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The Influence of Diethyl Sulphate on Vegetative, Flowering Growth,Chemical Composition and ISSR Genetic Markers of *Celosia argentea* L. Plants

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THE experiment was carried out during the years of 2021 and 2022 at the Nursery of Floricultural and Ornamental Plants, Faculty of Agriculture, Alexandria University, Egypt. Seedling roots of *Celosia argentea* L. var. spicata were soaked with different Diethyl Sulphate (DES) concentrations**,** i.e.0, 200, 400 and 600 ppm for 30 and 60 minutes to study the effect of DES on the morphological characteristics, phytochemical composition as well as the possibility of mutations induction using ISSR marker technique.
The concentration of 200 ppm for 30 min. increased significantly the anthocyanin concentration in the leaves in the first generation and the peroxidase activity in the second one, while 200 ppm for 60 min. increased significantly the alkaloids concentration in the leaves in the second generation. DES treatment at 400 ppm for 60 min. increased significantly the alkaloids concentration in the leaves in the first generation and the phenols concentration in the second one. The DES concentration of 600 ppm for 30 min. increased significantly the anthocyanin concentration in the inflorescence in the first generation. Using all concentrations DES for 60 min. increased significantly the leaf area. The anthocyanin concentration in the inflorescence was increased significantly in the second generation using the concentration of 600 ppm for 60 min..
Some variations in the habit of growth, leaf form and inflorescence structure were observed in both generations. ISSR marker- PCR technique was able to detect mutations in a plant *Celosia argentea* L. plants.

Keywords: *Celosia argentea*, Anthocyanin, Alkaloids, Phenols, Peroxidase, ISSR marker.

Introduction

Celosia argentea L. is an annual, herbaceous, erect, branched and popular as cut flower (Patil, et al., 2020). The plant belonging to the family Amaranth aceae (Kanu, et al. ,2017) Synonyms of this plant is plumed cockscomb, silver cockscomb, white cockscomb, flamingo feathers, wheat celosia, Garkha, garke, Kurdu kurda (Patil, et al., 2020). The *Celosia* genus consists of sixty species world-wide, but only two species in China, C. argentea and *Celosia cristata*, while in other countries and regions, *C. cristata* is still grouped in the *C. argentea* as a variant (*C. argentea* var. *cristata*). As for the consanguinity to *C. argentea*, *C. cristata* is usually used as an adulterant consciously or unconsciously (Tang, et al., 2016). *Celosia* is one of the most important medicinal plant. It is known as anti-inflammatory, immunostimulating, anticancer, hepatoprotective, antioxidant, wound healing, antidiabetic and antibacterial activities, anti - infection, anti- tumor, anti-diarrhea, anti-diabetes, anti-oxidant, treating eye diseases (Nidavani, et al., 2013 and Patil, et al., 2020).

Celosia argentea L. is distributed all over the world mainly in subtropical and tropical regions of Sri Lanka, South Asia, Africa, America, Karnataka, Andhra Pradesh, Tamil Nadu, West Africa, from Sierra Leone to Nigeria, Ethiopia, Somalia, Kenya, other parts of East Africa, Mexico and Central Africa (Verma and Demla, 2012 and Nidavani, et al., 2013).

Celosia argentea L. possess flavonoids betavulgarin in the aerial part; amino acids: celogenamide A, celoentins A, moroidin, aspartic acid, thereonine, glutamic acidin, saponins and cycpeptide Moroidin (peptide) in the seed and glycosides: citrusin C, indicant, phenol and ascorbic acid in the leaf, also, fatty acids, minerals (Tang, et al., 2016 and Patil, et al., 2020).

For breeding purposes, it seems necessary to induce artificially new genetic variability by means of the effective mutagens . Much attention has been

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given to chemical mutagens and the number of these chemicals is very great and is continuously increasing . However , for practical purposes of mutation induction, most of these chemicals are belong to the special class of alkylating agents as Ethyl Methane Sulphonate (EMS: CH_3 SO_2 OC_2 H_5, Diethyl Sulphate DES: $SO_2(OC_2$ $H_5)_2$, Dimethyl Sulphate and Sodium Azide (SA : NaN_3). These compounds have one or more reactive alkyl groups which can be transferred to other molecules at positions where the electron density is sufficiently high. All these substances react with DNA by alkylating the phosphate groups as well as the purine and pyrimidine bases.(International Atomic Energy Agency, Techn. Reports Seriers, 1977).

Diethyl sulfate react with the DNA structure through the phosphate group and nitrogen bases ,which used to induce mutation by alterations in DNA molecules as transitions, transvers ions, deletions, insertions, inversions, DNA single and double strand breaks, and DNA recombination (Acquaah, 2007).

To successfully identify mutations and study the genetic diversity of different medicinal plants species and crops can be used simple sequence repeat (ISSR) markers (Farajpour, et al. 2011)

Kannan et al. (2002) showed positive effect of 30 mM ethyl methane sulfonate over control on the reproductive traits of *Jasminum sambac* cv. Gundumalli.

El- Shennawy (2005) treated seeds of *Centaurea cyanus* with different doses of diethyl sulphate (DES) at 0.0, 0.2, 0.4, 0.6 ad 0.8 % and their combination. Individually treatments did not affect the plant height significantly whereas the combined treatments reduced the plant height compared with the control. The maximum reduction in plant height was recorded at 0.8 % DES.

El - Nashar (2006) found that treating seeds with 4000 ppm DES increased most traits of *Amaranthus caudatus* L. and *A. hypochondriacus* L.and all concentrations of DES and SA caused leaf abnormalities in the first and second generations.

Mung bean cv. Vamban seeds treated with 0.07% DES gave 50% lethality and increased plant height, fresh and dry weights of plant in M_2 generation (Mullainathan et al., 2006).

Bhat et al. (2007) showed that both mutagens DES and SA elicited various chromosomal aberrations in meiosis and reduction in seed germination and in seedling survival of *Vicia faba* L. in M_1 generation, such effects were dose dependent and positively correlated with seedling survival. However, the induction of meiotic aberrations was observed to be higher in DES than SA treatments, suggesting that DES could be more effective in inducing additional variability than SA, in *Vicia faba* L. cv. Major.

Dwarf plant and leaf and floret changes were obtained after treating seeds and seedlings of *Antirrhinum majus* cv. Snow flake with DES (0. 3, 0. 6 and 0. 9 %) and chilling (El-Torky et al., 2009)

Various meiotic abnormalities and reduction in chiasma frequency were obtained on *Capsicum annuum* in the DES-treated plants, the highest % recorded at 0.05% DES (Gulfishan et al., 2011).

El-Nashar and Asrar (2016) treated seeds of *Calendula officinalis* L. with five different concentrations of DES of 0, 1000, 2000, 3000, 4000, and 5000 ppm. The effects of the different concentrations of both mutagens on the seed germination trait were significant in both generations.

The comparison between the effect SA vs DES, revealed that DES at 0.3% showed higher mutagen efficiency on most traits of vegetative growth than SA and control treatments. Increased the doses of DES shortened the vegetative growth phase (VGP), enhanced flowering stage and induced various of leaf morphological changes in leaf size, shape, margin and petioles of *Borgo officinalis* plant (El-Khateeb el al., 2022).

Aim of the work is studying the effects of mutagenic reagent (diethyl sulphate,DES) on the vegetative ,flowering growth and phytochemical composition of *Celosia argentea* , as well as study the possibility of inducing mutations , which have wider landscape value and identificate them using ISSR marker technique.

