MATERIALS AND METHODS
Plant materials:

The scrub plants selected for the present study are in the following decreasing order of dominance: *Corisca spinarum* L., *Paba burifolia* Cl., *Chomelia asiatica* O. Koe., *Gymnosporia emarginata* Laws., and *Dodonaea viscosa* L. In a climax stand of the scrub the plants are showing about 1.5 to 2 m height.

Herbicide treatment:

The chemical herbicides used in the present study were, a bipyridylium compound, paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) and a chlorophenoxy compound, 2,4,5-T (2,4,5-trichlorophenoxy) acetic acid. Paraquat (Gramoxone®) supplied by the Albali and Chemical Corporation of India Ltd., and 2,4,5-T (Weedone®) was from the Agromore Ltd., India. Based on the preliminary studies, the concentrations of paraquat at 100 mg l⁻¹ and 2,4,5-T at 2000 mg l⁻¹ were used as a foliar spray to the drip point at 9 A.M. without any wetting agent with a manually operated Gator rocking sprayer. The untreated plants received deionised water as foliar spray.

Leaf samples:

The leaf samples were collected at two day interval following the foliar application upto six days and they were used for the studies on the cuticle, epicuticular waxes, the levels of individual sugars and enzymes of the carbohydrate
and nitrogen metabolism. To study the changes in the levels of carbohydrate fractions, nitrogen fractions and protein content, the leaves were oven dried and the dry powders were used. The leaves were thoroughly washed first with tap water followed by deionised water and were blotted dry. The leaf samples were collected one hour prior to experimentation and they were kept in a cold chest at 0-2°C.

Stem and Root samples:

The stem and root samples were collected at an interval of 20 days following each foliar spray and continued up to four consecutive foliar sprays on the same plant each time. (A spray was given 5 days after refoliation). After defoliation of the growing leaves, as a result of foliar application, the stem sampling was done at 3/4 M down from the apex. Root samples were collected simultaneously at a depth of 1/2 M to 1 M and at 1/2 M behind the root tip. Thus collected root and stem samples were oven dried and blend in a mixer to fine powder, sieved and used for analysis. Composite mixture of equal quantities of dry powders of the stems and roots of three individual plants was used for the analysis of carbohydrates and proteins.

Chlorophyll stability index:

The chlorophyll stability index (CSI) was determined by adapting the method of Poloyareva (1958). One gram sample of
healthy leaves were placed in a large tube one inch in diameter with 10 ml of distilled H₂O and heated in a waterbath at 56° ± 1°C for exactly 30 mins. At the end of this time the leaves were removed from the tubes and were ground in a waring blender for 5 min. with 100 ml of 80% acetone solution in H₂O. The chlorophyll extract was filtered and the filtrate examined immediately for light absorption with photoelectric colorimeter using a red filter. Pigment was extracted from one gram sample of unheated leaves and the light absorption was also measured. The difference between the two readings was defined as the CSI.

Leaf anatomy:

The leaf material was fixed for microscopy in formalin: acetic acid:50% v/v ethanol (5:15:90) for 24 h washed in 50% ethanol and was dehydrated progressively into absolute ethanol. The dehydrated material was treated with xylene and paraffin wax (v. p. 50°C). Transverse sections of 10 microns in thickness were cut using a rocking microtome. The sections were cleared with xylene and were stained with safranine (Gurr, 1965) for the overall mesophyll region.

Cuticle thickness:

For the measurement of cuticle thickness, the sections were stained as per the method of Norris (1974) in a saturated solution of Sudan III in 95% ethanol, rinsed with water and mounted in 1.0% phenol in 50% glycerol and 50% water. Cuticle
thickness measurements were made with a calibrated eye-piece micrometer in the region of the cuticle overlying the centre of the periclinal epidermal cell wall. Measurements were obtained from sections of five different leaves, using 8 measurements per section.

