CONCLUSIONS
AND
SUMMARY
The nature has gifted the health, has also provided numerous natural resources, to maintain it. There is not a single aspect of human life, where plants do not play any direct or indirect role. Plants have been used for nourishment, protection, defence, medicine, ornaments and for other various usage by human beings with the dawn of civilization. With the growth of civilization, the multifarious use of plant products began to unfold and in the course of time, their usage in different fields have been developed exhaustively. Generally, vegetation of a particular place depends upon its climatic conditions. Indian peninsula is situated at the equator and its geographical positioning has rewarded India all three seasons i.e. the rainfall, winter and summer. Its mountain ranges, rivers, great seas and associated topography and geography has given us innumerable species of plants and animals. The tribal inhabitants and aboriginals using their indepth, thorough knowledge of surrounding flora and fauna have utilized the biodiversity optimally without disturbing the ecological parameters or the existing ecosystem, thus adopting sustainable developmental approach.

In the course of this exploratory work, above theme was a guiding and inspiring factor, hence the Genus *Calotropis* (Linn) R.Br was studied carefully, using tribal and folklore information and the traditional medicinal uses of the genera were surveyed and are presented in the text of the present work, the aim of this study is to know the laws and ways of nature for making the optimum sustainable use of the plant resources gifted to man by mother nature.
The present work entitled "Ecophysiological, Ethnobotanical and Phytochemical studies on Genus Calotropis" (Linn) R.Br contains an investigation on the following aspects:

1. Ecophysiological observation including the study area, phenological behaviour, seed collection and seed germination studies etc.

2. Ethnobotanical survey of various selected localities of Sagar district.

3. Phytochemical analysis of different plant parts.

The observations have been grouped into different chapters followed by references.

The first chapter deals with introductory theme of the work. The first phase of observations focusses on study sites. Sagar district of Madhya Pradesh occupies almost the central position in the country, situated at 23°10' and 24°10'N latitude and 78°40' longitude on an average height of 583 meters above mean sea level. The main geological formation of the district is basalt and vindhyan sandstone. The climate of the region is broadly characterized by three distinct seasons. The temperature varies between 8°C to 44°C and the average rain fall is about 1316.70 mm. The relative humidity varies from 10.25 to 47.63, out of total 10246 sq.km. area of the district 3014 sq.km. area is covered with tropical dry deciduous forests. Out of total 16,46,198 population of the district 1,42,903 persons belong to various tribes including mainly Kol, Gond, Bharia, Kanwar and sora etc.

The phenological events of both the species were observed throughout the year i.e. from May 1995 to May 1996 at different selected sites of Sagar district, for
recording leafing, leaf fall, flowering, fruiting, fruit maturation, dehiscence and dispersal of seeds. Leafing and leaf-fall occurred throughout the year, however, minimum leafing occurred in summer and maximum in the month of August to January in both the species i.e. C. gigantea and C. procera. Similarly, leaffall occurred throughout the year but it was maximum in summer. Plants never get completely naked. Flowering also occurred throughout the year moderately, however, maximum flowering occurred from the last week of December to the end of March and these flowers produced maximum number of capsules. The fruit ripening, dehiscence and dispersal of seeds occurred mainly during last week of March to the end of May. In both species of Calotropis foliation and flowering is nearly a continuous process, however their number may differ, depending on the moisture availability and season of the year. These phenological events are in contrast to the findings of other workers of this area under study where these events were restricted only to a particular period of the year.

Seed collection was done during different stages of maturation by hand plucking i.e. (i) first time, when fruits fully formed and mature but dehiscence did not take place (ii) second time, when fruits were just ready for dehiscence by slight hand jerk (iii) Third time, from open pods, when dehiscence was taking place and parachutes were just about to come out. Seed extraction was done manually. All extracted seeds were stored in sealed polythene bags at room temperature in the laboratory.
Morphological parameters like the length, width, shape and size of fruits/seeds were studied. Wrinkled damaged and infected seeds were separated from the total crop. Seed health was studied by blotter method and healthy seed percentage was calculated from the seedlots.

Effect of storage conditions and storage periods on seed germination was studied and result indicated that storage temperature shows direct bearing on seed germination percentage. Temperatures too low and too high were found unsuitable. The decline in germination percentage was rapid with increase in storage period i.e. from 4 to 24 months old seed with variation in temperature of storage. The constant temperature suitable for storage of seeds was found to be 28(±1)°C if seeds are to be stored for a period of one year. At 90% relative humidity and 45(±2)°C percentage germination declined rapidly and it was nil after 8 months storage period. If seeds are stored at partial vacuum, there was less decline in percentage germination with increase in storage period. It was observed that in both the species best storage temperature was between 15(±1)°C to 28(±1)°C to retain germiantion percentage for longer period of storage.