Materials and Methods

The present investigations were carried out during two successive generations in two experimental seasons of 2021 and 2022, at the Nursery of Floricultural and Ornamental Plants, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Seeds of *Celosia argentea* L.var. *spicata* were obtained from the Flowers and Ornamental Plants Research Gardens of the Faculty of Agriculture, University of Alexandria.

Seeds were sown on 25 th March 2021- in 30 cm clay pots containing a mixture of sand and peat moss (1:1 v/v). The seedling roots were soaked completely in chemical fresh solutions of Diethyl Sulphate (DES) - for 30 or 60 minutes at the different concentration of 200, 400 and 600 ppm, the seedling roots of control plants were soaked in distilled water as the same manner. Chemically treated and non - treated seedlings were individually transplanted into pots containing the soil mixture, on open field. One

month later, complete fertilizer 19-19-19 was top dressed at the rat of 1/2 g /l.

For growing the second generation, seeds were collected from each treatment and then sown on 25 th March 2022. The procedure of sowing and transplanting were made likewise the first generation.

The experimental layout was factorial experimental layout of Randomized Complete Block Design with 3 replicates (Gomez and Gomez, 1984).

Each replication contained eight treatments and every treatment consisted of five plants.

The following data were measured in both of the two generations :

Vegetative growth

Survival percentage for first generation

The percentage of the survived plants was measured for each treatment in each replicate as the percentage of the survived plants which continued around the experiment relative to the number of transplanted seedlings according to the following formula :

$$\text{Survival \%} = \frac{\text{Number of survived plants}}{\text{Number of transplanted seedlings}} \text{X100}$$

Seed germination percentage for second generation

Germination percentage of every treatment was calculated after 30 days from sowing according to the following formula :

$$\text{Seed germination \%} = \frac{\text{Number of germinated seed}}{\text{Number of total seeds}} \text{X 100}$$

Plant height (cm)

It was measured in centimeters from the soil surface in the pot to the highest point of the plant at the end of first and second - experiments.

Stem diameter (cm)

It was measured in centimeters at the soil surface in the pots at the end of the flowering.

Number of branches: Number of the branches per plant was counted during the vegetative growth.

Number of leaves / plant

The total number of leaves per plant was counted at the end of the first and second generations experiments.

Fresh weight of vegetative growth (g)

The fresh weight of the plant was recorded in grams for each treatment in each replicate.

Dry weight of vegetative growth (g)

Plants of each treatment per replicate were dried in oven at 70 ˚C for 72 hours to a constant weight, then left to cool inside the oven and then weighted in grams.

leaf area (cm^2)

Leaf area (cm^2) was determined after Koller (1972) by weighting two matured leaf blades taken from the 4 th nodes of two plants at each treatment. Two squares with known areas taken from the leaf blades were weighted. The leaf area was calculated using the following equation then the average was calculated for one leaf.

$$\text{Leaf area } (cm^2) = \frac{\text{Leaf weights x Square areas}}{\text{Square weights}}$$

Flowering characteristics

Flowering date (days)

Flowering date was expressed as the number of days from sowing to the appearance of the first inflorescence on the plant.

Number of inflorescences per plant

Expressed as the number of inflorescences per plant.

Length of the inflorescences (cm)

It was measured in centimeters from the main stem of each plant.

Flowering period

Flowering period were expressed as the number of days of flowering from the appearance of the first inflorescence on each plant until the appearance of the last one in each treatment for each replicate.

Chemical analysis

Anthocyanin determination in the leaves and inflorescences were done according to Fuleki and Francis (1968). Total leaf chlorophyll contents a and b (mg/100 g fresh weight) were determined according to Moran (1982), total leaf carotenoid content was determined according to Guan et al. (2005), total leaf soluble carbohydrates content (%) was determined according to (Hedge and Hofreiter,1962). Alkaloids content in the leaves was determined according to (Luo et al., 2005). Phenols content in the leaves was determined according to (AOAC, 2000), antioxidant enzymes activity in the leaves (peroxidase (POD)) was assessed as described by (Mukherjee and Choudhuri, 1983).

Abnormal characters

All plants of the different treatments in both generations were examined to search for the abnormalities and changes in the vegetative or flowering growth were recorded. These changes included :

a) Habit of growth.

b) Abnormal leaves (colour and form).

c) Abnormal inflorescences (colour and form).

DNA isolation and Inter-Simple Sequence Repeat (ISSR) analysis were done as described by (Bhatia et al., 2011) and (Shaw et al., 2009) . The primers name, sequences and fragment sizes (bp) were illustrated as shown in Table 1.

Statistical analysis

Averages were calculated for all characters of each treatment either for the first or second experiment. Means were compared using the least significant difference test (LSD) at 5% level (Snedecor and Cochran, 1967).

For the percentages of seed germination and plant survival, angular transformation was settled, the statistical analysis was carried out using values resulting from transformation.

Results and Discussion

Survival percentage

The result of the survival percentage of the first generation of *Celosia argentea* L.as affected by DES was obtained in Fig.(1). Survival percentage of treated seedlings was recorded after a month of DES treatment. There was significant difference between the highest survival percentage (91.67%) for the treatment of 200 ppm DES for 60 min. compared to the control for 60 min. (62.50 %).

On the other hand, there was - no significant – differences between the treatments of 400 ppm DES for 30 min. (70.45%) and the control for 30 min - (75.00 %).

The highest dosage of DES (600 ppm) for 30 and 60 min. decreased the survival % to 70.00 and 46.67 %, respectively. Similar results were reported by Bhat et al. (2007).

Physical and chemical mutagens revealed that survival of plants to maturity depends on the nature and extent of chromosomal damage may be responsible for reduction in germination ability, plant growth and survival (Khan and Goyal, 2009).

Seed germination percentage

Figure 2. shows the results of the seed germination % of the second generation of *Celosia argentea* as affected by DES treatment. It was noticed that the highest average of seed germination was recorded at the treatment of 400 ppm DES for 60 min (64.00 %) compared to the control for 60 min. (51.00 %) with a significant difference between them. On the other hand, there was - no significant difference between the treatment of 600 ppm DES for 30 min. (63.00%) and the control for 30 min. (45.00 %).

The high seed germination obtained in the second generation may be related to stimulation effect and could be due to the environmental effects; such as, seed moisture contents as well as the mutagen chemical concentrations (Hussein et al., 1974 and Abd El- Maksoud and El- Mahrouk, 1992). These results were similar to those reported by El - Nashar (2006) on *Amaranthus*.

The stimulating effects of low and intermediate concentrations of DES on seed germination may be due to enzymatic activation and awakening of meristematic cell division. Disturbance in the formation of enzymes involving in the germination process, particularly at higher doses, could be considered one of the physiological effects caused by DES.

Plant height

Regarding to the first generation, the main effect of DES soaking duration revealed significant differences among the different doses as shown in Table (2). The treatment of 200 ppm for 30 min. and 60 min. had the tallest plants (108.33 and 104.73 cm; respectively) compared to the control for the same duration (74.75 and 71.67 cm; respectively).

Meanwhile on the second generation, the treatments of 200 and 400 ppm for 60 min. recorded the tallest plants (101.33 and 100.17 cm; respectively) compared to the control for 60 min. (82.33 cm). On the other hand, the highly significant differences were found between the treatments of 200 ppm DES for 30 min., 400 ppm DES for 30 min. and 600 ppm DES for 30 min. (80.75 , 84.50 and 78.90 cm; respectively) compared to the control for 30 min. (63.85 cm). These results were in conforming with El - Nashar (2006) on *Amaranthus*.