Cuticle content:

Quantitative estimation of cuticle was done by following the method of Holloway and Baker (1963). Leaf discs (cm²) which had been dewaxed by washing in chloroform were immersed at room temperature in a solution containing 1 g of zinc chloride in 1.7 ml of concentrated hydrochloric acid using 4 to 5 ml of reagent per disc. The reagent reacts immediately with the discs and the solution often become highly pigmented. Membranes begin to separate after 1 hr and the reaction is considered complete if the membranes are released on placing a test disc into a large excess of distilled water. The released membranes were dried and weighed.

Epicuticular waxes

Quantitative analysis

The extraction and quantitative analysis of leaf epicuticular waxes were carried out as per the method of Perera et al., (1977). The epicuticular wax content was estimated by the development of the colour change produced due to the reaction of wax with acidic K₂Cr₂O₇. The reagent was prepared by
mixing 40 ml of deionized water with 20 g of potassium dichromate. The resulting slurry was mixed vigorously with 1 litre of conc. sulphuric acid and heated below boiling until a clear solution was obtained.

The individual sample consisted of five leaf discs having a total area of 5 cm². Each sample was immersed in 15 ml of redistilled chloroform for 15 sec. The extract was filtered and evaporated on a boiling water bath, until the smell of chloroform was not detected. After adding 5 ml of reagent samples were placed in boiling waterbath for 30 min. After cooling 12 ml of deionized water was added. Samples were left for full colour development and then cooled, the optical density of the sample was read at 590 nm. The quantities of wax of different species were determined using standard curves prepared with Brassica oleracea wax. Waxes were dissolved in redistilled chloroform and 15 ml of aliquots containing a range of concentrations were carried out, the resulting standard curves were linear throughout the concentrations used.

Qualitative analysis:

The qualitative analysis of the wax components was carried through TLC on silica Gel-G plates as described by Wilkinson (1974). Each sample was dissolved in petroleum ether, an aliquot was applied to previously prepared TLC plates coated with 0.25 mm of silica Gel-G activated for 1 hr at 110°C and the
plate was developed in absolute benzene. The individual spots were visualized by spraying of 5% \( K_2Cr_2O_7 \) in 40% \( H_2SO_4 \) reagent and heated at 150°C (Ratcliff and Thorn, 1965).

**Incorporation of sodium acetate-\( 1^{14}C \):**

**Inoculation and Isolation of products:**

Fresh young expanding leaves near the plant apex were collected and placed in 50 ml Erlemeyer flasks containing sodium \( 1^{14}C \) acetate (10 µCi) in a total volume of 1 ml of water. The contents were thoroughly mixed, and the leaves were spread at the bottom of the flasks, incubated for 3 hrs at 30°C at 20,000 lux light with continuous shaking. At the end of the incubation period, the leaves were transferred into a sintered glass funnel, then the leaves were extracted with chloroform for 20 sec. The chloroform was evaporated and fractionation was made on thin layer chromatography.

**Fractionation of waxes:**

Surface waxes were dissolved in 0.5 ml of chloroform and 10 µl were used for determination of radioactivity. 50 µl were subjected to TLC on 0.25 mm layers of silica gel G and developed in absolute benzene solvent. Each time duplicate plates were run and one of the plates was sprayed with \( K_2Cr_2O_7 \) solution in order to aid the visualization of the components. The corresponding area of each fraction from the unsprayed plate was taken, the radioactivity was determined with scintillation counting system.
Potential transpiration rate:

The potential transpiration rate of stem cuttings was determined by using a simplified potometer (Rao et al., 1977). The rate of transpirational water loss of the twig cut under water was determined indirectly by measuring the rate of absorption with an assumption that the absorption balances the water lost in transpiration. The apparatus consisted essentially of a water reservoir (U-tube) in which the twig cut under water was sealed on to one side and the other side closed with a rubber cork. The water reservoir was having graduation both sides. The whole apparatus was kept under field conditions for 3 h and the volume of waterloss (ml) was calculated per unit leaf area. The potential transpiration rate was expressed as g cm$^{-2}$ hr$^{-1}$.

