different home gardens and has become an economically important floral crop (Kolawole and Peter, 2011). The crop is predominantly produced in Nigeria by resource-poor farmers and compound gardens where it is intercropped with other staple crops (Akinyemi and Tijani-Eniola, 1997). This leafy vegetable is an essential component of people's diet in Nigeria and other parts of West Africa such as Benin and Congo. The leaves are eaten as a vegetable due to its high vitamins A and C, iron and calcium. Poultice of the leaves smeared with honey, are being used as cooling agent that can be applied to inflammated areas and painful affections such as buboes and abscesses (Sudha and Mathanghi, 2012). Medicinal uses of this plant have also, been reported by Ramesh *et al.* (2000), where its leafy vegetative part has been used in treatments of various disorders such as fever, diarrhea, mouth sores, itching, wounds, jaundice, gonorrhea and inflammation. Sato (2003) also reported that *Celosia argentea* is rich in anti-oxidants. Despite the tremendous benefits of the plant, *Celosia argentea* is one of the 24 vegetable species identified by IBPGR in 1979 which showed genetic erosion and are of local importance or rank second in priority on global scale (Grubben, 2004) therefore, there is an urgent need to redesign breeding strategy to improve this economic plant. To ensure fast and effective improvement of this crop, an attempt has been shifted to mutation breeding.

Mutation is a sudden, rare, discrete change in the genetic material of an organism which results in a permanent change in the expression of the genes (Asencion, 2005). These sudden heritable changes are tools used to study the nature and function of genes which are the building blocks and basis of genetic variation, thereby producing raw materials for genetic improvement of economic crop. Mutagenic effects of sodium azide have been reported to be effective in inducing mutations in vegetable plants, thereby broadening the genetic base of the crop for selection (Adamu *et al.*, 2007). However, Anchalee and Kitti (2011) who worked on the effects of acute gamma radiation on seed germination and morphological characters of *Celosia argentea* reported that, mutation induction in this plant had never been investigated. The knowledge of availability of genetic variability, inter-relationships between different morphological and economic traits are prerequisite for plants genetic improvement (Azhar *et al.*, 2009). Also, multivariate analysis such as cluster diagram has been reported to be an effective alternative tool for differentiating induced mutants since it takes into consideration several traits simultaneously (Deka *et al.*, 2016). In affirmation of this statement, the use of multivariate analysis for identification, classification and separation of mutants have been reported by various authors (Chandra *et al.*, 2007, Muduli and Misra, 2008, Deka *et al.*, 2016). In view of this and on account of alleged usefulness of this plant, this study was aimed at inducing mutation in the seeds of *Celosia argentea* for the improvement of some of its morphological and yield parameters using sodium azide.

MATERIALS AND METHODS

Collection of materials

Healthy and dry Seeds of *Celosia argentea* were collected directly from local farmers in Ilorin, Kwara State and the mutagenic chemical (Sodium azide) was obtained from the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. The experiment was carried out at the laboratory and Botanical Garden, Department of Biological Sciences, Federal University of Technology, Minna from middle of July to early November.

METHODOLOGY

Sodium azide (NaN_3) treatment

The seeds were treated with sodium azide using modified procedures of Adamu and Aliyu (2007). Prior to treatment, the Air dried seeds were pre-soaked in distilled water for fourteen hours to initiate physiological activities in their embryo. The pre-soaked seeds were then, treated with different concentrations of sodium azide (2.0, 4.0 6.0 and 8.0mM) in 0.1M phosphate buffer pH 3 for 4 hrs with periodically agitation at room temperature. The control seeds were soaked in buffer solution pH 3 for the same hours. The seeds were thoroughly rinsed in tap-water for to remove excess mutagens.