Stem diameter

Plants treated with 600 ppm. for 30 mn. gave the largest stem diameter (1.887 cm.) in the first generation compared to the control for 30 min. (1.320 cm), while the treatment of 400 ppm. for 60 min. gave the thickest stem (1.642 cm.) in second generation compared with the control (1.129 cm) as presented in Table (2).This result confirmed with El-Khateeb *el al.* (2022) on *Borgo officinalis*.

All these results may be due to the simulative effect of the mentioned treatments or concentrations on the plant height and stem diameter which could be related to the physiological activation of plant

metabolism as a results of DES application , El - Torky (1992) and Abd El- Maksoud and El-Mahrouk (1992 and 1993), and the reductions in the plant height and stem diameter may be due to the physiological damage produced by the DES and its hydrolysis products as reported by Hussein et al. (1974), Abd El- Maksoud and El-Mahrouk (1992 and 1993), El- Torky (1992) and El - Nashar (2006).

Number of branches per plant

There were no significant differences found among the first generation treatments as presented in Table (2). On the other hand, the second generation showed significant differences among treatments, where the treatment of 400 ppm for 60 min. gave the highest number of branches per plant (29.7) compared to the control (17.7). There was no clear trend for the effect of the DES mutagen on branching and this was similar to the result of El-Khateeb *el al.* (2022) on *Borago officinalis*.

Soaking seedlings on DES at 200 and 400 ppm for 30 min decreased significantly the formation of branches per plant compared to control.

The reduction in the branching may be also due to the effect of the chemical mutagens on growth regulators such as gibberellins and cytokinins , which play important roles in controlling cambial activity. The effects of mutagens resulted in a reduction in these growth regulators which was detrimental to the mitotic activity of the cambial cells, consequently suppressed the branching (El-Nashar, 2006).

Number of leaves per plant

In the first generation, treating with 600 ppm. for 30 min. recorded the highest number of leaves per plant (82.3) compared with the control for 30 min. (65.7), with no significant differences as shown in Table (2), followed by the treatment of 200 ppm. for 60 min.

In the second generation, soaking in 400 and 600 ppm.DES for 60 min. recorded the highest number of leaves per plant (85.7) compared with the control for 60 min. (45.3) and showed significant differences among them, while the treatment of 600 ppm. for 30 min. increased number of leaves per plant (51.3) compared with the control for 30 min. (38.7), with no significant between them.

Clear significant effects on the number of branches and leaves per plant were also recorded. These results are in accordance with the findings of Hussein et al. (1974) on *Salvia splendens* and El-Nashar (2006) on *Amaranthus*. The physiological effects of DES and their hydrolysis products could also be the reason for increasing the number of branches and leaves in each plant.

Fresh and dry weight of vegetative growth

Table (3) showed in the first generation, that the greatest fresh and dry weight of vegetative growth was recorded at the treatment of 200 ppm. for 30 min. compared with for the control 30 min. with significant difference. While the treatment of 200 ppm. and 400 ppm for 60 min. increased fresh and dry weight of vegetative growth, respectively compared with the control for 60min. with between them.

In the second generation, the greatest fresh and dry weight of vegetative growth was recorded at the treatment of 400 ppm. for 60 min. compared with the control for 60 min. with significant difference. While the treatment of 600 ppm for 30 min. increased fresh and dry weight of vegetative growth compared with the control for 30min., with out significant difference. The proportional increases and decreases in the fresh and dry weights, with increasing DES concentrations reported in this study, were similar to those findings of Abd El-Maksoud and El-Mahrouk (1992) on *Asparagus densiflorus*. The difference in responses at various doses could be attributed to the environmental factors, such as temperatures and/or nutrition that prevailed during the growth period of the plants.

Another trend was reported by Mullainathan et al. (2006) on the second generation of mung bean treated with DES, where they found increases in the plant fresh weight.

Leaf area

In the first generation, treatment of 400 ppm. for 60 min. gave the largest leaf area (694.69 cm^2) compered the control for 60 min. (281.41 cm^2) as shown in Table (3) with significant difference. While treatment of 200 ppm(30 min) recorded the larger leaf area (433.61 cm^2) compared with the control(60 min) giving 164.32 cm^2 , with significant differences among the different treatments.

In the second generation, all concentrations for 60 min. increased significantly leaf area (692.71, 672.47 and 699.12 cm^2) compared to control for 60 min. (298.08 for cm^2), with significant differences. Also, treatment of 600 ppm. for 30 min. gave the largest leaf area (575.00 cm^2) compared to control for 30 min. (157.15 cm^2), with significant difference.

Generally, the low concentrations of DES increased the leaf area .These results were in agreement with that reported by El -Nashar (2006) on *Amaranthus*. This result may be due to chemical mutagens could be lead to increases and reductions in the leaf area of the mutagen treated plants by affecting the number and/or length of cells which can alter the leaf characters of the plants following the chemical treatment. Large leaf area means increase in cell number and small leaf area means decrease in cell number and size.

Flowering date

Concerning to flowering date, the treatments of 400 ppm and 600 ppm for 60 min. delayed flowering (127.3 and 128.0 days ,respectively) in the first generation compared the control for 60 min. (123.0 day) as shown in Table (3) with no significant differences. Also, no significant difference were found among the treatments of 400, 600 ppm. for 30 min. and control for 30 min.

In the second generation, the treatments of 200, 400 and 600 ppm for 60 min. delayed flowering (188.3, 189.7 and 190.3 days, respectively) compared to control for 60 min. (184.3 days), with significant differences. Also no significant differences were found between treatments of 400 , 600 ppm. for 30 min. and control for 30 min. The delaying effect of some DES treatments on flowering onset during the first and second generations was in harmony with the results stated by Hussein et al. (1974) and El-Nashar (2006).

On the other hand, elevated concentrations seemed to inhibit the cell growth, decrease growth rate, and delay the flowering date, as reported by Badr et al. (2000) and El-Nashar (2006).

Number of inflorescences/plant

The treatment of 200 ppm for 30min. in the first generation gave the highest number of inflorescences per plant (26.0) compared to control for 30 min.(17.0) and there were significant differences among the different treatments as shown in Table (4). The treatments of 400 and 600 ppm for 60 min. in the second generation gave the highest number of inflorescences (31.0 and 33.0, respectively) compered the control for 60 min. (18.3) and there were significant differences among them.

These results were supported with those reported by El-Nashar and Shetta (2015) on *Leucaena leucocephala*.

Length of inflorescence

Table (4) showed that the tallest inflorescences were produced by the treatment of 400 ppm for 30min. in the first generation compared to control for 30 min. with significant differences among the different treatments. Also, the treatments of 200 ppm for 60 min. increased significantly the inflorescence length in the first generation compared to control for 60 min.

Regarding the second generation, the tallest inflorescences were produced by the treatment of 200 ppm for 60 min. compared to control for 60 min. with significant difference, while the tallest inflorescence produced by the treatment of 600 ppm for 30 min. compared to control for 30 min. with significant difference between them.

Similar results were reported by Kannan et al. (2002) on *Jasminum sambac* treated with EMS.

Flowering period

In the first generation, highest flowering period (88.7 day) recorded at treatments of 200 and 600 ppm for 30 min. and did not differed significantly to the control (88.3 days) but the treatment of 600 ppm for 60 min. (84.7 day) decreased flowering period compared to control for 60 min. (85.7 days) with no significant differences as shown in Table (4).

On the other hand, in the second generation, the treatments 200 ppm and 600 ppm for 30 increased inflorescences period (86.7 and 87.7 day, respectively) compared to control for 30 and 60 min. (85.7 days) with no significant differences. These result are in agreement with the finding of El - Nashar (2006) on *Amaranthus*.