The potential cuticular transpirational rate of leaves was measured with the same apparatus by cementing the lower side of the leaf with vaseline (Possingham et al., 1967). The water loss in this set up occurs only through the upper leaf surface.

Penetration tests:

Penetration tests were made by following the method of Baker and Sukovac (1971).

The isolated epicuticular waxes from the untreated and treated leaves were deposited on Whatman No.42 filter paper. A volume of 0.02 ml chloroform containing not more than 20 µg
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FIG. 1 APPARATUS FOR PENETRATION TESTS

(From: BAKER & BUKOUAC, 1971)
of wax was used for deposition. Stock solutions of the waxes were prepared so that 0.22 ml deposited contain 5, 10, 15, 20, 25 µg/cm². The impregnated papers were dried over night at room temperature before use. The apparatus was shown in Fig. 1. The impregnated paper discs (A) were mounted between the rubber washers (B) clamped by metal plates (C) between the glass flanges (D). The side of the disc that received the wax faced the donor arm (F), constructed by fusing a 5 ml pipette bulb perpendicular to a flanged glass tube extending upward and away from the receiver arm (E) extending downward. The cross sectional area of the disc available for penetration was cm². Distilled water was added to the donor arm to a point of 0.5 cm above the zero mark. The time required for the miniscus to pass from the zero to the 1 ml was recorded. Non-impregnated discs were used as controls.

Quantitative determination of carbohydrates:

200 mg of a oven dried powdered material was extracted in 15 ml of 95% ethyl alcohol according to the method of Highkin and Frankel (1962). The extraction was repeated thrice to ensure complete extraction and all the aliquots were pooled then evaporated to 10 ml on a waterbath and cooled to room temperature. It was then centrifuged and the supernatant was used for estimating reducing and non-reducing sugars while the sediment was used for starch estimation.
The purification of the alcoholic extract was carried out by taking a fraction of (5 ml) extract to which saturated aqueous neutral lead acetate was added to precipitate the proteins. The excess lead was removed with the addition of 10 ml of saturated disodium phosphate. About 0.5 g of activated charcoal was added, shaken at intervals of 30 min and filtered. Then the filtrate was made up to 100 ml in a volumetric flask (extract A) and used for estimation of reducing and non-reducing sugars.

Reducing sugars:

Reducing sugars were estimated with Pehling's solution according to the method of Snell and Snell (1957). 6.0 ml of alcoholic extract A was mixed with 3.0 ml of freshly prepared Pehling's solution in a centrifuge tube and heated in a boiling waterbath for 15 min. The solution was cooled and centrifuged. The precipitated currous oxide was washed twice with deionised water and dissolved in 0.0 ml of 1:150 nitric acid: water. 1.8 ml of conc. ammonia was added and the total volume was made up to 7.0 ml. The blue colour developed was read at 620 nm using a Bausch and Lomb Spectronic-20 Photoelectric colorimeter. The reducing sugar content was calculated making use of a standard curve prepared with known quantities of glucose.

Non-reducing sugars:

Total soluble sugar content was determined in the above alcoholic extract-A to get the non-reducing sugars following the method of Scott (1960).
10.0 ml of the alcoholic extract—A was hydrolysed with 10.0 ml of 3 N HCl for 30 min at 100°C in a waterbath, and was neutralised with 30% NaOH. The total soluble sugars in the hydrolysed extract were estimated with Fehling’s solution following the standard method described in the reducing sugars. The non-reducing sugars were calculated in the manner indicated by Loomis and Shull (1937):

\[(\text{Total reducing sugars} - \text{free reducing sugars}) \times 0.95 = \text{non-reducing sugars}\]

