PART - II

CHAPTER - I

Effect of diethyl sulfate (DES) a Chemical mutagen on *Crotalaria juncea* Linn.

A. Introduction :

Developing plant receives its blue print from a fertilized egg (Zygote) in which its morphological, physiological mode of reproduction, growth and development are predetermined. However, how plant looks when it grows, how does it mainfest, its characters both morphological and physiological are not only dependent upon the blue print that it receives from a fertilized egg but on the interaction between environment and genetic material, that it has in it. If the genetic material is modified or altered by one or the other means in the sense by artificial means as chemical mutagenic agents, at an early stage of development such as in embryonic stage or at the seed level this is readily manifested and such manifestations, if is permitted by the nature may be perpetuated further.

The generation and recovery of mutants in order to braden the genetic base of crop plants has been termed mutation breeding. Whenever the seeds are treated with chemical mutagenic agents as Diethyl sulphate its immediate effect if any is manifested firstly in the germination process, morphology, secondly at the physiological level of the seedlings. Again physiological manifestation may or may not be of permanent nature and depends on the type of mutation that has been brought by the agents in the semi autonomous organells such as chloroplasts and mitochondria. The type of mutation brought by the mechanism of action, and the type of modification brought differs in different chemical mutagens. Therefore attempt is made to study the nature of morphological and physiological changes brought by Diethyl sulphate a chemical mutagen in Crotalaria juncea L. commonly known as sunnhemp.

B. Material and methods :

1. Morphological parameters

(a) Germination and survival

The seeds of Crotalaria juncea L. (sunnhemp) were collected from the Agriculture College, Kolhapur, for the study of the effect of Diethyl sulphate on different morphological and physiological parameters in M_1 and M_2 generation.

For the treatment the seeds were initially flooded with water to remove growth inhibitors and soaked for 3 h to completely hydrate the system. The water was changed periodically to ensure complete elimination of inhibitors if existed in traces. Weight amount of DES was dissolved in distilled water to prepare the solution of 0.1%, 0.2%, 0.25% and 0.3% concentractions.

The presoaked seeds were divided into four lots of 200 seeds each, including control and were soaked in respective mutagen solution of different concentractions for 3 h. at 20°C. While doing so care was taken to permit, on an average 1 ml. of mutagen solution perseed. In order to circumvent the brief half-life of DES due to rapid hydrolysis, (Heiner et al., 1962 and Konzak et al,. 1965) freshly prepared solution of the mutagen was added after every half an hour of treatment. Comparable control was maintained by soaking the presoaked seeds in distilled water. After completion of treatment the seeds were rinsed 4-5 times with distilled water and kept in it for half an hour. This would have enabled leaching out of any unreacted mutagen which would otherwise increase the physiological damage. 100 seeds of each of the treatment were sown in different pots for further investigation of seedlings and 100 seeds of each treatment were kept for germination in germination papers. Control was also sown and kept for germination. The rate of germination was scored. LD_{50} was determined based on 96 h of germination; and survival percentage was calculated after 15 days.

(b) Morphological peculiarities :

Morphological variation if any such as nature of cotyledon, leaf shape and it's margin, growth habit etc. were scored time to time as the emergence took place.

C) Growth parameter :

The seeds of Crotalaria juncea treated with various concentrations of DES were sown in earthen pots ~~were~~ filled with riverbed soil mixed with farmyard manure. In each pot about 25 seeds were sown depending upon the respective concentration of DES. The growth parameter such as height, leaf area, number of leaves, stomatal frequency, pollen fertility, were recorded timely. The morphological peculiarities were noted right from the cotyledonary stage till the emergence of the flowers.

(d) Leaf area :

Leaf area was determined by multiplying the maximum length, with maximum breadth. The total photosynthetic area was determined by multiplying the average leaf area with total number of leaves.

(e) Stomatal frequency :

The method followed for this study is of Stoddard (1965). Total number of stomata was estimated in the precalibrated microscope on films obtained by nail polish application. Nail polish was applied to the middle portion of the leaf on the lower as well as upper surfaces. After drying the nail polish films were removed. To avoid errors, maximum care was taken to select green and mature but identical leaves on the plants. Every time two impressions for each surface were taken.

(f) Pollen fertility :

Pollen fertility was estimated on the basis of acetocarmine stainability technique. The pollen grains stained by 1% acetocarmine were considered to be fertile and unstained ones as sterile. 1000 pollen grains were stained and scored from each treatment and based on that fertility percentage was determined.

2. Physiological Parameters :

(a) Moisture percentage :

The moisture percentage was determined on the basis of fresh and dry weights. 5 g of fresh plant material leaf/ stem / root was oven dried at $80^{\circ}C$, till the constant dry weight ~~is~~ obtained. The loss in weight is the moisture present and is calculated for 100 g of plant material.

(b) Total Chlorophylls :

Total chlorophylls were estimated by the method of Arnon (1949). Chlorophylls were extracted in 80% acetone from 500 mg of the plant material. The extract was filtered through Buchner's funnel using Whatman No. 1 filter paper. Residue was washed repeatdly with 80% acetone collecting the washings in the same filtrate. The volume of the filtrate was made to 100 ml. with 80% acetone. The absorbance was read at 663 and 645 nm for chlorophylls "a" and "b" respectively.

Chlorophylls (mg/100 g fresh tissue) were calculated using the following formula.

Chlorophyll "a" = 12.7 X A 663 - 2.69 X 645 = X

Chlorophyll "b" = 22.9 X A 645 - 4.68 X A 663 = Y

$$\text{Chlorophyll "a"/"b" (mg/100 g fresh tissue)} = \frac{X/Y \times \text{Vol. of extract} \times 100}{1000 \times \text{wt. of the material (g)}}$$

(c) Nitrogen :

Nitrogen was estimated colorimetrically by the method of Hawk et al., (1948). 0.5 g over dried material was digested in a Kjeldehl flask with sulphuric acid (1:1 dilution) and a pinch of microsalt (mixture of anhydrous copper sulphate and potassium sulphate in the proportion of 1:40) till a colourless liquid is obtained at the bottom of the flask. It was then cooled to room temperature and transferred ~~quantitatively~~ to the volumetric flask and volume was adjusted to 100 ml with distilled water. Then it was filtered next day through dry filter paper. The filterate was used for the estimation of nitrogen. 2 ml of this filtrate was taken in Nesslor's tube (35 and 50 ml marked). In other tubes different concentrations of standard ammonium sulphate (0.05 mg nitrogen/ml) were taken. One tube was kept as a blank without ammonium sulphate. To these tubes was added a drop of 8% potassium bisulphate and 1 ml H_2SO_4 (1:1, whereever needed). The volume of all the three tubes was adjusted to 35 ml with water. 15 ml of Nesselor's reagent was then added to each tube. Nesslor's reagent is a mixture of reagent A (7 g KI and 10 g HgI_2 dissolved in 40 ml distilled water) and B (10 g NaOH dissolved in 50 ml of water) in the proportion of 4:5. The colour intensity of the orange brown product ($NH_4Hg_2I_3$), produced by the reaction between NH_3 liberated from the sample and the reagent was measured at 520 nm spectrophotometrically. (Electronic Corporation of India). The amount of nitrogen in the sample was calculated from the standard curve of ammonium sulphate.

C. Results :

1. Morphological parameters :

(a) Germination and survival :

The effect of DES on the rate of germination, overall germination percentage after 72 hours and survival percentage after 15 days is depicted in Table 1 and Fig. 1 and 2.

It is clear from the result that the DES effect is registered in two ways, firstly by damping down the germination rate and percentage and second by delayed germination. This effect went on increasing as the concentration of DES increased.

It is evident from Table 1 and Fig. 1 that, the DES has drastic effect in M_1 generation as survival percentage is concerned. As the concentration increased, the survival percentage decreased abruptly compared to the germination percentage. In M_2 generation the survival percentage decreased as the concentration increased (72%, 68%, and 66% in control, 0.1% DES and 0.2% DES treated respectively).