Raising of M_1 generation (Planting of seeds):

The treated seeds and control were planted both in the laboratory and on the field. In the laboratory, 100 seeds per treatment were planted on pre-soaked cotton in petri-dishes under laboratory condition. After 7 days of sowing, number of seeds germinated were counted and expressed in percentage (Songsri *et al.*, 2011). Seedling heights were estimated by measuring root and shoot lengths of 5 randomly selected seedlings from each treatment as well as control. Percentage root and shoot injury were considered as measured of reduction in root and shoot length calculated. In the Garden, 5 randomly selected seeds of each treatment were planted in 7 litres of experimental pots; filled to 5 litres mark with sandy-loamy soil. The pots were then arranged in a completely randomized block design (CRBD); with each treatment replicated five times to obtain the seedlings (Adamu and Aliyu, 2007 and Premjyoti, 2006). The plants were watered once daily between 6.30-7.30 am using bore-hole water in the absence of rain. The seedlings were then tinned to 2 seedlings per pot after 3 weeks of planting and sprayed with an insecticide after three (3) weeks of the thinning to prevent insect attack and diseases. No fertilizer was applied. The morphological parameters such as plant height (cm), number of leaves/plant, stem diameter (cm), leaf length and width (cm^2) were measured at 2 weeks interval for 2 months and/or number of spike/plant and length of middle spike/plant (cm) at maturity stage.

Data Collection and Analysis

Data collected were subjected to analysis of variance (ANOVA) using SPSS version 20 to compare the means and Duncan multiple range tests (DMRT) were used to separate the means. A hierarchical cluster analysis of the genotypes based on arithmetic average of the morphological traits was done using past software 2016 version. LD_{50}values was determined based on mortality rate following Probit analysis (Sharma, 1998). Other data obtained were expressed in percentage.

Germination

$$\text{Germination (\%)} = \frac{N0.\ of\ seeds\ germinated}{N0.of\ seeds\ sown} \times 100$$

Percentage Survival and Lethality

$$\text{Survival (\%)} = \frac{N0.\ of\ plants\ at\ maturity}{N0.of\ seed\ germinated} \times 100$$

$$\text{Lethality (\%)} = \frac{Control-treatment}{Control} \times 100$$

RESULTS

Effects of Sodium Azide on Seedling Morphometric Variation

The result of percentage germination showed that there was a reduction trend in the germination percentages with increased in concentration of sodium azide of *Celosia argentea*. The highest percentage germination (94%) recorded in 2mM was equivalent to that recorded in the control. Similarly, percentage survival of the seedling among the mutants decreased with increased in concentration of the mutagen. However, the highest percentage survival (86.67%) recorded in 2mM, was higher than that of control (84.33%) (Table 1).

Significant decrease in the root and shoot length with increased in concentration was observed among the treated seedling. The highest root (2.68cm) and shoot (1.84cm) length were recorded in 2mM and the least 1.60cm and 1.48cm respectively were obtained in 8mM. These values were significantly different from one another and from the control values 2.76cm (for root length) and 1.96cm (for shoot length) Table 1.

Percentage lethality on seedling root showed increase in lethal effect of the mutagen (sodium azide), with increased concentration. The highest lethal effect (4.48%) was recorded in 8mM and the least 3.08% was recorded in 2mM. Contrarily, the lethal effect of the mutagen on shoot length decreased with increased in concentration (Table 1). The highest shoot lethal effect (24.56%) was recorded in 2mM and the least in 8mM (5.33%).

Table 1.0: Effect of Different Concentrations of Sodium Azide on Seedling Traits

Parameters	% Germination	Root Length	shoot Length	% Root Lethality	% shoot Lethality	% Survival
Control	94.00	2.76±0.34c	1.96±0.05b	-	-	83.33
2Mm	94.00	2.68±0.45b	1.84±0.13b	3.08	24.56	86.67
4Mm	64.00	2.20±0.14b	1.80±0.17ab	3.40	11.22	60.00
6mM	62.00	1.68±0.16a	1.72±0.10a	4.04	7.89	46.67
8Mm	44.00	1.60±0.13a	1.48±0.10a	4.48	5.33	40.00

Values are Mean ± S.E, Mean followed by the same superscript are significantly different (p≥0.05) tested by DMRT

Effects of Sodium Azide on Morphological Features

Results of plant height at the first four (4) weeks after thinning revealed that there was no significant differences ($p \geq 0.05$) in height of all the treated plants and the control. A decrease trend in plant height of the treated plants was observed at the end of the sixth week, with the control plant having significantly highest height of 58.78cm Among the mutant plants, 2mM treated plant had the highest plant height (31.56cm) followed by plants treated with 4mM (20.06cm) and the lowest was recorded 8mM treated plants with the mean value of 16.20cm. However, there was no significant ($P > 0.5$) difference among 4mM (20.06cm), 6mM (22.90cm) and 8mM (16.20) Table 2.