**Starch**

The residue, left behind after alcoholic extraction of the original material was taken for starch estimation according to the method of McCready et al., (1950). Starch was solubilised with 52% perchloric acid for 30 min filtered and was made up to 100 ml. 1.0 ml of the PCA extract was diluted to 5.0 ml with deionised water in a test tube and 10.0 ml of fresh anthrone reagent was added. The tube with its contents was heated for 7.5 min at 100°C on a waterbath. The tube was cooled rapidly at room temperature and the colour intensity was measured at 630 nm using Baush and Lomb Spectronic–20 Photoelectric colorimeter. A standard curve was prepared with known amounts of glucose and the starch content was calculated by multiplying the glucose equivalent present in the sample with 0.9.
Preparation of the reagents:

Fehling's solution was prepared by mixing equal volumes of solution-A and solution-B just at the time of use to prevent spontaneous reduction of copper.

**Solution-A:** 34.64 g of copper sulphate (CuSO₄·5H₂O) was dissolved in a clean beaker with 25.0 ml of warm deionized water and the contents were transferred to a 500 ml volumetric flask with two washings, allowed to cool and made up to 500 ml with deionized water. After 48 h the solution was filtered and used for the experiment.

**Solution-B:** 1.73 g sodium potassium tartrate (Rochelle salt) and 50.0 g of NaOH were dissolved in deionized water and made up to 500 ml in a volumetric flask.

Anthrone reagent:

200 mg of anthrone was dissolved in 100 ml of cold 95% sulphuric acid.

Sugars:

For extraction of sugars 1 g of freshly harvested leaf material from each sample was cut into small pieces and extracted with hot 80% ethanol on a waterbath. The extract was decanted, continued the same process for 2 to 3 times and the residue was ground and reextracted several times with additional amounts of ethanol. All the extracts were combined,
centrifuged and the supernatant evaporated to dryness. The dried residue was redissolved exactly in 1 ml of 80% ethanol and was stored in a tightly stoppered vial at 4°C until further use (Das and Rao, 1963).

The ascending, unidimensional paper chromatographic technique was employed for the identification of soluble sugars by using Whatman No.1 filter paper. Duplicate chromatograms were developed each time in n-butanol-acetic acid-water solvent system (4:1:1) (v/v/v). The chromatograms were air dried and the individual sugars were detected by spraying aniline phthalate and by heating at 105°C to 110°C.

The identification of sugars was done by running the authentic samples along with the extract solution and by subsequent cochromatography. The sugar spot on the paper was identified by the development of a parallel strip with spray reagent. The individual sugar spot was cut out from unsprayed chromatogram, the sugar was eluted and the eluate quantitatively was analyzed with anthrone reagent.

PREPARATION OF THE SPRAY REAGENT:

Aniline Phthalate (Patridge, 1945): The reagent was prepared by adding 0.30 mg of aniline and 1.6 g phthalic acid to 100 ml of water saturated n-butanol.
QUANTITATIVE ASSAY OF ENZYMES:

Extraction:

Weighing approximately 1.5 to 2.0 g leaf material was cut into small pieces and ground vigorously in a prechilled mortar and pestle for 90 sec with 4 vols. of extraction medium along with a pinch of sand. The extraction medium was 50 mM tris-HCl buffer, pH 7.8, containing 5 mM dithiothreitol (DTT); 1 mM EDTA; 2 mM MgCl₂, and 10 mM 2-mercaptoethanol. The extract was filtered through four layers of muslin cloth and an aliquot was set aside for the estimation of protein content by the method of Lowry et al. (1951). For spectrophotometric assays the extract was cleared by centrifuging at 10,000 x g for 10 min. All the above operations were done at 0 ± 2°C. In vivo enzyme activities were assayed as given below:

β-amylase: The assay of β-amylase was carried out as per the method of Bernfeld (1955). The reaction mixture contains 1% soluble starch, 100 µmoles tris-HCl buffer (pH 7.0) and the enzyme in the total volume of 2 ml. The samples were incubated at 57°C for 1 h. The enzyme reaction was then interrupted by the addition of 2 ml of 1,5-dinitrosalicylic acid reagent. The tube was heated for 5 min in a boiling waterbath and cooled under running tap water. The optical density of the solution containing brown reduction product was determined colorimetrically at 540 nm after the addition
of 20 ml of water. Control tube was run without enzyme. Activity was expressed in terms of maltose liberated in 1 h per mg protein.