(b) Morphological peculiarities :

The seedlings obtained after seed treatment

Table 1 : EFFECT OF DES ON SEED GERMINATION AND SURVIVAL OF *Crotalaria juncea* (M_1 generation).

Treatment	Hours after germination				Percentage of germination.	Percentage of survival after 15 days.
	12	24	48	72		
Control	30	90	100	100	100	94
0.1% DES	-	76	84	88	88	69
0.2% DES	-	65	74	76	76	20
0.25% DES	-	38	54	58	58	0
0.3% DES	-	10	12	20	20	0

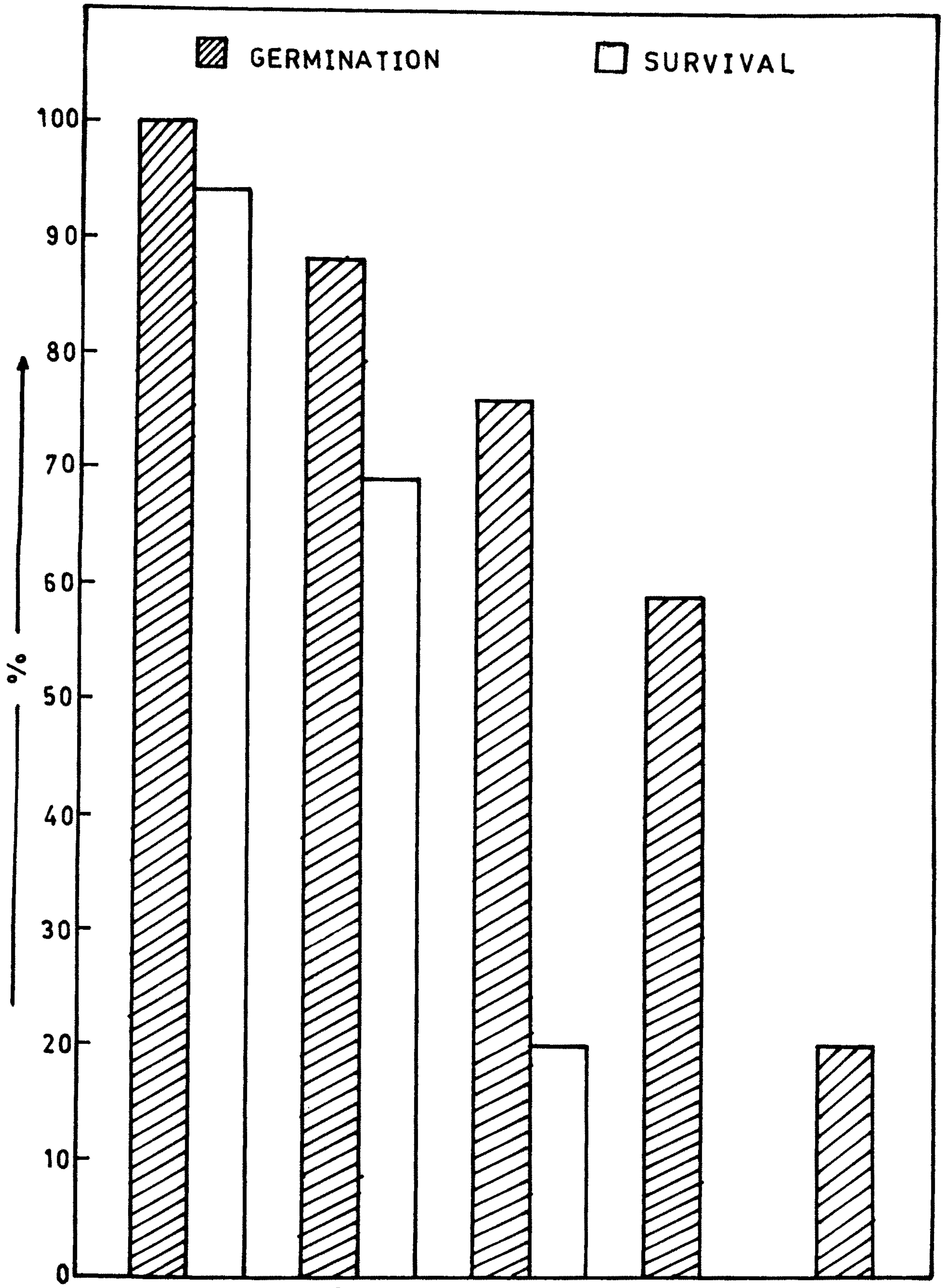


FIG. 1 : EFFECT OF DES ON GERMINATION AND SURVIVAL PERCENTAGE OF *Crotalaria Juncea* L. (M_1 GEN.)

with various concentrations of DES had their colytedons with malformed shapes and chiamers. In 0.1% DES treated plants, some of them have with twisted leaf, some with very small and some of them with apical portion crumpled. Where as in control the abnormality recorded in cotyledons (tricotyledons Fig. 3) is an exceptional one, otherwise all the plants are normal in nature.

In 0.2% DES treated plants number of morphological pecularities have been noted in the present investigation. Tricotyledonary seedling, with cotyledon slightly abnormal in shape (Fig. 4). Variation in pigmentation in stem, leaves and distorted nature of leaves is a common feature. Leaves are generally with crumpled appearance (Fig. 5) chiameric (Figs. 6,7,8 and 9), with dicotomy (Fig. 6,9) with wavy margine (Fig. 6,9). Sometimes leaves of variants have shown trilobed tendency with very irregular margin. Weaker stem is one of the significant characteristic of the 0.2% DES treated plants in general.

In M_2 generation few abnormalities have been recorded in 0.1% and 0.2% DES treated plants. Tricotyledonary seedlings, crumpled leaves, abnormal leaf shape, twisted lamina, incision in leaf margins, dicotamous nature of leaf, chimeric plants with weaker

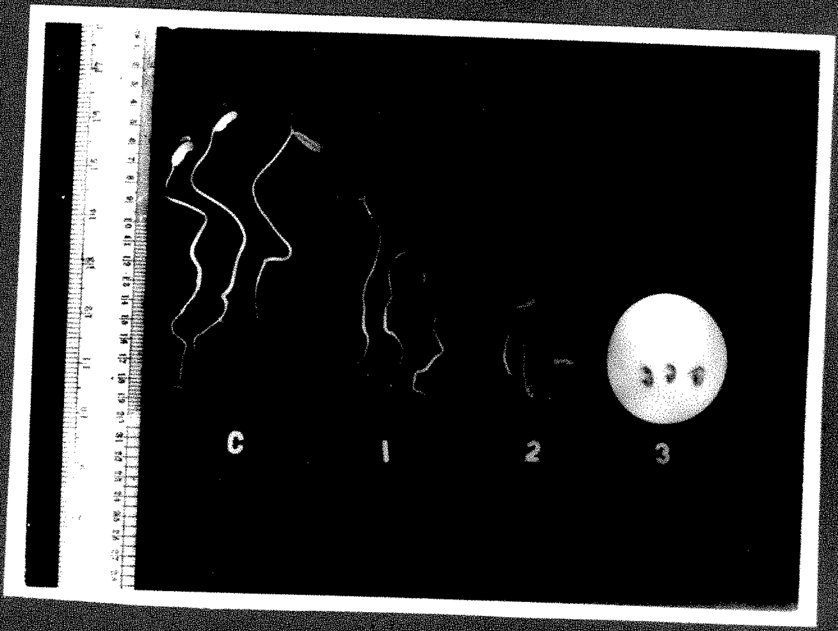


Fig. 2.