With the exception of week 4 where the control had the highest number of leaves per plant (56.33 leaves), the highest number of leaves per plant was recorded in 6mM plants throughout the study period with the values of 36.00, 75.00 and 103.33 leaf per plant at week 2, 6 and 8 respectively. For stem diameter, with the exception of 2mM (2.87cm) at week 2 and the control plant (8.54cm) at week 4, there were no significant differences in stem diameters of all the treatments and the control for the study period. The highest stem diameter (10.30cm) was recorded in treatment 6mM in week 8. The least stem diameter was obtained in 8mM treated plants throughout the study period (Table 2).

The result of the leaf length showed that there was significant difference ($p \leq 0.05$) among all the treated plants and the control throughout the experimental period. Treatment 6mM had the highest leaf length (46.67cm) in week 4 and the Control had the highest length in week 2, 6 and 8 after transplanting. Leaf width also showed that with the exception of week 4, there was no significant different among the leaf width of the treated plant and the control. However, the leave of the 6mM had the highest width throughout the period Table 2.

TABLE 2: EFFECTS OF SODIUM AZIDE ON MORPHOLOGICAL PARAMETERS

	SAMPLE	WEEK 2	WEEK 4	WEEK 6	WEEK 8
PLANT HEIGHT (cm)	CONTROL	20.00±3.88^{c}	35.66±5.08^{c}	58.78±3.74^{c}	71.78±3.28^{b}
	2mM	5.78±1.31^{a}	12.42±2.87^{b}	31.56±6.71^{b}	57.72±8.85^{ab}
	4mM	2.25±0.41^{a}	6.60±1.12^{a}	20.06±2.79^{a}	44.18±5.79^{a}
	6mM	6.68±2.33^{b}	11.08±3.42^{b}	22.90±4.52^{ab}	37.46±3.06^{a}
	8mM	2.25±0.51^{a}	5.60±1.15^{a}	16.20±3.31^{a}	37.20±4.71^{a}
NUNBER OF LEAVES	CONTROL	33.33±9.61^{b}	56.33±7.54^{c}	66.67±1.17^{c}	83.00±9.87^{b}
	2mM	22.67±2.91^{ab}	28.33±1.76^{a}	53.00±3.46^{b}	67.33±6.96^{a}
	4mM	19.00±5.57^{ab}	30.33±6.17^{b}	58.67±8.67^{b}	79.33±5.55^{ab}
	6mM	36.00±4.19^{ab}	46.67±1.97^{bc}	75.00±2.01^{d}	103.33±1.78^{c}
	8mM	12.33±3.18^{a}	25.33±9.82^{a}	45.67±2.81^{a}	61.67±4.25^{a}
STEM DIAMETERS (cm)	CONTROL	6.34±0.97^{b}	8.54±0.24^{b}	8.78±1.17^{a}	9.50±1.11^{a}
	2mM	3.92±0.54^{b}	6.04±0.59^{a}	8.24±0.52^{a}	9.12±0.41^{a}
	4mM	3.18±0.79^{b}	5.18±0.70^{a}	7.28±0.50^{a}	8.85±0.58^{a}
	6mM	4.83±1.45^{b}	5.98±2.13^{a}	8.04±1.74^{a}	10.30±2.13^{a}
	8mM	2.87±0.59^{a}	4.60±1.05^{a}	7.23±1.13^{a}	9.67±0.71^{a}
LEAF LENGTH (cm)	CONTROL	8.63±0.12^{c}	9.33±0.45^{ab}	10.53±0.19^{c}	12.13±0.35^{b}
	2mM	7.40±0.20^{ab}	9.53±0.09^{ab}	9.60±0.06^{b}	10.27±0.03^{a}
	4mM	7.03±0.09^{a}	9.70±0.15^{b}	8.87±0.38^{ab}	9.90±0.27^{a}
	6mM	8.60±0.66^{c}	9.67±0.12^{b}	9.83±0.18^{b}	10.27±0.17^{a}
	8mM	8.17±0.23^{bc}	8.47±0.18^{a}	9.20±0.21^{a}	10.27±0.19^{a}
LEAF WIDTH (cm)	CONTROL	2.83±0.52^{a}	3.53±0.18^{a}	3.77±0.07^{a}	4.00±0.26^{a}
	2mM	2.57±0.27^{a}	3.57±0.15^{a}	3.73±0.07^{a}	3.77±0.09^{a}
	4mM	2.23±0.07^{a}	3.47±0.12^{a}	3.73±0.12^{a}	4.07±0.09^{a}
	6mM	3.07±0.15^{a}	3.97±0.09^{b}	4.20±0.15^{b}	4.27±0.18^{a}
	8mM	2.97±0.17^{a}	3.43±0.03^{a}	3.67±0.03^{a}	3.80±0.06^{a}