**3,5-dinitrosalicylic acid reagent:**

1 g of 3,5-dinitrosalicylic acid in 20 ml of 2 N NaOH and 50 ml of distilled water, 50 g Rochelle salt were added and finally made upto 100 ml.

**Invertase:** Invertase activity was determined by measuring quantitatively the hexose formed by the method of Dodge and Hofreiter (1946) with the modified Nelson’s reagent. The assay system contained 100 μmoles, sodium acetate buffer (pH 5.6); 25 μm sucrose and enzyme extract in a total volume of 2 ml. Blank tubes contained boiled enzyme. Incubation was carried out at 57°C for 1 h and the reaction was stopped by the addition of 1 ml of 1.5 M dibasic sodium phosphate and heating in a boiling water bath for 2 min. Precipitated protein was filtered and aliquots were assayed for the liberated reducing sugars.

**Determination of reducing sugars with Nelson’s reagent:**

To 1 ml of the aliquot, an equal volume of the (low alkalinity) alkaline copper reagent was added, heated for 10 min on a boiling waterbath and then cooled. 1 or 2 ml of arseno molybdate reagent was added. When cuprous oxide was completely dissolved, the solution was diluted and allowed to stand for 15 min but not more than 40 min. Absorbance as
read at 500 nm. The amounts were calculated using standard curve prepared under identical conditions.

**Low-alkalinity copper reagent:**

Rochelle salt (12 g) and anhydrous sodium carbonate (24 g) were dissolved in about 250 ml of water. A solution of 4.0 g of cuprous sulphate pentahydrate in water was added with stirring, followed by 16 g sodium hydrosilicate. A solution of 180 g of anhydrous sodium sulfate in 500 ml of water was boiled to expel air, then the two solutions were mixed and diluted to 1 litre. After one week, the clear supernatant solution was used for determinations.

**Arsenomolybdate reagent:**

To 25 g of ammonium molybdate in 450 ml of water was added to 21 ml of 0.6% $\text{H}_2\text{SO}_4$ followed by 3.0 g of disodium hydrogen arsenate heptahydrate dissolved in 25 ml of water. The solution was incubated for 24 h at 37°C and stored in a glass stoppered amber colored bottle.

**Phosphorylase:** Phosphorylase activity was determined by the method of Whelan (1965). Assay mixture consists of 5% starch, 0.5 M citric acid- NaOH buffer (pH 6.0), 0.1 M Glucose-1-phosphate and enzyme extract in a total volume of 3 ml. Incubation was carried out at 35°C for about 10 min and the reaction was terminated by 5 ml of 5% TCA. The mixture was centrifuged and the supernatant was taken for the inorganic phosphate estimation.
Estimation of inorganic phosphate:

Inorganic phosphate was estimated according to the method of Chen et al., (1956). 0.7 ml of mix 'C' was added to 0.5 ml of phosphate solution in a small test tube and incubated for 20 min at 45°C. Blank was run with water instead of phosphate solution and read at 620 nm.

Reagent A: 10% ascorbic acid.

Reagent B: 0.42% ammonium molybdate 4 H₂O in 1 N H₂SO₄.

Reagent C: One part of A to 6 parts of B.

Phosphoglucomutase: Phosphoglucomutase enzyme activity was determined according to the method of Tosi et al., (1970). The assay mixture consisted of 60 μmoles triphosphate (pH 7.4); 5 μmoles MgSO₄; 5 μmoles cysteine; 4 μmoles glucose-1-phosphate and the enzyme extract in a total volume of 1 ml.