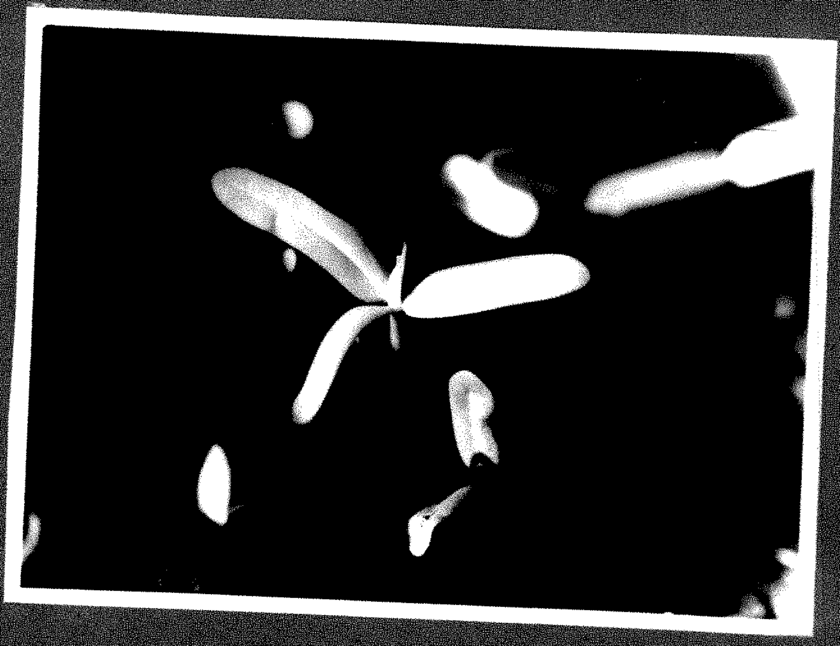


Fig. 3.

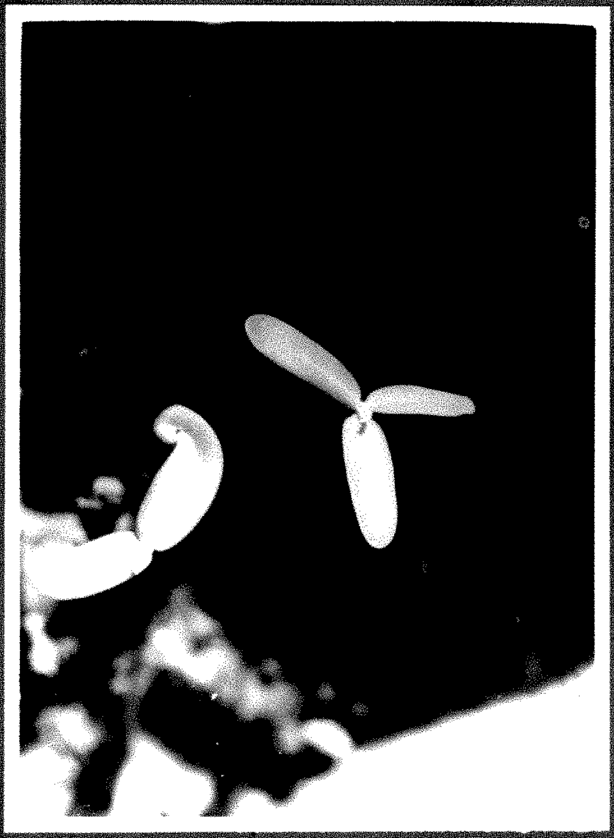


Fig- 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

stems are some notable morphological characteristics. Where as in variants which are showing maximum vigour are devoid of above abnormality features. On the other hand they are stout, healthy showing high vigour over the control.

(c) Growth parameters :

The growth in cm as recorded every week, after the seedling emergence of DES treated plants of M_1 and M_2 generation are represented in Table 2, Fig. 10 and Table 3 and Fig. 11 respectively. Plants obtained after DES treatment and showing morphological vigour are studied in details. These variants are analysed for morphological and physiological parameters. The results depicted in table for 0.1, 0.2% DES treatment are that of variants and not the mean of the whole population.

In control plants and in seedlings of M_1 generation after DES treatment, growth pattern in 0.1% DES treated is more or less same upto fourth week. Whereas in the ~~seedlings of~~ 0.2% DES and 0.3% DES treatment the growth of plants is in the lag phase, and is much low as compared to control plants upto 4th and 2nd week respectively. After 4th week the rate of growth declined slowely in 0.1% DES treated plants as compared to control plants (Fig. 10). In the seedlings of 0.2% DES treatment, after fourth week, there is significant deviation in growth pattern than the control, (Fig. 10). The 0.3% DES treatment seedlings remained in lagphase and perished after 2nd week, indicating that the dose is lethal. The overall growth of 68 cm height is attained by control plants on the seventh week, whereas 64 cm height is attained by 0.1% DES treated ones. The maximum height of 89 cm is attained by 0.2% DES treated plants. The Fig. 10, 12 clearly indicates that there is reduction of seedling height at lower doses and enhancement at higher doses (viz. 0.2% DES) in which the survival is affected significantly.

The growth pattern in the seedlings raised from DES treated M_2 generation seeds is different from those of M_1 (Table 3 and Fig.11). In M_2 generation there is gradual increase in growth as the concentration of DES increases (Fig. 11,13). The overall growth of 70 cm height is attained by control plants on the seventh week, whereas 74 cm and 85.5 cm height is attained by 0.1% DES and 0.2% DES treated ones respectively. In general, the delayed senescence of cotyledons and initiation of flowering (Table 4+5) has been noticed in 0.1 and 0.2% DES treated plants over control in M_1 and M_2 generation of C. juncea.

Table 2 : EFFECT OF DES ON GROWTH (HEIGHT) OF *Crotalaria juncea* (M_1 generation).

Treatment	Height (cm) at week intervals.						
	1	2	3	4	5	6	7
Control	6.1	14.0	29.0	46.0	57.5	62.0	68.0
0.1% DES	5.0	12.5	26.5	43.5	52.5	57.5	64.0
0.2% DES	2.5	7.0	17.0	40.0	65.0	77.5	89.0
0.3% DES	1.5	1.5	-	-	-	-	-

Table 3 : EFFECT OF DES ON GROWTH (HIGHT) OF *Crotalaria juncea* (M_2 generation).

Treatment	Hight (cm) at week intervals.						
	1	2	3	4	5	6	7
Control	6.5	12.0	20.0	32.0	50.0	62.0	70.0
0.1% DES	7.0	16.0	24.0	35.0	51.5	65.0	74.0
0.2% DES	9.0	18.0	29.0	41.0	63.0	75.5	85.5

