MATERIALS AND METHODS

Seeds (scarified by 20-min \(H_2SO_4\) treatment and followed by thorough washing in water) of Cassia sophera var. purpurea were used in this study. The herbicides employed were MCPA (4-chloro-2-methylphenoxyacetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichloro-phenoxyacetic acid), 2,4,5-TP (2-(2,4,5-trichlorophenoxy)-propionic acid), 2,4-DB (4-(2,4-dichlorophenoxy)-butyric acid), MCA (Mono-chloroacetic acid), DCA (Dichloroacetic acid), TCA (Trichloroacetic acid), Dalapon (2,2-dichloropropionic acid) and MH (Maleic hydrazide or 1,2-dihydro-3,6-pyridazinedione). Aqueous solutions of these herbicides were made to give 0.01, 0.1, 1, 10, 100, 250, 500, 750 and 1000 ppm (mg/litre) of the active ingredient. The pH of the solutions were adjusted to 7.0.

Work in the Laboratory

Experiments were carried out by scattering 10 seeds or seedlings of Cassia sophera var. purpurea in sterile Petri-dishes (7-cm dia) upon a single sheet of Whatman no. 1 filter paper moistened with 2 ml of water or test solution. These dishes were kept in a germinator which provided constant temperatures of 20°, 25°, 30° and 35°C. All dishes were incubated both in continuous light and dark conditions. Illumi-
nation was offered from a single cool-white fluorescent lamp (30 cm long) and darkness was obtained by keeping the dishes under double thickness of black cloth. There were four replications per treatment. Unless otherwise stated, the results were expressed as percentage of the corresponding controls.

Treatment of seeds: All observations were made after 5 days from the treatment (Fig. 3). Concentrations of herbicides used were between 0.01 to 500 ppm in Tables 1-6. Germination counts were taken. Lengths of root and hypocotyl were measured (in cm) as well as the fresh and dry weights of seedling determined. Symptoms of toxicity were also noted in treated seedlings.

In order to find out the effect of higher concentrations of all herbicides (Table 7), germination, seedling growth and symptoms of toxicity were observed at 30°C (dark) in concentrations of 100 to 1000 ppm.

Scarified seeds were soaked in various concentrations (100-1000 ppm) of herbicides at room temperature for 24 hr (Table 8). Then the seeds were washed thoroughly and allowed to germinate in water at 30°C (dark). Germination count, root length, hypocotyl length, and symptoms of toxicity were noted.

Treatment of Seedlings: Scarified seeds were germinated under
favourable conditions and seedlings of appreciable size were obtained within 2 days. On the third day, 20 seedlings were selected at random and their radicle and hypocotyl lengths measured. On the same day, experiments (Table 9) were then carried out with 10 such seedlings in the same way as were done for Table 7. As usual, percentage decreases in root and hypocotyl lengths and symptoms of toxicity were noted (Fig.4).

**Work in the Field**

All experiments were carried out in earthen pots and in four replicates.

**Pre-soaking treatment:** Scarified seeds were soaked in various concentrations (0.01-500 ppm) of herbicides at room temperature for 24 hr. Then the seeds were washed thoroughly in water, sown in potted soil (pot dia 25 cm) and watered regularly. Periodic observations were made to note germination, survival and symptoms of toxicity (Table 10a and 10b). For recording survival, observations had to be made up to 7 weeks.

**Pre-emergence soil application:**

(a) Petridish experiment. - In each Petridish (11 cm dia), 50 gm of dry soil were taken and 25 seeds were sown. Afterwards, in each dish 20 ml water or test solution (100 and 500 ppm) was poured. Four replicates were maintained. After 5 days germination count, root length and hypocotyl
lengths were measured (Table 11).

(b) Pot experiment. - Earthen pots of 15-cm dia and 14-cm height were filled with garden soil. In each pot, 25 seeds were sown. Over the free surface of the soil, 200 ml of water or test solution (100 and 500 ppm) was poured uniformly. The pots were kept in the open and watered regularly for 45 days. Delay in germination, total germination count, survival percentage, percentage of abnormal germination and symptoms of toxicity were observed (Table 12).

Post-emergence treatments:

(a) Soil application. - Seeds were sown in earthen pots of same diameter and height as in the pre-emergence soil application. After a sufficient number of seedlings emerged, they were thinned down to 20 seedlings per pot. When the plants were 15 days old, herbicides were applied to the soil at concentrations of 100 and 500 ppm. Repeated applications, in each case with 200 ml of solution were carried out thrice at intervals of one week. Percentages of seedling mortality and symptoms of toxicity all after each application, were observed and recorded (Tables 13a and 13b). Moreover, studies were continued to record the number of days for 100 percent mortality.