Chlorophyll- a ,- b and total carotenoid content (mg/g)

Plants treated with 600 ppm for 30 min. gave the highest chlorophyll - a ,- b and total carotenoid content with no significant difference to the control for 30 min. for chlorophyll - a and- b, but with significant difference for total carotenoid content in first generation. While the lowest chlorophyll- a ,- b and total carotenoid content was obtained in plants treated with 600 ppm for 60 min., with significant difference to the control for 60 min. as shown in Table (5).

In the second generation, plants treated with 600 ppm for 60 min. gave the highest chlorophyll - a,- b and total carotenoid content with significant differences to the control for 60 min. While the lowest chlorophyll - a , total carotenoid content and chlorophyll - b was found in plants treated with 200 and 400 ppm for 30 min. respectively, with no significantly difference to the control for 30 min.

The entire range of chlorophyll mutations occurred due to a deficiency in chlorophylls, carotenoids or combination of both in plastid genes causing variegation, as reported by Kirk and Tilney-Bassett (1978). Similar harmful effects were mentioned by Hussein et al. (1974) in Salvia splendens and El-Nashar (2006) on Amaranthus; based on these previous studies, the effect of DES, which produced chlorophyll mutants, can be attributed to an enhancement of chloroplast differentiation or any previously mentioned reasons.

Similar results were reported by El-Nashar and Shetta (2015) on *Leucaena leucocephala*. and El-Khateeb *el al.* (2022) on *Borago officinalis*.

Total soluble carbohydrates content

Table (5) showed that the highest total *soluble* carbohydrates content was recorded at the treatment of 400 ppm for 60 min. (8.10 %), with significant

difference to the control for 60 min.(6.70 %) in first generation. While the lowest total carbohydrates content was recorded at the treatment of 400 ppm for 30 min. (2.70 %), with significant difference to the control for 30 min. (4.05 %).

In the second generation, the highest total carbohydrates content was recorded at the treatment of 400 ppm for 60 min. (7.80 %), with significant differences from the control for 60 min.(6.40 %).While the lowest total carbohydrates content was recorded at the treatment of 400 ppm for 30 min. (3.10 %), with significant differences to the control for 30 min.(4.20 %).

Similar results were reported by El-Khateeb *el al.* (2022) on *Borago officinalis*.

Anthocyanin in the leaves

The anthocyanin concentration in the leaves was decreased significantly by all treatments in the first generation compared the control (Table 6). In the second generation the highest anthocyanin concentration in the leaves was recorded by 200 ppm for 30 min. (34.30 mg mL^{-1}), with significant differences to the control for 30 min. (27.30 mg mL^{-1}). While the highest anthocyanin concentration in the leaves recorded by 600 ppm. for 60 min.(24.80 mg mL^{-1}), with significant differences to the control for 60 min. (7.50 mg mL^{-1}).

This result agrees with the results of El - Nashar (2006) on *Amaranthus*.

Anthocyanin in the inflorescence

In the first generation the highest anthocyanin concentration in the inflorescence recorded by 600 ppm for 30 min.(64.60 mg mL^{-1}), with significant differences to the control for 30 min. (61.33 mg mL^{-1}). While the anthocyanin concentration in the inflorescence was decreased significantly using all other treatments for 30 min. is presented in Table (6).

In the second generation the highest anthocyanin concentration in the inflorescence recorded for the treatment of 600 ppm.for 60 min.(58.50 mg mL^{-1}), with significant differences to the control for 60 min. (15.70 mg mL^{-1}). While the anthocyanin concentration in the inflorescence was decreased significantly using all other treatments for 30 min.

Alkaloids in the leaves

In the first generation all treatments increased significantly the alkaloids concentration in the leaves (Table 6), the highest alkaloids concentration in the leaves recorded by 400 ppm for 60 min.(1.50 mg mL^{-1}), with significant difference to the control for 60 min. (0.28 mg mL^{-1}), while the lowest alkaloids concentration in the leaves was recorded by 200 ppm for 60 min.(0.30 mg mL^{-1}), with no significant difference to the control for 60 min.(0.28 mg mL^{-1}).

The highest alkaloids concentration in the leaves was found at 200 ppm for 60 min. with significant differences to the control for 60 min. in the second generation. While the lowest alkaloids concentration in the leaves recorded by for 30 min. at 200 ppm , with significant differences to the control for 30 min.

Increased of alkaloids in the leaves confirmed with Mostafa (2009) on *Balanites aegyptiaca* due to the application of dimethyl sulphate.

Phenols in the leaves

Plants treated with 600 ppm for 30 min. gave the highest phenols concentration in the leaves, but did not differ significantly from the control for 30 min. in first generation is presented in Table (6).

Plants treated with 400 ppm. for 60 min. gave the highest phenols concentration in the leaves (6.5 mg mL^{-1}) and different significantly from the control for 60 min. in second generation. While all other treatments decreased significantly the phenols concentration in the leaves in for 30 min.

Peroxidase (*POD*)

Peroxidase activity decreased significantly with all treatments in the first generation compared to the control (Table (6). In the second generation the highest peroxidase activity was recorded in plants treated with 200 ppm for 30 min. which different significantly from the control for 30 min. While the lowest peroxidase activity was recorded for 60 min soaking time at 200 ppm DES and different significantly from the control for 60 min.

Effect of diethyl sulphate (DES) on the induction of variations (Aberrations)

a-Growth habit changes

Some treatments caused changes in the habit of growth is some plants resulting in forms.

The dwarfed growth in the first generation may be due to physiological damage resulted in the alteration from normal to dwarf growth (Abd El-Maksoud and El- Mahrouk , 1993). In addition El-Torky (1992) postulated that the chemical mutagen caused a kind of damage during the mitotic cycle of the plant seedling which resulted in this type of chlorophyll deficient . he change in the habit of growth during the second generation of the first season may be attributed to the effect of the chemical mutagens on the genetic factors controlling the growth habit of the plants (Abd El-Maksoud , 1988).

Leaf changes

The treatments of 200,400 and 600 ppm DES for 30 and 60 min. caused changes in the leaf in the M_1- Second generation compared with the control (Fig.5 and 6).

These results were in agreement with those reported by El-Nashar (2006) on *Amaranthus,* Mostafa(2009) on *Balanites aegyptiaca* and by El-Khateeb *el al.* (2022) on *Borago officinalis*.

Chromosomal disturbances may be the reason of the changes of leaf form or shape which appeared in the second generation plants and referred to the layer rearrangements as a result of the chemical mutagens effects (Abd El-Maksoud , 1988 and El-Nashar, 2006).

b-Inflorescences changes (*Modified form of inflorescence*)

The treatments of 200,400 and 600 ppm DES for 30 and 60 min. produced modified inflorescences leaf in the first and second generation compared with the control (Fig.7 and 8).

These results were in agreement with those reported by El-Nashar (2006) on *Amaranthus,* and Mostafa, (2009) on *Balanites aegyptiaca*. Aberrations in inflorescence shape could be attributed to the effect of low and high concentrations of chemical mutagens o the cell umber and cell length. Cell number and cell length may be altered in the inflorescence of treated plants. Large inflorescence had larger florets with an increase in cell number and / or cell size. Small inflorescence had smaller florets with a decrease in cell number and / or cell size (Badr and Etman,1976).

Polymerase chain reaction (PCR) analysis

Inter simple servence repeat was used to identify six mutants (their description and treatments as shown Table 7) from control plants. Four primers were used and a total of 67 bands were amplified with 33 polymoric bands and 48.9 % polymorphis as shown in Table (8).

The primer 4 gave the largest number of polymorphic bands (10) followed by the primer 3 (9 polymorphic bands).

The highest genetic distance between control and mutants was found with the mutants 7, 5,4 and 8 (30.8,30.1,29.3and 28.2, respectively).