The assay mixture was incubated at 30°C for 30 min and enzyme activity was terminated with the addition of 1.0 ml of 5 N H₂SO₄. 5 ml of water was then added and kept on a boiling waterbath for 10 min to hydrolyze the residuary Glu.-1-P. The inorganic phosphate released after acid hydrolysis was determined by the method of Chen et al., (1956).

Hexokinase: Hexokinase activity was determined as per the method of Devlin and Gallaway (1968). The assay system contained 0.2 M tris-HCl buffer (pH 7.5); 0.1 M MgCl₂; 0.1 M ATP; 0.0015 M NADP; 0.04 M glucose and enzyme extract in a total
volume of 3 ml. The increase in absorbance was measured after 5 min of reaction at 340 nm.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.47): Glu.-6-P dehydrogenase activity was determined by following the method of Devlin and Galloway (1968) by measuring the increase of absorbance at 340 nm after five minutes of the reaction starting at room temperature. The assay system contained 0.2 M tris-HCl buffer (pH 7.5); 0.1 M MgCl₂; 0.0015 M NADP; 0.04 M Glu.-6-P and the enzyme extract in a total volume of 3 ml.

Isocitric dehydrogenase: Isocitric dehydrogenase activity was determined adopting the method of Kornberg (1955). The assay mixture contained 100 μmoles, Phosphate-buffer (pH 7.0); 1.5 μM NADP; 5 μM MgCl₂; 6 μM DL-isocitrate and the enzyme extract in a total volume of 3.0 ml. The increase in O.D was measured at 340 nm.

Succinic dehydrogenase (EC 1.3.99.1): The enzyme assay was based on the use of phenazine methosulphate to couple electron transfer between succinate and 2,6-dichlorophenol indophenol (Lord and Ferrett, 1971). The reaction mixture contained in a final volume of 3 ml 15 mM potassium phosphate buffer (pH 7.6); 0.1 mM 2,6-dichlorophenol indophenol; 4 mM KCN; 2 mM sodium succinate, the enzyme and 0.1 ml of 1% phenazine methosulphate. The reaction was started by the addition of PMS and was measured by following the decrease in the extinction at 600 nm.

Nitrogen fractions:

Total nitrogen:

Total nitrogen was estimated according to the method of
About 100 mg samples of leaf powder was taken in a
25.0 ml microkjeldahl flask to which 100 mg of catalyst,
(1.0 g copper sulphate + 9 g potassium sulphate + 1.0 g
selenium dioxide) was added. 3.0 ml of fuming concentrated
sulphuric acid (H₂ free, analar grade) and 1.0 ml of hydro-
gen peroxide were also added to the sample and was digested
on a hot plate until a clear colourless solution was obtained.
The volume of the solution was made to 25 ml in a volumetric
flask after digestion.

5.0 to 10.0 ml aliquots of the digest were transferred
to the distillation unit and 10 ml of 40% sodium hydroxide
was added and the contents were distilled for 20 min. The
ammonia liberated was absorbed into boric acid indicator
mixture kept below in a volumetric flask. The completion
of the distillation was recognised by the change in pH of
the indicator in the receiver. The indicator solution was
titrated against N/100 HCl until pink colour reappeared.
The amount of nitrogen present in the sample was calculated
using the factor 1.0 ml of N/100 HCl = 0.14 mg of nitrogen.
The results were expressed on unit dry weight basis.

The indicator solution was prepared by mixing 0.33 mg
of bromoresol green and 0.666 mg of methyl red in 100 ml
absolute alcohol. The boric acid indicator mixture was
prepared by adding 10 g of boric acid and 200 ml of absolute
alcohol, 20 ml of indicator solution in a litre with deionised
water. The pH of the solution was then adjusted to 5.0 to 5.1.
Protein nitrogen: Protein nitrogen was estimated by the method of Thimann and Looe (1957). Approximately 1.5 to 2.0 g dried plant material was macerated with 10.0 ml of 10% TCA at 4°C and was centrifuged at 2000 g for 30 min. The precipitate was washed with 5.0 ml of 5% TCA. Again 5.0 ml of 5% TCA was added to the precipitate and was incubated for 30 min at 30°C to remove nucleic acids. The precipitate now obtained after centrifugation was analysed by microkjeldahl method as mentioned above for total nitrogen.