Values are Mean ± S.E, Mean followed by the same superscript are significantly different ($p \geq 0.05$) tested by DMRT

The results of the spike parameters at maturity showed that, there were significant differences ($p \leq 0.05$) in the number of spike and length of middle spike produced. The highest numbers of spike per plant (30.00 spikes) and length of middle spike (20.50cm) were recorded in treatment 4mM plants followed by 6mM with the value of 28.00 spikes and 19.97cm for number of spike and length of the middle spike respectively (Table 3, Plate 1). The lest number of spike per plant (15.80 spikes) was recorded in the Control. However, statistical analysis revealed that there were no significant different in the length of middle spikes obtained from the treatment (2, 4 and 6) mM, but they differ significantly ($p \leq 0.05$) from the other treatment. The results of the effects of the sodium azide treatment on the *Celosia argentea* resulted in production of dichotomous branching (Plate 2). The highest percentage branching (50%) was observed in 4mM followed by 6mM (40%) with the lowest recorded in 2mM (22.22). The Control treatment showed no branching system. Statistical analysis however, showed that there was significant difference ($p \leq 0.05$) in the numbers of spike produced by the treatments Table 3.

The result of the survival showed that there was a decreased in the survival rate with an increase in the concentrations of the mutagenic agent, with the highest mortality recorded in 6mM and eventually improved in 8mM. The result of the LD_{50} value determined based on the mortality rate from the probit analysis curve equation ($y = 90.47x - 10.95$, $R^2 = 0.806$) showed that the optimum concentration that causes maximum mutation with minimum damage to the plants was 4.74mM (Figure 1).

TABLE 3: EFFECTS OF SODIUM AZIDE ON HARVEST PARAMETERS

Sample	Number of Spike	Length of Spike (cm)	% Branching	% Survival
Control	15.80 ± 1.07^a	14.56 ± 0.39^a	-	100.00
2Mm	26.80 ± 2.18^{bc}	19.70 ± 0.29^b	22.22	90.00
4Mm	30.00 ± 4.76^c	20.50 ± 0.61^b	40.00	40.00
6Mm	28.00 ± 1.48^{bc}	19.77 ± 0.20^b	50.00	20.00
8Mm	21.25 ± 1.49^{ab}	14.40 ± 0.47^a	33.33	30.00

Values are Mean ± S.E, Mean fol lowed by the same superscript are significantly different ($p \geq 0.05$) tested by DMRT

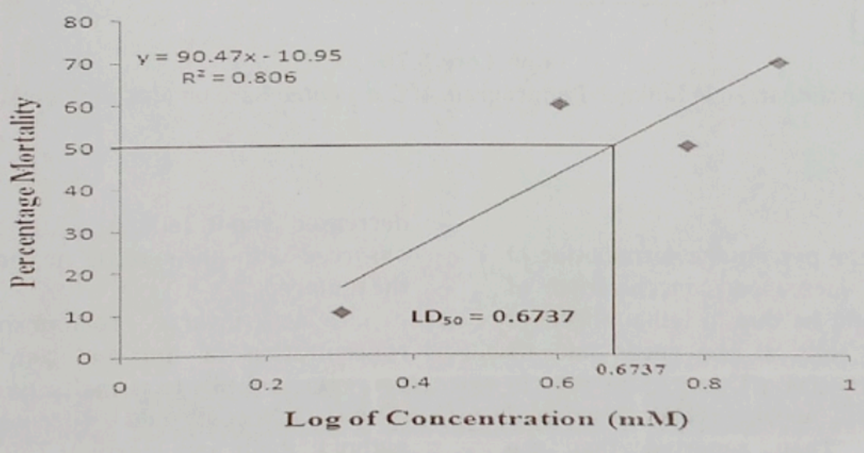

Figure 2: Probit regression of induced *C. argentea* with different sodium azide Concentration

A Euclidean dendrogram generated to study the genetic relationship among the induced mutants and the control using morphological data grouped all mutants and the parent (control) into 2 major clusters (Figure 2). The first cluster consists of a distinct mutant of 6mM treated plant.