The dendrograme tree (fig 14) showed that the mutant 7 (600 ppm for 30 min.) was more genetically distinct from the control and other mutants. These findings referred to the morphological characters while obtain as the mutagenic effect of diethyl sulphate treatments as an alkylating agents. Diethyl sulphate introduce chromosome aberrations and amino acid changes (Khan and Tyadi,2009).

Conclusively

The obtained results indicated that the different concentrations of diethyl sulphate caused some morphological variations in the vegetative, flowering growth as well as increased chemical composition of *Celosia argentea* L.var. spicata plants. Using ISSR - PCR technique was able to detect mutations in a plant *Celosia argentea* L. plants.

TABLE 1. ISSR primers, sequence, size of amplified fragment (bp), used to analyze genetic relationships among the control and treated plants of *Celosia argentea var. spicata* samples.

Primer	Sequence (5´→3´)	Size range (bp)
ISSR-1	(CAC)3GC	200-1000
ISSR-2	(GT)6CC	100-800
ISSR-3	(TG)7G	200-900
ISSR-4	(CTC)5TGC	100-1000

TABLE 2. Mean values of the plant height, stem diameter, number of branches / plant and number of leaves/ plant of *Celosia argentea* L. as affected by Diethyl Sulphate (DES) treatments in the first and second generations.

DES ppm	Time (min.)	Plant height (cm)		Stem diameter (cm)		Number of branches / plant		Number of leaves/ plant	
		Fir.	Sec.	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.
0	30	74.75^{cd}	63.85^{c}	1.320^{cd}	0.981^{d}	15.3^{a}	16.0^{bcd}	65.7^{a}	38.7^{c}
	60	71.67^{cd}	82.33^{b}	1.462^{bcd}	1.129^{cd}	12.3^{a}	17.7^{bcd}	67.0^{a}	45.3^{bc}
200	30	108.33^{a}	80.75^{b}	1.770^{ab}	1.321^{abc}	18.0^{a}	8.0^{e}	65.7^{a}	47.0^{bc}
	60	104.73^{a}	101.33^{a}	1.819^{a}	1.469^{ab}	20.0^{a}	20.0^{bc}	77.3^{a}	69.7^{ab}
400	30	96.10^{ab}	84.50^{b}	1.439^{cd}	1.426^{abc}	17.7^{a}	11.3^{de}	26.7^{b}	41.3^{c}
	60	83.67^{bc}	100.17^{a}	1.570^{abc}	1.642^{a}	19.0^{a}	29.7^{a}	64.3^{a}	85.7^{a}
600	30	100.80^{ab}	78.90^{b}	1.887^{a}	1.318^{bc}	15.7^{a}	15.3^{cde}	82.3^{a}	51.3^{bc}
	60	62.80^{d}	85.40^{b}	1.203^{d}	1.332^{abc}	22.3^{a}	22.7^{ab}	56.3^{ab}	85.7^{a}
LSD $_{0.05}$		17.39**	12.00**	0.322**	0.323**	NS	7.3**	33.8**	26.1**

Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D at 0.05 level of probability.
** =Highly significant at 0.01 level of probability& N.S.= Not significant.

TABLE 3. Mean values of the fresh and dry weight of vegetative growth (g), leaf area (cm^2) and flowering date of *Celosia argentea* L. as affected by Diethyl Sulphate (DES) treatments in the first and second generations.

DES ppm	Time (min.)	Fresh weight of vegetative growth (g)		Dry weight of vegetative growth (g)		Leaf area (cm^2)		Flowering date (days)	
		Fir.	Sec.	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.
0	30	22.6^{d}	30.0^{b}	3.9^{c}	5.2^{b}	164.32^{d}	157.15^{c}	120.0^{b}	184.3^{b}
	60	26.5^{d}	34.1^{b}	6.1^{c}	6.1^{b}	281.41^{cd}	298.08^{c}	123.0^{ab}	184.3^{b}
200	30	72.8^{a}	39.0^{b}	10.8^{a}	7.2^{ab}	433.61^{bc}	373.75^{bc}	119.0^{b}	183.0^{b}
	60	48.0^{c}	47.1^{ab}	5.6^{c}	7.8^{ab}	572.92^{ab}	692.71^{a}	125.0^{ab}	188.3^{a}
400	30	57.4^{bc}	26.6^{b}	6.9^{bc}	4.8^{b}	322.12^{cd}	394.26^{bc}	120.3^{b}	183.7^{b}
	60	47.8^{c}	68.8^{a}	6.4^{c}	10.9^{a}	694.69^{a}	672.47^{a}	127.3^{a}	189.7^{a}
600	30	63.8^{ab}	45.1^{ab}	10.0^{ab}	7.3^{ab}	416.56^{bc}	575.00^{ab}	120.0^{b}	183.7^{b}
	60	24.9^{d}	46.0^{ab}	3.8^{c}	7.0^{b}	607.78^{ab}	699.12^{a}	128.0^{a}	190.3^{a}
LSD $_{0.05}$		12.3**	23.9*	3.3**	3.8*	241.58**	239.53**	6.3**	3.8**

Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D at 0.05 level of probability.
*, ** = Significant at the 0.05 & Highly significant at 0.01 level of probability.

TABLE 4. Mean values of the number of inflorescences / plant , length of inflorescences and flowering period and of *Celosia argentea* L. as affected by diethyl sulphate (DES) treatments in the first and second generations

		Number of inflorescences/ plant		Length of inflorescences		Flowering period (days)	
DES ppm	Time (min.)	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.
0	30	17.0^{bc}	19.4^{bc}	4.45^{d}	7.50^{d}	88.3^{a}	85.7^{ab}
	60	9.7^{d}	18.3^{bc}	4.93^{d}	8.80^{bcd}	85.7^{ab}	85.7^{ab}
200	30	26.0^{a}	12.0^{c}	9.37^{ab}	6.60^{d}	88.7^{a}	86.7^{a}
	60	17.7^{bc}	20.7^{b}	8.23^{abc}	11.70^{a}	82.3^{b}	79.7^{bc}
400	30	20.3^{abc}	18.0^{bc}	10.40^{a}	8.00^{cd}	84.7^{ab}	78.7^{bc}
	60	20.0^{abc}	31.0^{a}	7.43^{bc}	10.50^{abc}	81.7^{b}	79.7^{c}
600	30	22.7^{ab}	18.7^{bc}	8.13^{abc}	11.10^{ab}	88.7^{a}	87.7^{a}
	60	14.0^{cd}	33.0^{a}	6.07^{cd}	9.00^{bcd}	84.7^{ab}	78.7^{c}
$LSD_{0.05}$		6.6^{**}	8.3^{**}	2.45^{**}	2.61^{**}	4.2^{**}	6.9^{*}

Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D at 0.05 level of probability.
*, ** = Significant at the 0.05 & Highly significant at 0.01 level of probability.

