Chapter III  Materials and Method
MATERIALS AND METHODS

Phytosociology:

Sampling procedures were broadly followed after Misra Joshi (1952). Sampling for phytosociological studies were done by using quadrats of 50 x 50 cm laid down randomly. Phytosociological data were collected in different fields in different crop seasons, from second week of July to October and from January to March.

First the species were listed and then quantitative and qualitative characters of species were recorded by counting the individuals, species wise. The analytical characters i.e., frequency (Raunkiaer, 1934), density and abundance (Oosting, 1958) were determined and the relative values for these were calculated by using following formulae (Phillips, 1959).

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\text{\% Frequency} = \frac{\text{Number of qu. in which species occurs}}{\text{Total number of quadrats sampled}} \times 100
\]

\[
\text{Density} = \frac{\text{No. of individuals of the species in all qu.}}{\text{No. of individuals of all species in all qu.}}
\]

\[
\text{Abundance} = \frac{\text{Total No. of individuals of a species in all quadrats}}{\text{Total No. of quadrats in which the species occurred}}
\]

Ecophysiology:

Followed by Mall (1968) method. Two agricultural fields
of both villages were selected for detailed studies and for periodic observation. Seed samples of *M. emarginata* were collected from these fields. The average length and breadth of seeds were measured by a vernier-caliper. Seed weight was determined by electric balance (Pathak, 1981). Seed output was determined by counting the seeds of five plants.

Effect of irrigation intervals from daily to 2, 3, 4, 5 and 6 days intervals was observed on the growth of radicle and plumule. Effect of depth of seed burial was studied. For this study seeds were sown from surface to 2, 4, 6 and 8 cm depth.

To study the effect of different soil types on seed germination 5 earthen pots having different types of soil were taken and seeds were kept for germination. After one week, length of radicle and plumule of germinating seeds was measured.

Since seeds of *M. emarginata* showed a long seed coat dormancy hence, they were treated with conc. sulphuric acid for one hour and then such seeds were taken for all the experimental studies. All methods of breaking dormancy followed by Babaley (1985). Seeds of *M. emarginata* were collected from both sites in April 1985 and were stored in air tight glass bottles at room temperature (15°-35°C). Germination studies were made during May and June in the same
year in the germinator.

Studies on germination were done in sterilized Petri-dishes lined with three layered filter paper moistened with distilled water. The criterion of germination was visual detection of radicles emergence.

Seed germination was studied by following methods:

(A) Physical treatments:

(i) Seeds were kept in tap water for 18 hrs in a beaker. After 18 hrs seeds were taken out and washed with running water. Later on they were kept on filter paper in germination trays.

(ii) Seeds were kept in boiling water for 15 minutes then allowed to cool down to room temperature. Later on they were placed on moist filter paper in germination trays to study the germination.

(iii) Seeds were placed on the magnetic stirrer and stirred for 30 minutes and then kept for soaked in running tap water for 18 hrs. Afterwards they were kept in germination trays on moist filter paper for germination in germinator.

(iv) To study the effect of temperature seeds were kept in oven at a temperature of 40° ± 2°C for periods ranging from 24, 48 and 72 hrs. Later on they were soaked in ordinary tap water as usualy for 18 hours, washed and kept for germination in germinator.
(v) Seeds were dipped in 1 percent solution of Potassium permagnet for 18 hours. Later on seeds were soaked in tap water for another 18 hrs. Lastly they were placed on a moist filter paper for germination.

(B) Chemical treatment:

(i) Seeds were placed in 1% solution of Indol acetic acid and Indol butaric acid for different time intervals of 15, 30, 45 minutes and 24 hours. Lateron they were kept on moist filter paper in the germinator for the germination studies.

(ii) Seeds were kept in 15, 30, 45, 60 and 90 percent of sulphuric acid for 15, 30, 45 and 60 minutes at room temperature. Afterwards they were washed and soaked in water for 18 hours and kept for germination.

In each treatment 300 seeds were used for germination. They were kept on three layers of moistened filter paper in germination trays in a germinator at a temperature of 30 to 35°C for breaking dormancy.

Absorption of water by ramates and parent plant roots:

Ten uprooted plants of *M. emarginata* were taken from both sites in an enamel tray containing saffarenin coloured water. Parent roots and ramate roots were dipped in this water from 24 to 72 hours. Later they were washed with running tap water and transverse section were cut to note the movement of saffarenin through xylem tissue.
Allelopathy:

Methods of allelopathy first given in central India by Pathak (1981) all method here following. The seeds of the crop in which M. smaragdinae is generally found as a weed were obtained from Govt. Agriculture department Sagar and stored at room temperature in stoppered glass bottles for the purpose of present investigation.

Five gms of oven dried (60°C) powder of stem, root and leaf were soaked separately in 100 ml of distilled water for a period of six days. After storage dark brown leachates were obtained which were used as standard. Further dilution 1:1, 1:10, 1:50, 1:100 and 1:250 were made by diluting with distilled water. All the solutions remained kept in glass stoppered bottles at room temperature for experimental purpose. Each time pH of the solution was measured by using an electric pH meter.

All experiments of allelopathy were conducted in sterilized conditions. Seed surface was sterilized with 1 percent mercuric chloride. Afterwards washing with mercuric chloride they were kept at a temperature of 25°-30°C at 90% humidity.

For each set 100 seeds of each crop species were taken. The criterion of germination was estimated by measuring plumoly and radicle of 10 seedlings.
Control of the weed:

Two herbicides were used for controlling the weed in the fields.

(i) 2-4 Dichloro acetic acid (2-4, D)
(ii) 2-5 Trichloro phenoxy acetic acid (2-5 T)
(iii) 1:3 Ratio of 2,4-D and 2,4,5-T.

Germination studies were conducted as usually and seeds were kept in germination at a temperature of 24 ± 2°C and 90 percent Relative humidity. For each set of germination experiments, 100 seeds were used as an experimental unit.

(i) Pre-emergence laboratory experiments:

All the herbicidal solutions were prepared in distilled water and their concentration ranged from 100 to 500 ppm. Experiments were carried out in sterilized conditions to study the effect of herbicides on seed germination and seedling growth of *M. emarginata*.

(ii) Post-emergence laboratory experiments:

Surface sterilized seeds of *M. emarginata* were kept for germination in 250 ml flasks with 2 percent agar medium. When seedlings become 8 days old foliar surface sprays of test solution were done by using a glass sprayer. Measurement of radicle and plumule growth were taken after 8 days.
(iii) **pre-emergence field experiments**:

Doses of herbicides which were found lethal in laboratory experiments for *M. emarginata* seed germination and seedling growth were tested in field condition. Experiments on pre-emergence treatment could not be successfully performed due to frequent rains in rainy season.

(iv) **Post-emergence field experiment**:

Spraying of selective doses of herbicides was done on 8, 15 and 30 days old plant of *M. emarginata* by using hand sprayer. Observations were made after 8 days of spraying and at the time of harvesting.