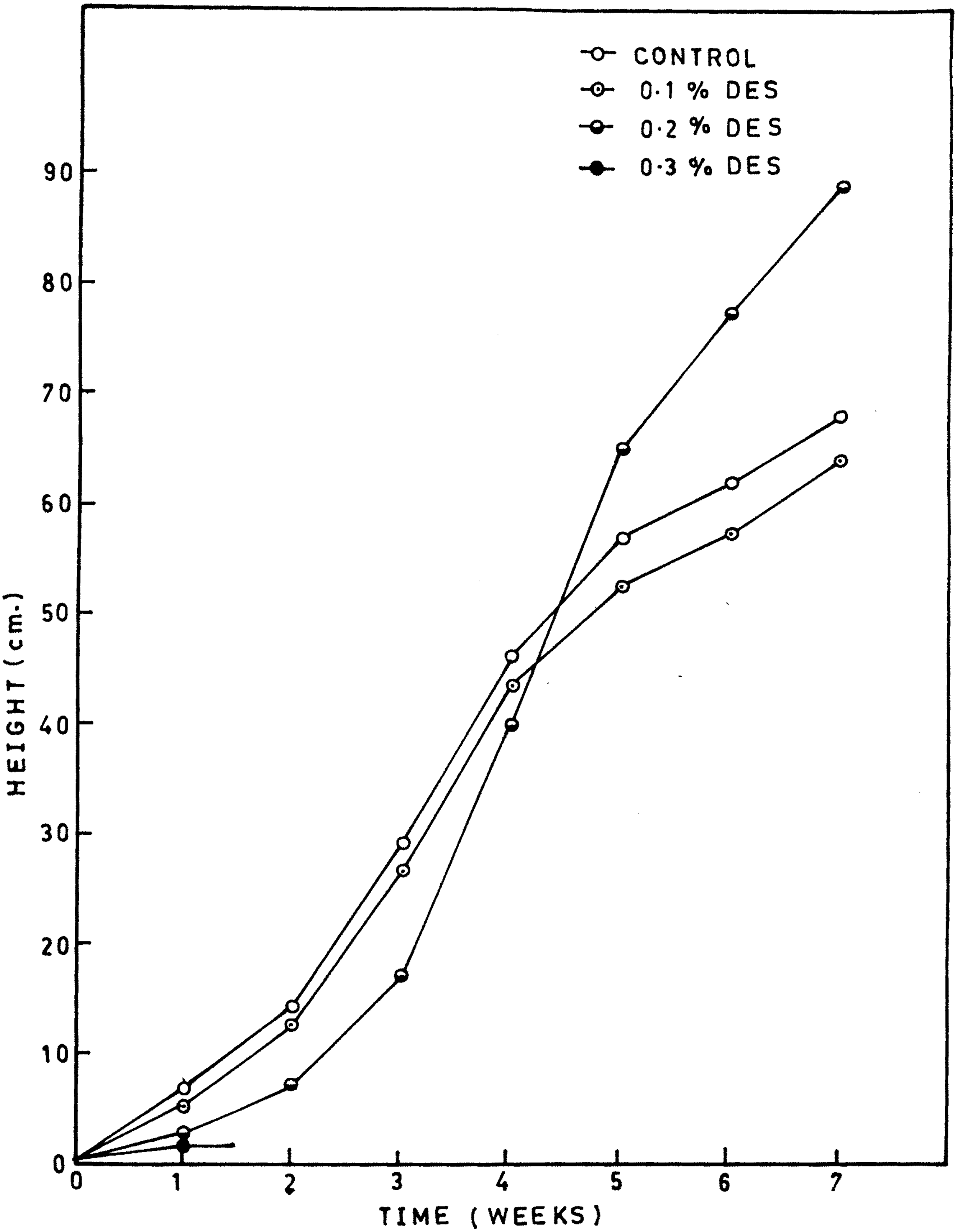


FIG. 10 : EFFECT OF DES ON GROWTH RATE OF <u>Crotalaria</u> <u>Juncéa</u> L. (M_1 GENERATION.)

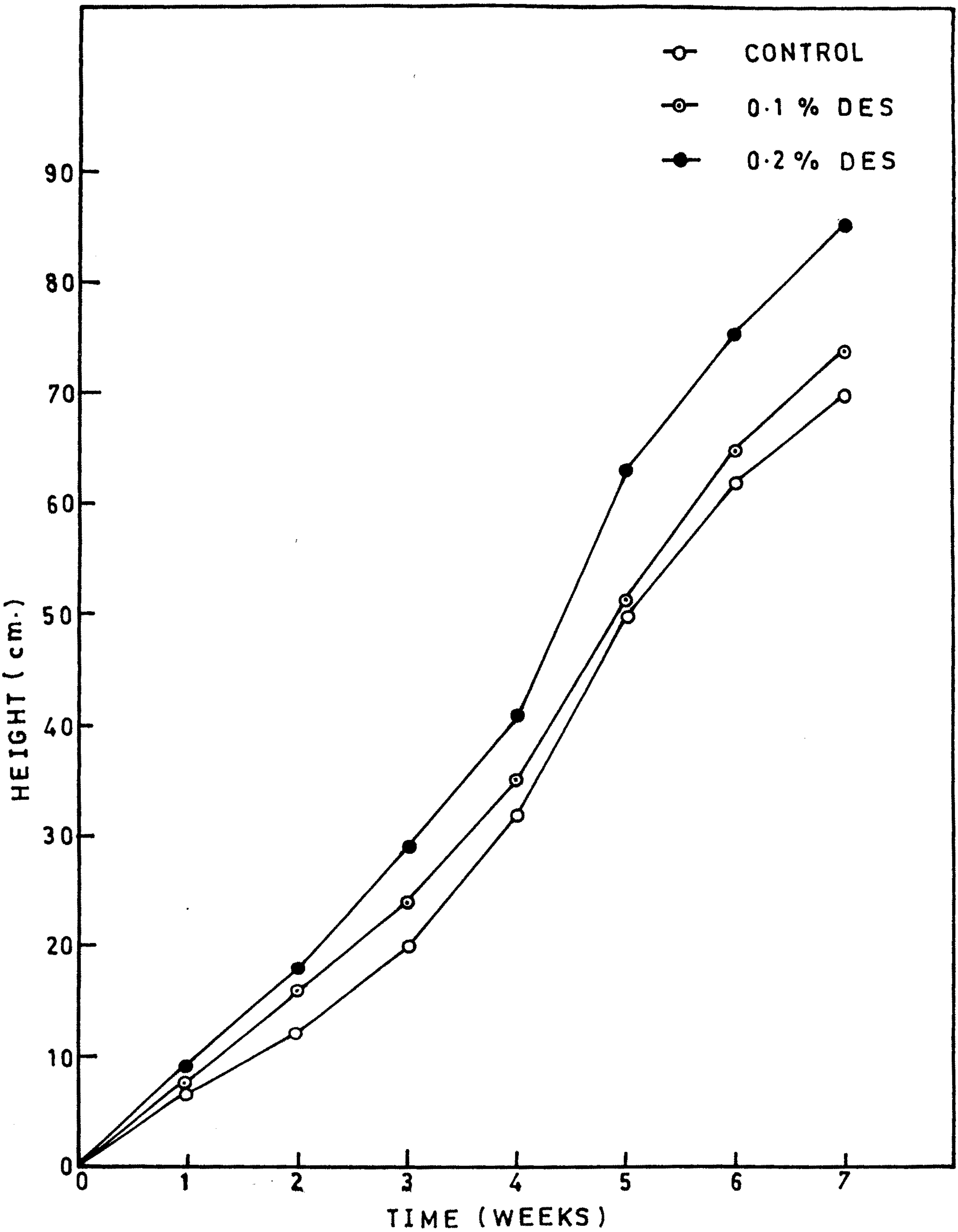


FIG. 11 : EFFECT OF DES ON GROWTH RATE OF

Crotalaria Juncea L. (M_2 GENERATION).

Fig. 12.

Fig. 13.

(d) Leaf area :

The average leaf area, average number of leaves per plant and the total leaf area per plant of DES treated plants in M_1 and M_2 generation is given in the Table 4 and Table 5 respectively. It is clear from the table that in M_1 generation the leaf area and also the total number of leaves per plant decreased in 0.1% DES treated plants and again increased in 0.2% DES treated plants with respect to control. The average leaf area is 12.9 cm^2 in control, 12.22 cm^2 in 0.1% DES treated and 15.42 cm^2 in 0.2% DES treated, and the total leaf area is 288.0 cm^2, 219.96 cm^2 and 462.6 cm^2 in control, 0.1% DES and 0.2% DES treated plants respectively. So in 0.2% DES treated plants the increase in total photosynthetic leaf area is almost double than the control.

In M_2 generation also the same situation is observed, the total leaf area being 778.24 cm^2, 676.8 cm^2 and 1304.24 cm^2 in control, 0.1% DES and 0.2% DES treated plants respectively.