TABLE 5. Mean values of the chlorophyll-a (mg/g), chlorophyll-b (mg/g),total carotenoid content (mg/g) and total soluble carbohydrates of *Celosia argentea* L. as affected by diethyl sulphate (DES) treatments in the first and second generations

		Chlorophyll-a (mg/g)		Chlorophyll-b (mg/g)		Total carotenoid content (mg/g)		Total soluble carbohydrates content (%)	
DES ppm	Time (min.)	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.
0	30	0.560^{abc}	0.390^{c}	0.165^{a}	0.136^{d}	0.140^{bcd}	0.108^{b}	4.05^{d}	4.20^{d}
	60	0.487^{bc}	0.534^{b}	0.138^{b}	0.175^{bc}	0.145^{bc}	0.131^{a}	6.70^{b}	6.40^{b}
200	30	0.501^{bc}	0.386^{c}	0.151^{ab}	0.147^{cd}	0.130^{cde}	0.104^{b}	3.70^{d}	3.90^{e}
	60	0.452^{cd}	0.628^{a}	0.133^{bc}	0.206^{b}	0.135^{cde}	0.143^{a}	5.06^{c}	5.30^{c}
400	30	0.590^{ab}	0.394^{c}	0.163^{a}	0.137^{d}	0.158^{ab}	0.108^{b}	2.70^{e}	3.10^{f}
	60	0.363^{de}	0.665^{a}	0.118^{cd}	0.252^{a}	0.120^{de}	0.144^{a}	8.10^{a}	7.80^{a}
600	30	0.648^{a}	0.426^{c}	0.170^{a}	0.144^{cd}	0.172^{a}	0.112^{b}	4.39^{cd}	4.12^{de}
	60	0.333^{e}	0.674^{a}	0.102^{d}	0.272^{a}	0.115^{e}	0.143^{a}	3.78^{d}	4.10^{de}
$LSD_{0.05}$		0.110^{**}	0.075^{**}	0.022^{**}	0.036^{**}	0.023^{**}	0.015^{**}	0.73^{**}	0.23^{**}

Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D at 0.05 level of probability.
** =Highly significant at 0.01 level of probability.

TABLE 6. Mean values of the anthocyanin in the leaves and inflorescences (mg mL^{-1}), alkaloids in the leaves (mg mL^{-1}),phenols in the leaves (mg mL^{-1}) and peroxidase (POD) of *Celosia argentea* L. as affected by diethyl sulphate (DES) treatments in the first and second generations.

		Anthocyanin (leaves) (mg mL^{-1})		Anthocyanin (inflorescence) (mg mL^{-1})		Alkaloids in the leaves (mg mL^{-1})		Phenols in the leaves (mg mL^{-1})		Peroxidase (POD) (mg mL^{-1})	
DES ppm	Time (min.)	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.	Fir.	Sec.
0	30	64.50^{a}	27.30^{b}	61.33^{b}	55.00^{b}	0.20^{f}	0.45^{d}	6.0^{abc}	5.8^{cd}	49.00^{e}	57.00^{b}
	60	41.80^{b}	7.50^{f}	66.30^{a}	15.70^{f}	0.28^{e}	1.46^{c}	5.5^{c}	5.5^{d}	66.00^{a}	31.00^{e}
200	30	15.60^{f}	34.30^{a}	55.80^{c}	42.60^{c}	0.84^{b}	0.28^{e}	6.3^{ab}	4.7^{e}	53.00^{d}	68.00^{a}
	60	41.80^{b}	4.50^{g}	60.00^{b}	10.80^{g}	0.30^{e}	2.80^{a}	5.8^{bc}	6.3^{ab}	61.00^{b}	26.00^{f}
400	30	18.80^{e}	19.70^{d}	35.00^{e}	53.30^{b}	0.78^{b}	0.46^{d}	6.3^{ab}	4.3^{f}	36.00^{f}	55.00^{b}
	60	8.30^{g}	7.10^{f}	42.50^{d}	23.30^{e}	1.50^{a}	2.00^{b}	6.2^{ab}	6.5^{a}	48.00^{e}	35.00^{d}
600	30	27.10^{d}	9.70^{e}	64.60^{a}	26.40^{d}	0.48^{c}	1.43^{c}	6.5^{a}	5.8^{cd}	58.00^{c}	32.00^{de}
	60	31.80^{c}	24.80^{c}	61.40^{b}	58.50^{a}	0.38^{d}	0.48^{d}	6.0^{abc}	6.0^{bc}	65.00^{a}	51.00^{c}
$LSD_{0.05}$		3.03^{**}	1.44^{**}	2.65^{**}	2.60^{**}	0.07^{**}	0.16^{**}	0.5^{**}	0.3^{**}	2.82^{**}	3.22^{**}

Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D at 0.05 level of probability.
** =Highly significant at 0.01 level of probability.

TABLE 7. The treatments which induced the mutation and description of the mutants of *Celosia argentea* L. plants in the second generation.

Number of plant	Treatment	Change
1	0 ppm for 30 min	control
2	0 ppm for 60 min	control
3	200 ppm for 30 min	Strange branching
4	200 ppm for 60 min	Taller plant
5	400 ppm for 30 min	Dwarfed plant
6	400 ppm for 60 min	fascinated inflorescences and leaves
7	600 ppm for 30 min	fascinated inflorescences and leaves
8	600 ppm for 60 min	fascinated inflorescences and leaves

TABLE 8. Number of bands, number of polymorphic bands and polymorphism % detected by ISSR marker in the second generation of *Celosia argentea* L.var. *spicata* plants by diethyl sulphate

Primer number	Sequence of ISSR primers	Total number of bands	Number of polymorphic bands	Polymorphism %
1	5′-(CAC)3GC-3′	17	8	47.06
2	5′-(GT)6CC-3′	15	6	40.00
3	5′-(TG)7G-3′	16	9	56.25
4	5′-(CTC)5TGC-3′	19	10	52.63
Total		67	33	
Average		16.7	8.2	48.99

TABLE 9. Genetic distance of DNA among second generation of *Celosia argentea* L. var. *spicata* plants treated by diethyl sulphate using ISSR marker

	1	2	3	4	5	6	7	8
1	0.0							
2	24.0	0.0						
3	20.1	30.8	0.0					
4	29.3	26.5	29.3	0.0				
5	30.1	19.1	23.9	36.5	0.0			
6	27.7	21.5	28.1	26.2	27.1	0.0		
7	30.8	26.2	34.7	32.4	24.5	23.9	0.0	
8	28.2	21.8	31.7	30.0	28.3	22.5	32.0	0.0

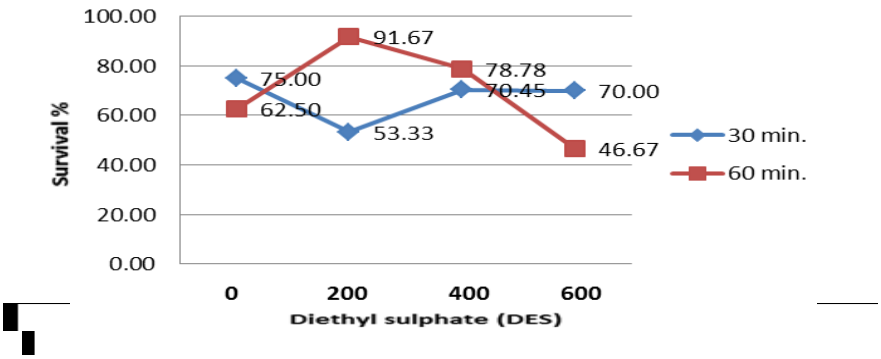


Fig. 1. Survival percentage of *Celosia argentea* L. as affected by diethyl sulphate at the first generation.

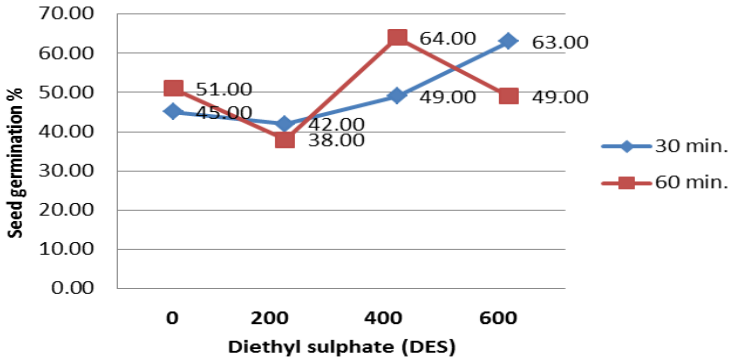


Fig. 2. Seed germination % of *Celosia argentea* L. as affected by diethyl sulphate in the second generation.

a b

Fig. 3. A Photograph depicts change in the growth habit (branches) as in the first (a) - second (b) generations of *Celosia argentea* L. plants after the treatment with diethyl sulphate at 200,400 and 600 ppm for 30 min. compared with the control.