(b) Foliar spray. - (1) At young stage. Scarified seeds were sown in earthen pots of 25-cm dia and 22-cm height.
After a sufficient number of seedlings emerged, they were thinned down to 10 per pot. When the plants were 30 days old, they were aerially sprayed with different concentrations (0.01, 0.1, 1.0, 10, 100 and 500 ppm) of herbicides up to the dripping point. Spraying was done once in the case of phenoxyx while the same was repeated thrice for aliphatics and MH.

Following the sprays, the mortality of plants (Table 14a) and toxicity symptoms (Table 14b) were recorded. Height of plants (shoot height) were measured in cm at specified intervals up to 90 days. Flowering and fruiting were also noted in terms of initiation of flowering, opening of flower, appearance and maturation of pods, all measured in terms of days after spraying (Table 15). Furthermore, the plants were checked as to the number of fruits per plant, number of seeds per fruit and per plant, seed weight (gm), viability of seeds (expressed as percentage germination) and development of seedlings to see whether any toxic effect might be transmitted to the next generation (Table 16).

Portions of both control and aerially treated plants were removed at intervals and anatomical changes in the stem examined. Free-hand sections were made, stained with Bismark Brown and studied under the microscope.

As toxicity symptoms were variable, an analysis was made
of transverse sections from the middle of the third internode of treated and untreated plants 7 days after the herbicidal application. Measurements of various histological zones, tissues and cells were taken with the help of a standardised ocular (Table 17). An average of 20 readings was taken for each aspect.

(ii) At adult stage. From 100-day old plants, ten individuals per pot (same dia and height as in the previous case) were selected and sprayed with 100 and 500 ppm concentrations of each herbicide up to the dripping point. Sprayings were repeated thrice, each at an interval of one week. Percentage mortality was recorded and symptoms of toxicity noted (Tables 18a and 18b). Fruits not fully grown at the time of treatment, fully-grown at the time of treatment and formed after recovery of the plants from herbicide toxicity were harvested following maturation and seeds from them were put to germination tests in Petridishes under laboratory conditions (Tables 19a-c).

Palynological analysis was carried out to confirm the toxic effect of herbicides on pollen-grains. Buds just about to bloom (4 days after treatment) were plucked from the plants and grains from mature anthers were dusted on a clean grease-free slide. To study their fertility, methods proposed by Sharma and Sharma (1972) and followed by Ladizinsky (1979) were employed. The grains were stained in Muntzing's mixture
of glycerol and 1% aceto-carmine solution. By ringing with paraffin, the preparations were stored for further microscopic examination. At least 500 pollen-grains were scored for both treated and untreated plants and were considered normal if they had rounded shape and darkly-stained cytoplasm (Table 20). This method gave an indication of their fertility percentage, since only empty grains are not able to germinate.

Persistence of herbicide toxicity in soil: Pots of 25-cm diam and 22-cm height were filled up with loamy garden soil. Over the free surface of the soil, 200 ml water or test solution (100 and 500 ppm of 2,4,5-TP, MCA and MH) was poured uniformly and regularly kept moist by watering. The pots were exposed to the natural conditions for 7 days. There were two replications for each treatment. Soil samples were taken out from the pot and inhibitory influence of the existing herbicides was bioassayed with seeds of G. sophera var. purpurea at definite intervals (1, 3 and 5 days) in sterile Petridishes (11 cm diam) containing 50 gm soil moistened with 20 ml distilled water. Twenty-five seeds were scattered in each Petridish. There were four replications for each test. Besides germination percentage, radicle and hypocotyl lengths were also taken (Table 21).

Estimation of total chlorophyll:

(a) Effect on chlorophyll biosynthesis. - Scarified seeds
were germinated at room temperature under darkness. Seedlings of proper size were obtained within 72 hr. These seedlings were transferred to Petridishes containing herbicidal solution and exposed to continuous light. After regular intervals of time (6, 12, 24 and 48 hr), chlorophyll content was measured from cotyledonary leaves. It was estimated in terms of mg/gm of fresh weight (Table 22a). According to Arnon (1949), the concentration of chlorophyll was determined by measuring in a 10-mm cell, the density of 80% acetone chlorophyll extracts with Beckman Spectrophotometer at 663 and 645 m\(\mu\) and using simultaneous equations as given by Mac Kinney (1941):

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C = 0.0202 D_{645} + 0.00802 D_{663}
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\(C\) = total chlorophyll in gms/litre

\(D_{645}\) and \(D_{663}\) = density values at 645 and 663 m\(\mu\) respectively.

Herbicides used were 2,4,5-TP, MCA and MH at 100 and 500 ppm concentrations.

(b) Effect on chlorophyll degradation. - Procedure was the same as above, but in this case only seedlings were grown under continuous light both before and after herbicidal treatment (Table 22b).