(e) Stomatal frequency :

The stomatal density of leaves treated with increasing concentrations of DES in this species of Crotalaria in M_1 and M_2 generation is given in the

Table 4 and Table 5 respectively. It is interesting to note that with increasing concentration of DES there is gradual increase in the stomatal density per unit area of leaf. The increase in number is not so significant in M_1 generation, but is slightly significant in the M_2 generation. In both M_1 and M_2 generation there is gradual increase in the stomatal frequency on both the upper and the lower epidermis. In 0.2% DES treated plants along with increase in the total photosynthetic area there is also increase in the stomatal density which excelarated the leaf function there by bringing about the vigour.

(f) Pollen fertility :

The pollen fertility is recorded in C. juncea in M_1 and M_2 generations treated with DES and is presented in the Table 4 and Table 5 respectively. From the table it is clear that pollen fertility decreases with increase in concentration of DES. DES treatment has no severe effect on the pollen fertility, because in 0.2% DES treated plants the pollen fertility is as high as 90.2%. Same is the case in M_2 generation also where the fertility percentage is 92% in 0.2% DES treated ones.

Table 4 : EFFECT OF DES ON DIFFERENT MORPHOLOGICAL PARAMETERS OF <u>Crotalaria juncea</u> (M_1 generation).

Parameters		Control	Treatment 0.1% DES	Treatment 0.2% DES
1. Leaf area :	i) Average leaf area cm^2	12.9	12.22	15.42
	ii) Number of leaves per plant.	20.0	18.0	30.0
	iii) Total leaf area per plant cm^2.	258.0	219.96	462.60
2. Stomatal frequency cm^2.	i) Upper epidermis	71	71	83
	ii) Lower epidermis.	131	143	179
3. Initiation of flower after sowing (number of days)		21	21	26
4. Percentage of pollen fertility.		93	92	90.2

Table 5 : EFFECT OF DES ON DIFFERENT MORPHOLOGICAL PARAMETERS OF <u>Crotalaria juncea</u> (M_2 generation).

Parameters		Treatment	
	Control	0.1% DES	0.2% DES
1. Leaf area : i) Average leaf area cm^2.	20.48	18.8	23.29
ii) Number of leaves per plant.	38.	36.	56.
iii) Total leaf area per plant cm^2.	778.24	676.8	1304.24
2. Stomatal frequency cm^2 i) Upper epidermis	30.0	90.0	95.0
ii) Lower epidermis.	150.0	160.0	180.0
3. Initiation of flower after sowing (number of days).	32.	36.	39.
4. Percentage of pollen fertility	95.	94.	92.

(2) Physiological parameters :

(a) Moisture percentage :

The effect of DES on moisture percentage in *C. juncea* in M_1 and M_2 generation is depected in Table 6 and Table 7 respectively. It is very clear from the table that the DES has no significant effect in M_1 generation, as far as the moisture percentage is concerned. In case of root the percentage decreased (72.36) in 0.1% DES treated, and again increased (74.78) in 0.2% DES treated ones over control (74.24). In stem the percentage went on decreasing as the concentration increased. Where as in case of leaf, in 0.1% DES treated there is increase (77.25) and again in 0.2% DES treated there is decrease (71.81) in the moisture percentage. If the total moisture percentage of the entire plant is considered, there is gradual decrease in the moisture percentage as the concentration increased i.e. 75.53, 72.99 and 71.58 in control, 0.1% DES and 0.2% DES treated respectively. However the trend of decrease in moisture percentage is steady and marginal one.

In M_2 generation there is increase in the moisture percentage as the concentration increased. This increase rate is also marginal i.e. 77.67, 78.91 and 79.45 in control, 0.1% DES and 0.2% DES treated respectively.

Table : 6 EFFECT OF DES ON DIFFERENT PHYSIOLOGICAL PARAMETERS OF *Crotalaria juncea* L. (M_1 generation).

Parameters		Control	Treatment. 0.1% DES	Treatment. 0.2% DES
1. Moisture				
a) Moisture percentage	i) Root	74.24	72.36	74.78
	ii) Stem	76.95	69.36	68.10
	iii) Leaf	75.41	77.25	71.81
b) Moisture percentage of the entire plant.		75.53	72.99	71.56
2. Chlorophyll *				
a) Chlorophyll "a"		122.4	123.6	139.0
b) Chlorophyll "b"		77.8	69.4	101.8
c) Chlorophyll "a"/Chlorophyll "b"		1.55	1.78	1,37
d) Total chlorophylls		200.2	193.0	240.8
3. Nitrogen +				
a) Nitrogen content of	i) Root	0.55	0.531	0.45
	ii) Stem	0.75	0.98	0.78
	iii) Leaf	1.925	1.625	2.0
b) Nitrogen content of the entire plant.		1.07	1.04	1.08

* Values expressed as mg 100^{-1} fresh tissue.

\+ Values expressed as g 100^{-1} dry tissue.

(b) Total chlorophylls :

The effect of increasing concentrations of DES on the total chlorophyll of *C. juncea* in M_1 and M_2 generation is given in the Table 6 and Table 7 respectively. It can be seen from table that the total chlorophylls decreased slightly in 0.1% DES treated and again increased considerabl in 0.2% DES treated in M_1 generation (Table 6).

In M_2 there is an increase in 0.1% DES treated and again fall in 0.2% DES treated plants. So the total chlorophyll being 200.47, 215.45 and 200.88 mg. per 100 g of fresh tissue in control, 0.1% DES and 0.2% DES treated plants respectively (Table 7).

(c) Nitrogen :

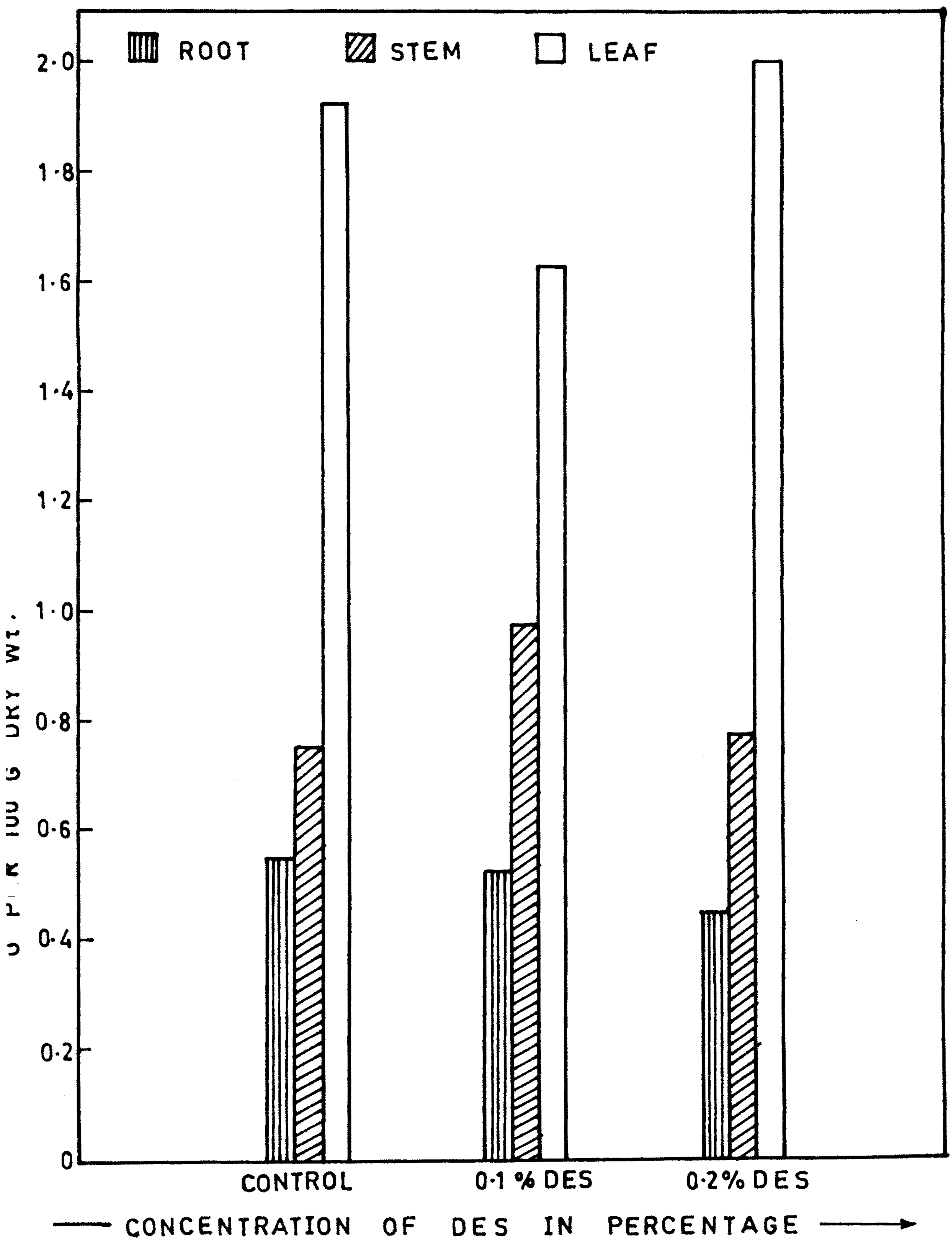
The nitrogen level in *C. juncea* treated with DES (M_1 and M_2 generation) is given in the Table 6 Fig. 14 and Table 7 Fig. 15 respectively. The table indicates that the DES has no significant effect on the nitrogen level of treated plants. In case of plants of M_1 generation in root the nitrogen level decreased as the concentration of DES increased, but it is very marginal i.e. 0.55, 0.525 and 0.45 g per 100 g dry tissue in control 0.1% DES and 0.2% DES treated respectively. Where as in stem, 0.1% DES treated plants showed highest nitrogen content than 0.2% DES treated and control plants. The nitrogen content of stem of 0.2% DES treated plants is slightly