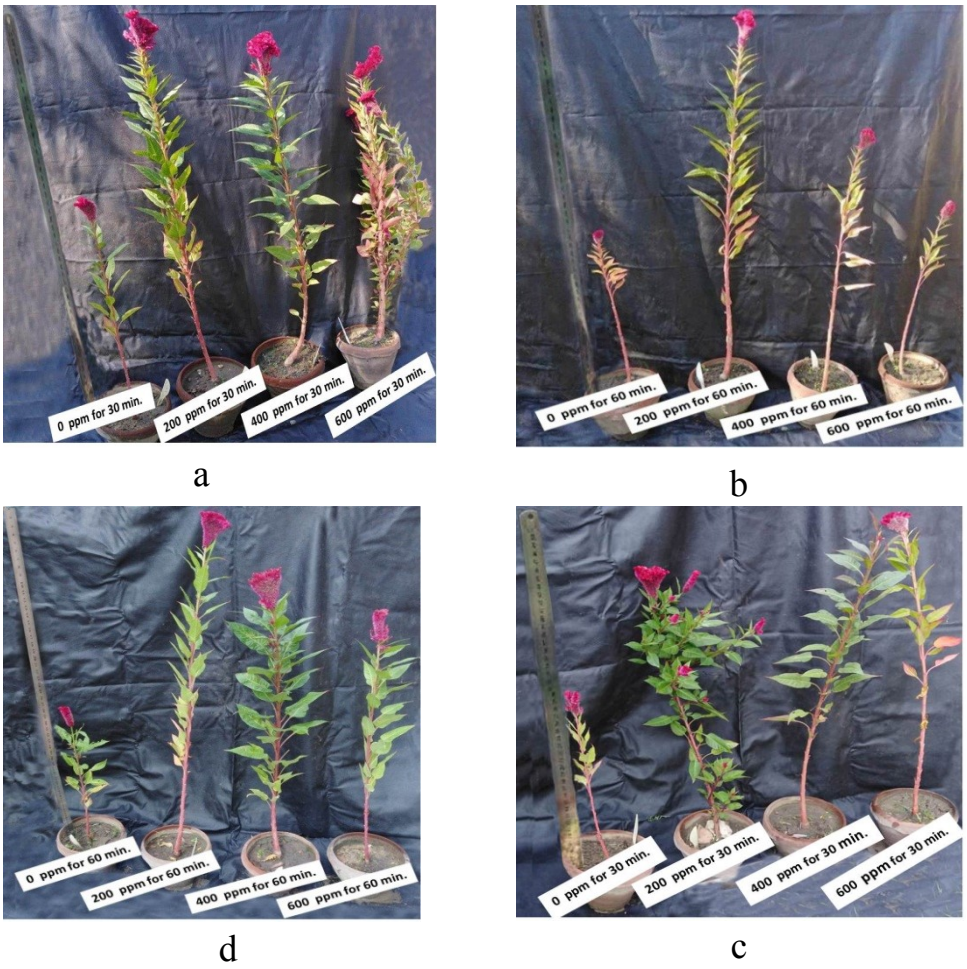
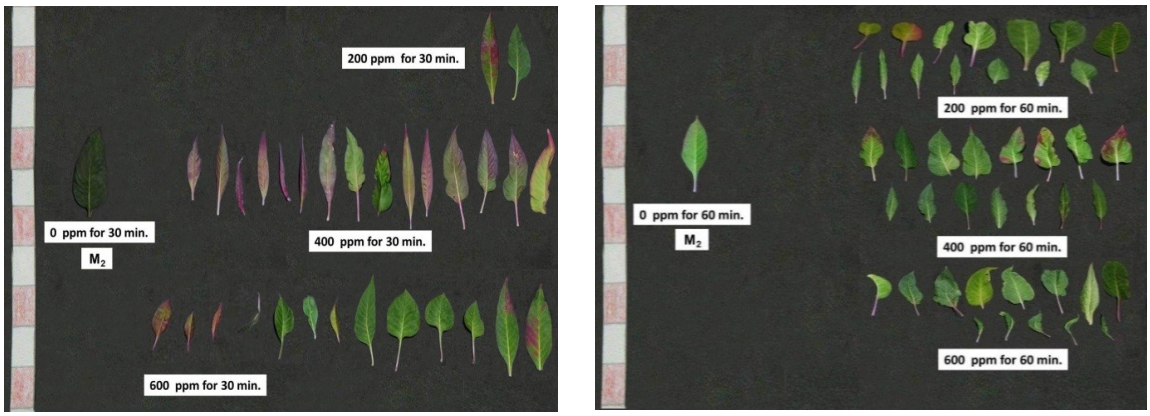


Fig. 4. A Photograph shows change in the growth habit as a greater growth in the (a & b) first- (c & d) second generation of *Celosia argentea* L. plants after the treatment with diethyl sulphate at 200,400 and 600 ppm for 30 and 60 min. compared with the control.

Fig. 5. A Photograph shows change in the growth habit as a shortest growth in the second generation of *Celosia argentea* L. plants after the treatment with diethyl sulphate at 200,400 and 600 ppm for 30 min. compared with the control.

a b

Fig. 6. A photograph depicts the leaf abnormalities in *Celosia argentea* L.plants produced after the treatment with diethyl sulphate at 200,400 and 600 ppm for 30 (a) and 60 (b) min in the first generation.



a b

Fig. 7. A photograph depicts the leaf abnormalities in *Celosia argentea* L. plants produced after the treatment with diethyl sulphate at 200,400 and 600 ppm for 30 (a) and 60 (b) min in the second generation.

a b

Fig. 8. A photograph shows changes in inflorescence in *Celosia argentea L. formed* after the treatment with diethyl sulphate at 200,400 and 600 ppm for 30 (a) and 60 (b) min. in the first generation.

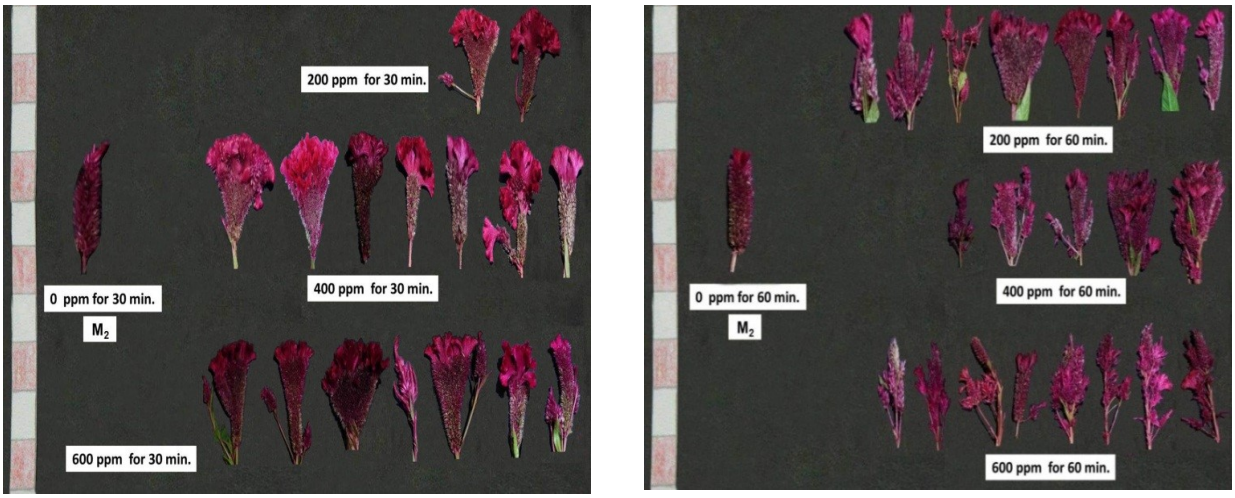


Fig. 9. A photograph shows changes in inflorescence in *Celosia argentea* L. formed after the treatment with diethyl sulphate at 200,400 and 600 ppm for 30 (a) and 60 (b) min. in the second generation.