The second cluster comprised 4 genotypes and was subdivided into two subclusters, II-A and II-B. Subcluster II-A comprised of mutant plants, 2mM, 4mM and 6mM genotypes, in which 8mM was distinct from the other two mutants. Subcluster II-B comprised of the control genotype.

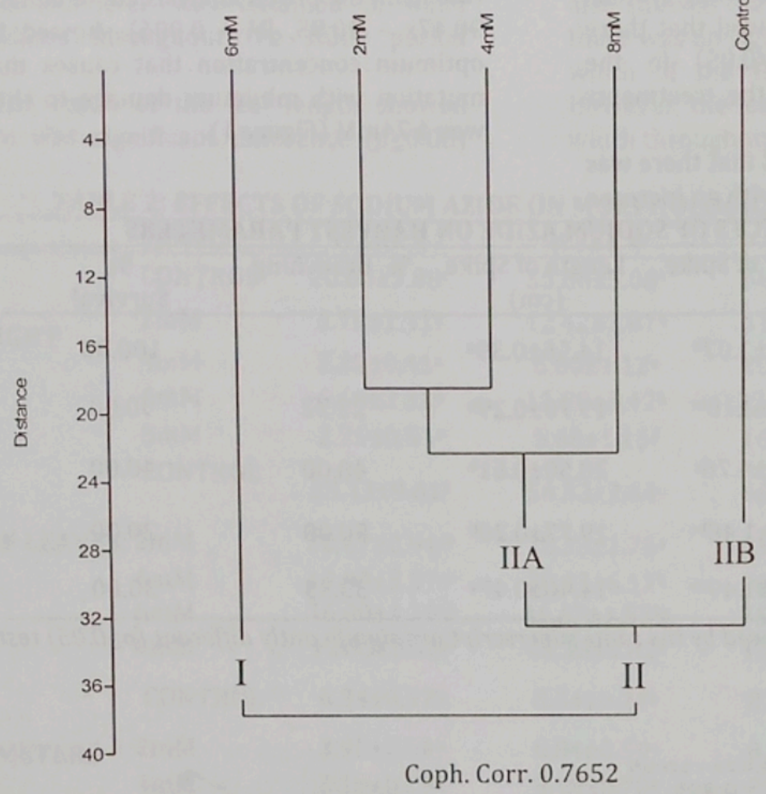

Figure 2: Euclidean Sigle Linkage Dendrogram of *C. argentea* Base on Morphological traits

DISCUSSION

The reduction in the percentage germination of seeds, with the increased concentration of sodium azide, might be due to lethal effects of mutagen on the cells of the seeds and its physiological activities. This result is in agreement with the work of Mahamune and Kotheka (2012). They reported that the decrease in germination is attributed to disturbances at cellular level and enzymes involved in the germination process. The gradual reduction in root and shoot length with increased concentration among the treated plants might be due to inhibition in the function of growth hormone such as auxins and cytokinins. Singh (1974) reported that reduced seedling growth might be attributed to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances. Contrary to this result Shagufta *et al.* (2013) who measured seedling height in terms of root-shoot length reported gradual increase in root injury with an increase in the concentration of the mutagen. In agreement with their result, decreased shoot lethality in *C. argenta* was observed with an increase in concentration of the mutagen.

An inverse relationship between concentration of the mutagen and survival percentage of plants at maturity was observed in this study, confirmed earlier work by various authors; Rajib and Jagatpati (2011), Rajendra and Rajendra (2012), Shagufta *et al.* (2013). Gaul (1964) opined that chromosomal and extra chromosomal injury might lead to disturbances at physiological and cytological levels resulting in decrease in survival percentage. Also, the reduction in seed germination, seedling growth and survival induced by mutagenic treatments has been ascribed to damage in cell constituents at molecular level or altered enzyme activity (Chowdhury and Tah, 2011).