Table 7 : EFFECT OF DES ON DIFFERENT PHYSIOLOGICAL PARAMETERS OF *Crotalaria juncea* (M_2 generation).

Parameters		Treatment	
	Control	0.1% DES	0.2% DES
1. Moisture			
a) Moisture percentage of i) Root	77.94	80.07	81.62
ii) Stem	78.03	80.11	79.29
iii) Leaf	77.05	76.57	77.45
b) Moisture percentage of the entire plant.	77.67	78.91	79.45
2. Chlorophyll.*			
a) Chlorophyll "a"	139.61	151.73	138.88
b) Chlorophyll "b"	60.86	63.72	62.0
c) Chl "a"/Chl "b"	2.29	2.38	2.24
d) Total chlorophylls	200.47	215.45	200.88
3. Nitrogen+			
a) Nitrogen content of i) Root	0.497	0.40	0.50
ii) Stem	0.95	0.93	0.98
iii) Leaf	2.15	2.10	2.25
b) Nitrogen content of the entire plant.	1.199	1.14	1.24

* Values expressed as mg 100^{-1} fresh tissue.

\+ Values expressed as g 100^{-1} g dry weight.



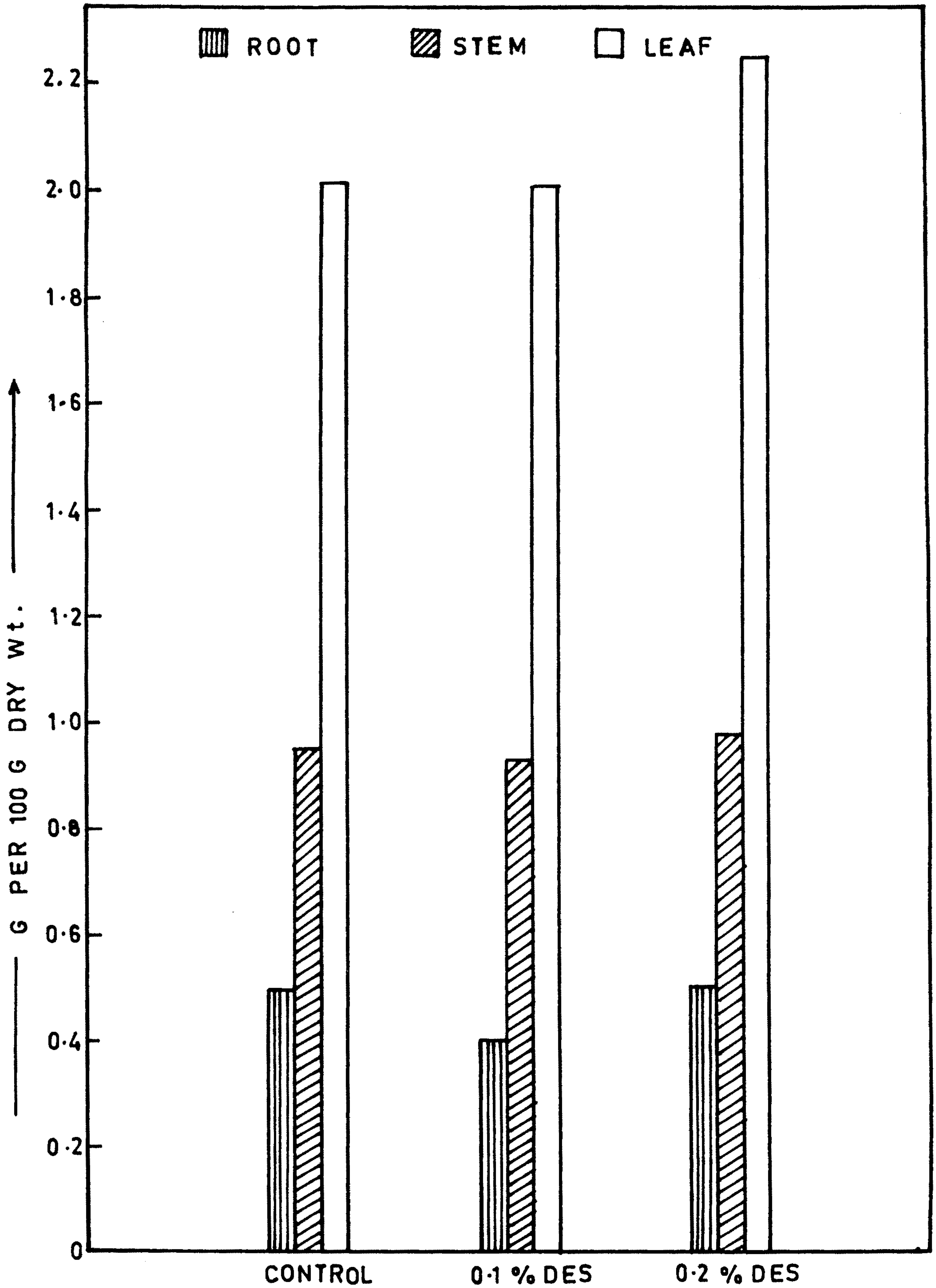
G. 14: EFFECT OF DES ON NITROGEN CONTENT OF *Crotalaria Juncea* L. (M_1 GENERATION).

more than control. In leaf the level decreased in 0.1% DES treated and again increased in 0.2% DES treated, and is more than the control. If the nitrogen content of the entire plant is considered there is no significant effect of DES at all, because the nitrogen content being, 1.075, 1.041 and 1.078 in control, 0.1% DES and).2% DES treated plants respectively in M_1 generation.

In M_2 there is decrease in nitrogen level in 0.1% DES treated and again increase in 0.2% DES treated plants in all parts i.e. root, stem and leaves over control. If the nitrogen content of the entire plant is considered there is decrease in 0.1% DES treated and increase in 0.2% DES treated plants. However, there is no significant increase or decrease in nitrogen content of C. juncea treated with various concentrations of DES in M_2 generation.