Fig. 10. A Photograph shows a fascinated inflorescences and leaves in *Celosia argentea* L. plants after the treatment with diethyl sulphate at 200 ppm for 30 in the first generation.

Fig.11. A Photograph showing a fascinated inflorescences and leaves in *Celosia argentea* L. plants after the treatment with diethyl sulphate at 200 ppm for 60 in the first generation.

Fig. 12. A Photograph showing a fascinated inflorescences and leaves in *Celosia argentea* L. plants after the treatment with diethyl sulphate at 600 ppm for 30 min. in the first generation.

Fig. 13. A Photograph showing control and mutants of *Celosia argentea* L. plants in the second generation.

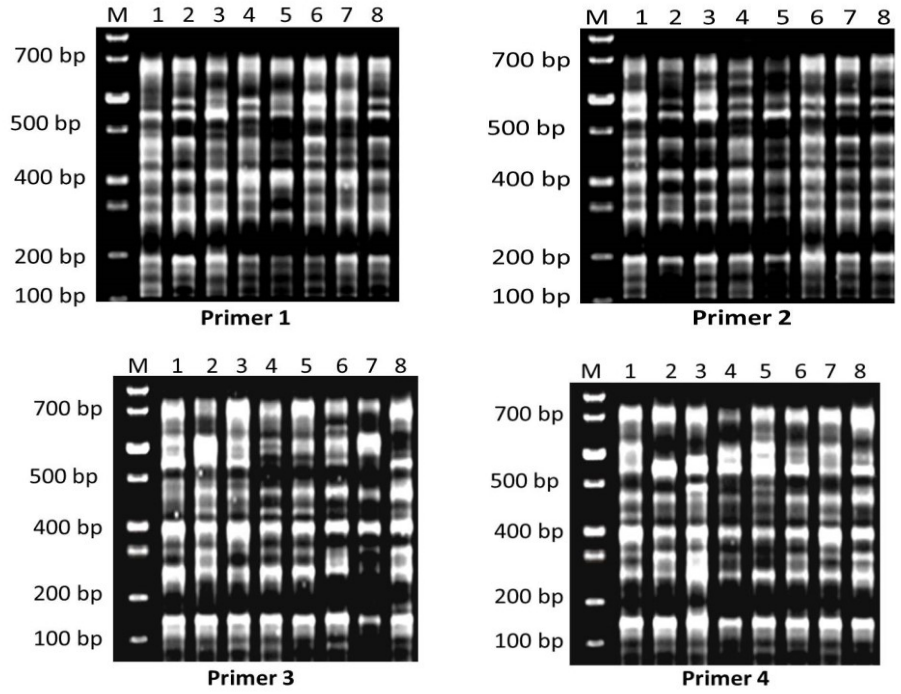


Fig. 14. Photograph showing PCR amplified fragments for the control (original parent) and variant plants of *Celosia argentea* L. in the second generation after amplification with the primer ISSR (1-4).

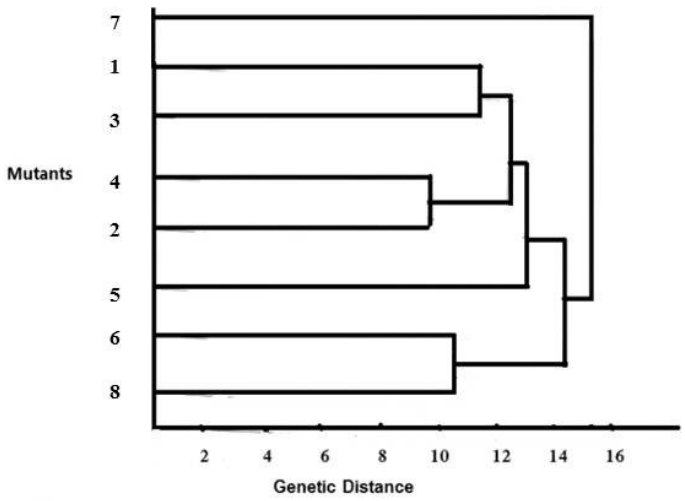


Fig. 15. Tree diagram for second generation of *Celosia argentea* L. var. *spicata* plants treated by diethyl sulphate on the basis of ISSR using profile four ISSR marker.

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Conflicts of interest

The author declared that present study was performed in absence of any conflict of interest.

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تأثيركبريتات ثنائي كحول الإيثيل علي النمو الخضري والزهري والتركيب الكيمائي والمعلمات الجزيئية ISSR لنباتات عرف الديك

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تم إجراء البحث في مشتل الزهورونباتات الزينة بكلية الزراعة- جامعة الإسكندرية خلال عامي 2021 و2022 . نقعت جذور شتلات نبات عرف الديك في تركيزات مختلفة من كبريتات ثنائي كحول الإيثيل وهي:0و200و400و600 جزء في المليون لمدة 30 و60 دقيقة وذلك بغرض دراسة تأثير كبريتات ثنائي كحول الإيثيل كمطفر كيماوي علي صفات النمو والتركيب الكيميائي لنبات عرف الديك وكذلك إنتاج الطفرات واستخدام المعلمات الجزيئية ISSR .
واظهرت النتائج أن تركيز 200 جزء في المليون لمدة 30 دقيقة له تأثير معنوي علي زيادة تركيز الأنثوسيانين في أوراق الجيل الأول ونشاط انزيم البيروكسيديز في الجيل الثاني، بينما تركيز 200 جزء في المليون لمدة 60 دقيقة كان له تأثير معنوي علي زيادة تركيز القلويدات في اوراق الجيل الثاني.
أدي إستخدام تركيز 400 جزء في المليون لمدة 60 دقيقة نقع إلي زيادة معنوية في تركيز القلويدات في الاوراق في الجيل الأول وتركيز المركبات الفينوليه في الأوراق في الجيل الثاني.
واظهرت النتائج أن تركيز 600 جزء في المليون لمدة 30 دقيقة له تأثير معنوي علي زيادة تركيز الأنثوسيانين في النورة في الجيل الأول. كل التركيزات المستخدمة لمدة 60 دقيقة زادت من مساحة الورقة و قد زاد تركيز الأنثوسيانين في النورة في الجيل الثاني معنوياً باستخدام تركيز 600 جزء في المليون لمدة 60 دقيقة. بعض تغيرات في طبيعة النمو وشكل الورقة وشكل النورة لوحظت في كلا الجيلين. تقنية المعلمات الجزيئية ISSR قادرة على اكتشاف الطفرات في نبات عرف الديك.

الكلمات الدالة: عرف الديك، الأنثوسيانين، القلويدات، الفينول، البيروكسيديز، المعلمات الجزيئية ISSR.