MATERIALS AND METHODS

The present research work deals with the morphological responses and changes in the efficacy of antioxidative metabolism of two oil yielding crops namely safflower (*Carthamus tinctorius* L.cv Varalakshmi) and sunflower (*Helianthus annuus* L.cv BSH-90) during early seedling growth, subjected to different concentrations of cadmium as Cd Cl$_2$ and Cd (NO$_3$)$_2$.

EXPERIMENTAL DESIGN

The seeds of safflower and sunflower were procured from Andhra Pradesh Seed Development Corporation, Kurnool, India.

Healthy and uniform seeds of safflower and sunflower were surface sterilized with 0.1% Hg Cl$_2$ solution and thoroughly washed with distilled water several times. Seeds of both the species were imbibed in distilled water over night. The seeds were allowed to germinate in petri plates lined with two
layers of filter papers. Distilled water alone served as control. While for treatment 50 ppm, 100 ppm, and 150 ppm of CdCl$_2$ and Cd(NO$_3$)$_2$ were used. The petri plates were maintained at 25°C. Care was taken to avoid contamination by changing the filter papers as and when necessary.

MORPHOLOGICAL PARAMETERS

Germination

Emergence of radicle is considered as germination. The number of seeds of two species germinated in CdCl$_2$ and Cd(NO$_3$)$_2$ solutions and control were counted and recorded every day after the initiation of germination and percentage of germination was calculated from 5 independent sets of experiments.

Root length and Shoot length

The length of root and shoot were measured and recorded on day 5, 7 and 9 after inducing cadmium stress. The results were the average of five replicates.

Fresh weight and Dry weight

For biomass determination, the roots and shoots were separated and blotted to dry with filter paper. The fresh weight of root and shoot of the two species in two salts were recorded on day 5, 7 and 9.
The roots and shoots were dried at 70°C for 48 hours and the dry weights were recorded.

ANTIOXIDATIVE ENZYMES

Superoxide Dismutase (SOD) (EC: 1.15.1.1)

Extraction:

Plant material was homogenated in 50mM phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidine. The homogenate was filtered and centrifuged in a refrigerated centrifuge at 15000g for 15 min and the supernatant obtained was used as a source of enzyme. All steps in the preparation of enzyme extract were carried out at 4°C. An aliquot of 0.1ml enzyme extract was used for the determination of the protein content.

Assay

The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich, 1971).

The reaction mixture 93ml consisting of 50mM phosphate buffer (pH 7.8) 13mM methionine, 75μM nitrotetrazolium, 0.1mM EDTA, 2μM riboflavin and 0.1ml of enzyme extract. Riboflavin was added lastly and the test tubes were shaken and placed 30 cm below a light source (40 W fluorescent lamps). The reaction was allowed for 30 min and then stopped by
switching off the lights. The tubes were covered with black cloth. The reaction mixture which was not exposed to light did not develop colour and served as control. The absorbance was measured at 560nm in a spectrophotometer (Shimadzu 1601). Log A560 was plotted as a function of the volume of the enzyme extract used in the reaction mixture. From the resultant graph the volume of the enzyme extract corresponding to 50% inhibition of the reaction was read and considered as one enzyme unit.

**Extraction for peroxidase and catalase**

The plant material was placed in a pre cooled mortar and ground with cold 0.05M Tris HCl buffer, pH 7.0. The extract was passed through cheese cloth and centrifuged at 1000 rpm to remove cellular debris. The supernatant solution was centrifuged again at 10,000 rpm for 20 min. The supernatant was used as crude enzyme source for the assay of catalase and peroxidase. All stages were carried out in a cold room.

**Peroxidase (POD): (EC.1.11.1.7)**

**Assay**

Peroxidase activity was assayed as per the method of Kar and Mishra (1976).

The reaction mixture containing 0.1M Tris buffer (pH 7.0), 0.01M pyrogallol and 0.005M \( \text{H}_2\text{O}_2 \). The reaction was started by adding enzyme
solution and the mixture was incubated at 25°C for 5 min. The reaction was stopped by adding 1.0 ml of 2.5N H₂SO₄. The amount of pyrogallol formed was estimated by measuring the absorbance at 425nm in a UV spectrophotometer (Shimadzu 1601). The enzyme activity was expressed as change in absorbance units, g⁻¹ fresh weight min⁻¹. (1 OD = 0.01 units)

**Catalase (CAT): (EC. 1.11.1.6)**

**Assay**

Catalase activity was assayed and estimated as per the method of Barber (1980).

The reaction mixture consisted of enzyme extract, 0.005M H₂O₂ and 0.05M Tris buffer (pH 7.0). After incubating it for 1 min at 20°C, the reaction was stopped by adding 1.0 ml of 2.5N H₂SO₄. A blank was maintained with the reaction mixture at zero time. Catalase activity was expressed as mg H₂O₂ oxidized g⁻¹ fresh weight min⁻¹.

**Glutathione-S-Transferase (GST) (EC:2.5.1.18):**

**Extraction**

The plant material was powdered in liquid nitrogen using a mortar and pestle and extracted in 2.0 ml of potassium phosphate buffer (pH 7.0). The homogenate was centrifuged for 10 min at 11000g in a micro centrifuge. The supernatant was passed through a Sephadex G-25 column; active fractions
were collected and used as enzyme source for the assay of glutathione-s-transferase (GST).

**Assay**

Glutathione-s-transferase activity was determined using the method of Habig and Jocoby (1981).

The reaction mixture consists of enzyme extract 100 mM potassium phosphate buffer (pH 6.5) containing 1.0 mM EDTA, 1.0 mM 1-chloro 2,4-dinitrobenzene (CDNB) and 1.0 mM reduced glutathione. The enzyme activity was measured at 340 nm. The activity of the enzyme was calculated using the extinction coefficient of the conjugate 9.6 mM⁻¹ cm⁻¹ and expressed as μmol GSH oxidized mg⁻¹ protein min⁻¹.

**Determination of protein concentration**

The total soluble protein concentration in all extracts was determined by the method of Lowry *et al.*, (1951).

1.0 ml of the sample solution was taken into a test tube. To this 5.0 ml of alkaline solution was added, mixed thoroughly and allowed to stand at room temperature for 10 min. Then 0.5 ml of diluted (1:1) Folin-Ciocalteu’s reagent was added rapidly with immediate mixing and kept for incubation for 30 min at room temperature. The colour developed was read at 750 nm against
reagent blank in spectrophotometer (Shimadzu 1601). Protein content was calculated from a standard curve prepared from bovine serum albumin.

Statistical analysis

Three repetitions as per treatment were made, and enzyme activities were assayed three times. The data obtained in all cases were subjected to Analysis of variance (ANOVA) and the mean values were compared by Duncan's Multiple range (DMR) test at 0.05% level (Duncan, 1955).