D. Discussion :

It is evident from the Table 1 and Fig. 1 that in Crotalaria juncea L. higher concentrations of DES have reduced germination percentage significantly, where as lower concentrations have little effect. It is also evident from the Table 1 and Figs. 1 ,2 that 0.3% DES treatment has an adverse effect on germination. At 0.1% DES treatment the germination is not significantly impaired. Provided there is a homogeneous treatment of



IG. 15 : EFFECT OF DES ON NITROGEN CONTENT OF *Crotalaria Juncea* L. (M_2 GENERATION).

seeds, observation on the reduction of germination may indicate the mutagenic activity. At 0.25% DES the germination is reduced almost to 50% of that of control, this reflects on the sublethal effect of the concentration which may be considered as LD 50 for this species of Crotalaria (Table 1). However to get the variants at further growth stages it is desirable to treat the seeds with 0.2% DES.

Survival percentage in *C. juncea* has also been decreased of 0.2% DES treatment, where as in 0.25% and 0.3% DES treatment the effect is lethal. Strong mutagen concentrations by inhibiting root growth produce a delayed death in the field unlike compounds that act by extragenic toxicity (Ehrenberg, 1954; Ehrenberg and Gichner 1967 and Osterman Golkar *et al.*, (1970)

Similar results have been obtained by number of workers in various systems. Pipie (1972) has observed in pea that higher concentrations of DES has lethal effect on germination and survival. Kloble *et al.*, (1973) has also observed decrease in germination and survival percentage with increase in dose concentrations of DES in soyabean. Filippetti *et al.*, (1977) reported higher frequency of mutations per M_1 progeny induced by DES than irradiation treatment in *Pisum sativum*. They have also reported the significant decrease in germination and survival percentage with increase in dose concentrations. Guimaraes (1978) reported the effect of DES on Rice seeds, he has shown that DES has an adverse effect on the survival at higher concentrations, however DES shows lower physiological damages at lower doses in M_1 plants.

Parameshwar and Shankara (1984) studied the effect of diethyl sulphate on cultivars of Gossypium hirsutum and observed decrease in germination and survival with increase in dose in all 4 varieties under investigation. They reported that the percentage of germination and survival is dependent upon genotype. Bairathi and Nathawat (1979) studied the effect of DMS on Crotalaria juncea and found that 0.1 -0.2% DMS stimulated root growth, increased root diameter and lateral root formation by increasing mitotic activity in endodermis and pericycle in the subapical region. Further they reported that there was early differentation and maturation of vascular elements close to the tip and it caused the deformities and indistinctness in the apical zonation and metacutization of apical tips making the roots temporarily dormant where as they observed that higher concentrations are toxic and caused permanent inhibition of root growth. Strong inhibition of mitosis and aberrant metaphase in root tips may be the cause of less survival at higher concentrations as observed in barley by Gichner et al., (1977) after DES treatment. These findings are in confirmity with the present investigation.

It is also observed in the present investigation that the percentage of survival is almost equal in 0.1 and 0.2% DES treated seeds of C. juncea in M_2 generation with that of control, thus indicating that variants obtained in the present investigation are desirable, may be due to less chromosomal aberrations.

Thus it is evident from the foregoing discussion that chemical mutagens have an adverse effect on germination and survival percentage of plants at higher level of concentrations. The mechanism of its interaction with genetic material is well reviewed by Hollaender (1976).

It seems from the present investigation that the immediate effect of higher concentrations of DES has drastic effect as morphological characteristics of Crotalaria juncea are concerned (Fig. 4,5,9).

Joshnu and Rao (1972) studied genetics of some leaf mutations in whitejute. They observed changed leaf pattern from oblong to dicotamous, tricotamous, with wavy margin and attributed minor interest in the study of evolution. Singh (1974) was of the opnion that, leaf character has been considered as the most drastically affected in mutational research. However Rahman (1972), Beletskil *et al.*, (1981), Lee and Halloran (1982) have observed morphological abnormalities in various plant systems after chemical mutagen treatment. Vardanyan (1976) while studying chlorophyll mutations in bean under the effect of chemical mutagen reported correlation of frequency of mutations directly with the mutagenic concentrations, but there was no co-relation between mutation frequency and morphological characteristics of the mutants. In the present investigation higher frequency of mutants has been obtained after 0.2% DES treatment, however the genotype should be considered as one of the important factor in mutation breeding. Parameshwar and Shankara (1984) observed morphological variants after DES treatment affecting vegetative and floral parts, the frequency was dependent on the genotype.

Dzhakeli (1983) used various chemical mutagens to induce mutations in tea plants. Morphological changes are of qualitative and quantitative nature, Morphological changes observed in M_1 were not inherited in M_2 generation significantly. Similar results have been obtained in the present investigation when seeds of *C. juncea* were treated with various concentrations of DES and screened for morphological peculiarities.

The nature of induced sectors on leaves of various plants e. g. soybean, tomato, maize, pea and tobacco has been studied and the origin of these sectors is interpreted by various workers (Blixt, 1972; Vig, 1973; Vig, 1973, 1974; Vig, 1975; Dulieu et al., 1975; Deshayes and Dulieu, 1974; Vig et al., (un pub), Conger/unpub). The presence of different mutated spots on soybean leaves allows one to differentiate among genetic events which originate from somatic crossing over, from possible point mutations, from non disjunction or losses of chromosome segments. The mutated spot on tobacco leaves can be attributed to somatic crossing over, possible point mutation, minute chromosome deletions and gene conversion, while mutated spots on maize leaves may be due to deletion or mutations. Peas provided a useful system for study of possible point mutation and chromosome losses. In all of the test plants, the chlorophyll deficient mutants are controlled by many & most of which are not mapped. Heterozygotes of certain chlorophyll mutants which are controlled by known loci as in barley can be distinguished from the homozygous mutants or normal genotype. These heterozygotes lead themselves to the specific locus technique of mutation detection. A greater understanding of the action of chemical mutagen and the genetic changes they induce in *Crotalaria juncea* will be possible after cytological and genetical studies. The scoring of chlorophyll deficient mutants is usually detected in M_2 seedlings and not in M_1. In the present investigation morphological changes are not of true chlorophyll, deficient mutants because the observations are recorded only in M_1 and not observed in M_2 generation, indicating the immediate effect of the mutagen on C. juncea.

In general the effect of DES on growth (height) of Crotalaria juncea in M_1 generation was inhibiting, however the results presented that of variants obtained after 0.1 and 0.2% DES treatment. The growth pattern of 0.1% DES treated plants (variants) was almost similar to that of control, where as significant increase in height of variants after 0.2% DES treatment is reported. In M_2 generation similar pattern of the growth has been observed indicating that variants are true breeding. However studies of the further generations is essential to confirm such findings.

In accordance with Zhatov (1979) lower doses of DES treatment is benificial to plants which is confirmed in the present investigation, where 0.25% and 0.3% DES treatment are found lethal to C. juncea. Height is the specific character of plants where fibre is obtained from stem, thus it is beneficial in C. juncea to procure a plant which shows heighest growth in respect of height. Thus 0.2% DES treatment in the present investigation has shown significant increase in height of the plants over control.

Yamashita et al., (1972) are of the opinion that chemical and physical mutagens have specific action to induce mutation in plants. Mutations induced by physical mutagens are not induced by chemical mutagens. But in the present investigation it has been observed that mutations induced in C. juncea after chemical and physical mutagens are of same nature as height parameter is concerned.

In M_1 generation the effect of 0.2% DES has shown the lag phase of growth in the early stages, while in the later stages the growth accelarated over the control (Table 2., Fig.10). Where as it is evident from the Table 3 and Figs. 11, 13 that in M_2 generation the variants obtained after 0.2% DES treatment are vigarous right from the seedling stage upto flowering.