Soluble nitrogen: TCA soluble nitrogen was obtained by subtracting TCA-insoluble nitrogen (protein nitrogen) from total nitrogen obtained previously. The results were expressed per unit dry weight.

Protein content: Protein content was estimated by multiplying the total nitrogen content by a correction factor of 0.25.

ENZYMES OF NITROGEN METABOLISM

Nitrate reductase (EC 1.7.1.1): The enzyme was extracted in 25 mM phosphate buffer (pH 8.8) containing 1 mM EDTA and 10 mM cysteine and was assayed with NADH as the electron donor as described by Hagerman and Bucklesby (1971). The basic assay mixture contained 50 μmoles, phosphate buffer (pH 7.5); 20 μmoles, potassium nitrate; 0.4 μmoles NADH and the enzyme extract in a final volume of 2.0 ml. The reaction was initiated by the addition of the enzyme. After incubation at 30°C for 15 min the reaction was terminated by the addition
of 1.0 ml of 1% (w/v) sulphanilamide in 1.5 N HCl, followed by 1.0 ml of 0.02% (w/v) N-(1-naphthyl) ethylene diamine hydrochloride. The colour developed for 30 min at 30°C was read at 540 nm. The activity was determined with the preparation of a standard curve with KNO₂ under similar conditions.

Nitrite reductase (EC 1.6.6.4): The enzyme was assayed using reduced ferredoxin as the electron donor as per the method of Losada and Paneque (1971) after extracting the enzyme in tris-HCl buffer as already described. The reaction mixture (2 ml) included the enzyme, 75 mM tris-HCl buffer, pH 8.0; 2 mM KNO₂; 0.1 mM ferredoxin and 0.5 ml (freshly prepared) 2.5% (w/v) dithionite solution in 0.29 M NaHCO₃. The reaction was initiated with the addition of dithionite solution. After 10 min the reaction was stopped by vigorous shaking so that the dithionite was oxidized. The reaction mixture was diluted 100 fold. To a 2 ml aliquot 1 ml 1% (w/v) sulphanilamide in 1.5 N HCl was added followed by 1 ml 0.02% (w/v) N-(1-naphthyl) ethylenediamine hydrochloride. The color was allowed to develop for 30 min and read at 540 nm. Calculations were made with the help of a standard curve with KNO₂ under similar conditions.

NADH-Glutamate dehydrogenase (EC 1.4.1.2): The enzyme was assayed as per the method of Raghavendra (1975) in the direction of reductive amination. The reaction mixture included 50 mM tris-HCl buffer (pH 8.0); 0.2 mM NADH; 80 mM,
ammonium acetate; 5 mM, ß-ketoglutarate and the enzyme extract. The reaction was started with the addition of ß-ketoglutarate and was followed by decrease in absorbance at 340 nm.

**NADPH-Glutamate dehydrogenase (EC 1.4.1.4):** The assay method was similar to NADH-glutamate dehydrogenase except that 0.2 mM NADPH replaced NADH in the reaction mixture.

**Leaf moisture content:**

Herbicide treated and untreated leaves were washed with deionized water, quickly blotted dry and were weighed. The leaves were then kept in a hot air electric oven maintained at 100°C for 48 hours. The oven dried leaf material was weighed to record the dry weight.

**Leaf area:**

Leaf area was estimated by plotting the out-line of the leaves on a cm graph sheet.

**Infra-red spectrum:**

IR-spectral recording of the dry powder of the epicuticular wax from the leaves of G. spinarum was obtained by the potassium bromide disc technique in a Perkin-Elmer 250 IR-Spectrophotometer.

In this connection, the author deeply feels for not able to take the IR-spectral recordings for the epicuticular waxes of other scrub plants due to out of order of the instrument working condition.