Similar to the results recorded in this study, Songsri *et al.* (2011) reported a significant differences in stem diameter, plant height and number of leaf per plant of gamma ray treated *Jatropha curcas*. Increase in the number of leaves with an increase in the concentration of the sodium azide until it researches the optimum value observed in this study might be attributed to creation of more positive traits in plant by the mutagen. The variation in spike length and formation of dichotomous branching observed might be attributed to excitation of endogenous hormones involved in cell division and growth. This confirmed the statement that, the changed in the plant condition might be due to hormonal imbalance resulting from the mutagenic treatment (Suman, 2012).

The moderate level of genetic diversity observed among the genotypes, suggests its moderate genetic base, which is possibly due to accumulation of novel gene combinations in response to dynamic pressures of the difference in concentrations of the mutagenic agent. Dyulgerova (2012) reported that the separation of mutants from parent cultivars showed the effect of the used mutagens in creating genetic diversity. Diversity in plant provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristic (Thul *et al.*, 2009) for selection.

5.0 CONCLUSION

Sodium azide had proven to be an effective mutagen for inducing useful mutation in *C. argentea* at high concentaration. *Celosia argentea* being a leafy vegetable, the highest number of leaves couple with highest percentage branching obtained in 6mM, indicate the effectiveness of the concentrations for inducing desirable mutations plant. However, further study would be carried out on the mutants, to determine the effectiveness in transmission of these desirable traits to the next generation.

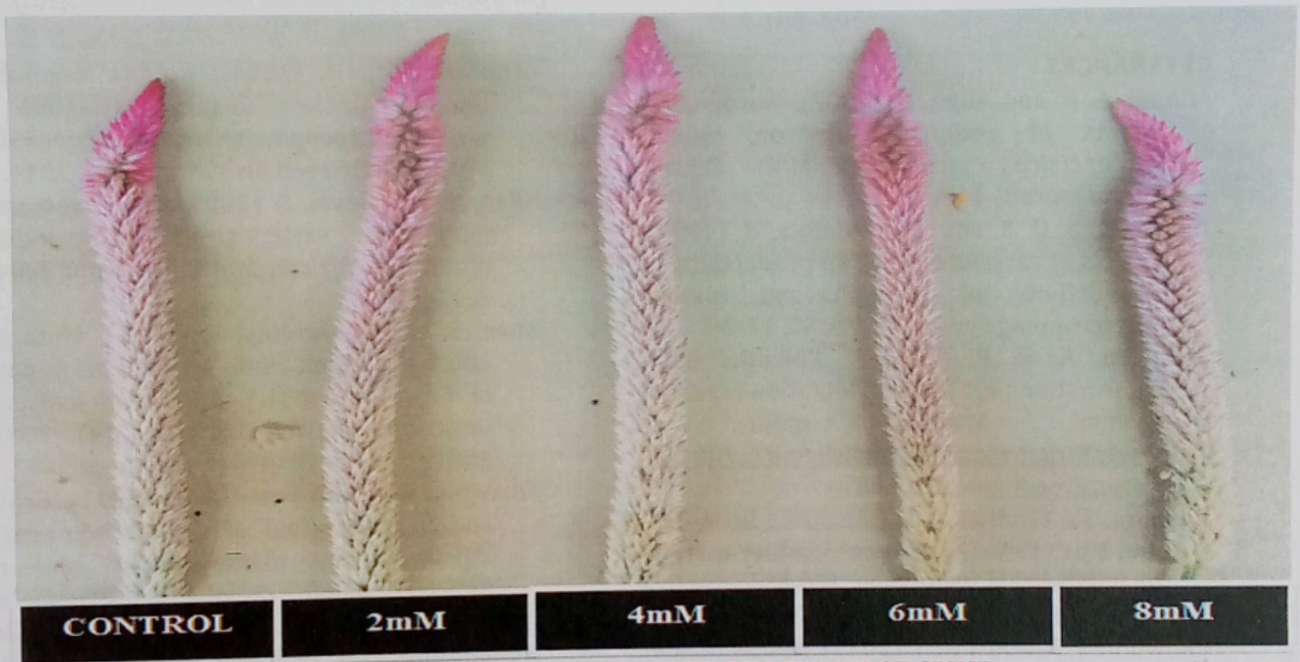

PLATE 1: EFFECT OF SODIUM AZIDE ON LENGHT OF SPIKE

PLATE 2: EFFECT OF SODIUM AZIDE ON DICHOMOUS BRANCHING FORMATION

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