Goua (1967), Constantin et al., (1976), Sander and Muehlbauer (1977), Mohammed and Josef (1979) and Parlina (1980), while studying the effect of various chemical mutagens have shown that growth was retarded at the heighest chemical mutagen concentrations and at longer duration of treatment. Similar results are obtained in the present investigation, suggesting higher concentrations of DES are lethal to C. juncea. However there are certain desirable mutants referred here as variants are obtained at sublethal doses of DES to C. juncea. Delayed senescence of cotyledonary leaves in 0.1 and 0.2 DES treated plants is a remarkable variation over the control. Abu-Shakra et al., (1978) documented nitrogen fixation and delayed leaf senescence in soybean, the activity of chlorophyll and RuBP case in leaves of soybean have been maintained, and acetylene reduction activity in root nodules through seed maturation. The incorporation of delayed leaf senescence into an agronomically desirable genetic background may help to increase seed yield and symbiotic nitrogen fixation during seed development. In C. juncea this feature has significant importance as it is used as a green manure. With symbiotic nitrogen fixation it survives, grows and dominates other vegetation as refractory sites subject to erosion, low fertility and similar adverse soil conditions. Crotalaria juncea can help spear head the fight to stop erosion now prevalent in the tropics and can help to rebuild the soils already damaged and degraded.

It is evident from the Tables 4 and 5 that average leaf area (cm^2), number of leaves per plant and total leaf area per plant (cm^2) is significantly higher in plant/plants obtained from 0.2% DES treatment to C. juncea (M_1 and M_2 generation) over control. Singh and Drolsom (1973) observed considerable variation in leaf area index in wheat mutants. Increase in biomass is a basic need when the plant is used as a green manure. According to David et al., (1977) under steady state conditions in vegetative growth phase (Gossypium hirsutum and Glycine max) the nitrogen absorption function of root is directly related to the photosynthate supplying function of leaf. There is balanced interdependance between nitrogen uptake and growth of plant parts which is dependent on carbohydrate supplied by the leaves and that nitrogen uptake, dependent only on existing root and soil characteristics. Their results suggested that plant growth models should be based on the balanced interdependance of the nitrogen absorbing and photosynthate supplying functions. It will be also interesting to study the photosynthesis and nitrogen metabolism in this variant to verify this hypothesis.

Stomatal frequency is also in correlation with leaf area of plants obtained after 0.2% DES treatment to C. juncea in M_1 and M_2 generations. Similar results have been obtained by Egmaberaive et al., (1971) in cotton after DES treatment.

Effect of chemical mutagen on pollen fertility/sterility has attracted attention of various workers (Goud et al., 1970; Tarar, 1979; Prasad, 1980; Premsekhar and Appadurai, 1981; Singh et al., 1982). However in the present investigation the pollen fertility is not significantly hindered even at higher dose namely 0.2% DES suggesting gene or point mutations, at less severe level. In general the fertility decreased with increase in DES concentrations in C. juncea which is in confirmity with the results of various workers Ehrenberg, (1973;), Prasad, (1980).

It is evident from the Table 6 that in M_1 generation of C. juncea the average moisture percentage is least affected in all doses of DES as compared with control. Crotalaria juncea yields 18-27 tons green matter per hector under normal agronomic conditions (Tropical Legumes, Resources for the Future, 1979). Moisture is one of the important factors in humus formation. Generally plants are ploughed into the soil for manure purpose when they are 2-2.5 months old, they get rapidly decomposed at this stage, maximum moisture content helps in decomposition. It seems from the present investigation that the variants obtained after DES treatment are good source for green manure like that of control.

In M_2 generation (Table 7) similar trend has been observed in total moisture percentage of C. juncea. It indicates that the plants obtained in M_2 generation are true breeding and having less drastic effect on moisture percentage of variants of C. juncea.

Sunnhemp, C. juncea is also recommended as raw material for fibre and textile industry. Higher percentage of moisture in freshly harvested plants indicates easy separation of bast fibres in retting process, in other words the tissue is soft and easily separable, require less efforts to separate phloem fibres. It is also considered that fodder having high moisture percentage with high nitrogen content is useful for dairy animals. However, it is reported that the use of C. juncea as fodder more than 10% is toxic to the live stocks (Tropical Legumes, Resources for the Future, 1979). Though the sunnhemp is mildly toxic it is essential to screen the variants in near future for its toxicity, alkaloid contents.

It is evident from the present investigation that, in M_1 generation the total chlorophylls increased in 0.2% DES treated plants (Table 6). In M_2 generation the pattern is same only in control, while in 0.1% and 0.2% DES treated plants it is rather different (Table 7). However the chlorophyll a/b ratio indicates that plants obtained after 0.1% DES treatment are efficient in photosynthesis in M_1 and M_2 generations. In M_2 generation overall increase in chlorophyll "a" in all treatment including control is observed in the present investigation. This effect may be of environmental in nature as seeds were sown in rabbi season to advance the generation. It is worth to study the same in Kharif to get a clear idea of increasing chlorophyll component.

Mallikarjunaradhya and Channabyredowda (1981) while studying the effect of chemical mutagen on chlorophyll level in Carthamus tinctorius showed that EMS alone and in combination treatment with γ-irradiation also produce more chlorophyll. However varieties respond differently in producing chlorophyll abnormalities. Trend increase in chlorophyll was marginal in both. The observations from the present investigation agree with the above findings.

Photosynthetic efficiency of the plant can be measured by studying chlorophyll content, namely chlorophyll "a" and "b" and its ratio. It is the opinion of the Holden (1973) that C_3 and C_4 dicots plants have certain ranges of chlorophyll "a" to "b" ratio. However the ratio does not give any correct idea about the nature of the plant, but it implies that C_4 plant shows less chlorophyll "b". Sestak (1966) has shown that C_4 plants are more efficient in photosynthetic activity. The level of chlorophyll "a" is more that of chlorophyll "b" in C_4 plants. About 30% more chlorophyll "a" content was recorded by Holden (Loc. cit.) in C_4 grasses. However the present investigation results shows that the plant is of C_3 nature with high amount of chlorophyll "a" (Tables. 6,7).

Crucial role of nitrogen in plant growth and development need not be emphasised. The nitrogen metabolism of the plant reflects the physiology of whole plant as well as its interactions with its surroundings (Strogonov 1964). If nitrogen metabolism is disturbed it has wide range of consequences. Sunnhemp established a symbiotic system with cow pea type rhizobia that nodulate it and are present in most soils. The roots nodulate freely and, give adequate phosphate, one hector of sunnhemp can add upto 300 kg. of nitrogen to the soil (Rao and Sadasivaiah 1968).

It is evident from Tables 6 and 7 that after DES treatment to C. juncea nitrogen level in M_1 and M_2 generation is not significantly hampered. Analysis of root, stem and leaf portion indicates that the difference is marginal. However it is necessary to analyse the seed in respect of nitrogen content. There are number of reports supporting as well as conflicting on the nitrogen value of fodder plants after chemical treatments. (Singh, 1970; Shukene and Vaichene, 1973; Sichkar, 1974; Riina and Orav, 1980; Singh and Chaturvedi, 1981) and Yankulov et al., 1982).

Riina and Orav (1980) have determined nitrogen content in mutants of barley induced by DAB and showed to have decreased protein content, but after MNU treatment they observed an increase in protein content. Singh and Chaturvedi (1981) showed values of protein after chemical mutagen treatment in Vigna radiata. Yankulov *et al.*, (1982) reported high protein mutants of winter fodder barley induced by chemical mutagen. In the present investigation the content of nitrogen in *C. juncea* in M_1 and M_2 generation is not much altered indicating mutation might have occured at the point level by which the symbiotic association of the bacteria and the host is not disturbed.