Imbibition of seeds was necessary to accelerate the seed germination. It was observed that, there was an enhancement in germination percentage as well as the speed of germination to a certain period of imbibition, after that it goes on decreasing. In both the species, an optimum period from 6 to 18 hours was suitable for obtaining maximum germination with in short period. The period of imbibition for optimum percentage germination varied which may depends on nature of seed coat offering
resistance to speed of water absorption. It was observed that moisture content percentage of less than 6-7% does not cause deterioration in germiantion power of a seed. Germination Index related to imbibition showed different trends in both species.

In the present study temperature was found to affect significantly the seed germination. At different temperature regimes, the germination time ranged between 2 to 9 days. The minimum germination time was 2 to 4 days at 30°C. Better germination occurred between the range of 30°C to 35°C but speed of germination in terms of G.V.I. was also found to be maximum at 30°C & 35°C. Germination percentage was less at 20°C and was nil at 10°C and 45°C in both the species. The optimum temperature for germination in both the species was found to be between 30 to 35°C. 30°C could be taken as optimum temperature. It appears that it is a optimum temperature for the enjmatic activity and all other physiological processes.

Seed colour differentiation within a species of *C. gigantea* has been observed. It was found that light coloured seeds were of immature or partially ripened and hence lacking nutrition. No colour differentiation has been found in *C. procera*.

Germination response in relation to different growth media in laboratory showed highest percentage germination in artificial growth media followed by towel paper, sandy loam, red soil and block soil in both the species. Result of different potting media also exhibited more or less similar trend.

The seed viability was tested by cutting test and biochemical test i.e. 2, 3, 5-triphenyl tetrazolium chloride (TTC) staining test. The results of cutting test for viability have provided reliable and accurate picture of healthy seeds correlated with
germination percentage. On the basis of staining pattern of seeds with (TTC) tetrazolium, seeds were differentiated into viable and non-viable. Seeds with little or no staining were kept in non-viable, category. Results of cutting and chemical test showed higher values as compared to their actual laboratory germination in both the species.

Results of the effect of different concentration of hormones on seed germination indicated that 2 to 10 ppm of IAA treated seeds showed better germination, while with increase in IAA concentration there was decline in germination percentage. IBA pre soaking treatment had also shown a stimulatory effect on seed germination at higher concentration of 5 to 20 ppm in both the species in comparison to IAA. There was insignificant effect of GA₃ on seed germination in these species. It showed a little impact at lower concentrations in comparison to control condition.

An attempt has been made to study the effect of different light conditions on seedlings growth in nursery. The effect of light conditions on root and shoot length indicated that root length was maximum in full sun light condition and shoot length was maximum in semi-shady condition. Root/shoot ratio showed following trend in both species i.e. full sun light condition > semishady condition > shady condition for maximum growth. This appears due to different needs of individual species with respect to the microclimatic requirements of humidity, temperature and soil conditions which are all affected due to light conditions.

For studying the effect of potting media on seed germination and seedling growth, seven potting media were taken. They revealed that that the best medium for
germination and growth of seedlings is red & black soil+sand+manure (1:1:1). Sandy loam soil+manure (1:1) media also found somewhat more suitable than pure sand or pure sand +manure medium. This appears due to increased aeration and water retaining capacity which favours germination.

Effect of sowing depth and pattern of sowing on seed germination indicated that 1.0 to 2.00 cm depth was found suitable for better germination. The seeds completely failed to germinate at a depth of more than 4cm. It appears that it depends upon the size of the seed, shape and size of plumule and particle size of the soil. When seeds were sown by different methods i.e. broad casting, strip and dibbling methods, percentage germination was found comparatively less by dibbling method.

Effect of irrigation interval on seed germination indicated that seeds of both the species failed to germinate in water logged conditions. The best germination was observed when polythene bags were watered moderately once a day.

Studies on vegetative propagation of these species by stem cuttings were undertaken and the effect of different hormones on the survival and sprouting of the cut stumps was considered. Cut stumps treated with IAA and GA₃ gave maximum survival values in comparision to IBA. Vegetative propagation was better during rainy and winter seasons respectively in comparison to summer season. Coppice growth from cut stumps was better during the months of July to October and later it declines.
Ethnobotanical Studies:

On account of increasing realization about the adverse side effects of many modern synthetic drugs on human health, the attention is now again being focused on traditional remedies. Herbal remedies are gaining renewed importance and therefore, ethnobotany especially dealing with various aspects of ethnomedicinal plants has become the subject of very active interest during past few decades.

The present ethnobotanical observations were carried out by periodic and extensive ethnobotanical survey of Sagar district for one complete year and collected numerous relevant information about the medicineal uses of different plant parts of these species by consulting the medicine men, villagers, tribals and local inhabitants for the treatment of various diseases. The first part of this study i.e. material and methods provide details of the field collection proforma and preservation of plant species in laboratory to make herbarium.

In the present investigation it was observed that about thirty various types of human and animal ailments i.e., Asthma, dysentery, piles, cataract, epilepsy, scabies, cuts, wounds, swelling, earache, stomachache, boils, rheumatism, scorpion bite, Snake, bite, leuckorrea, migraine, whooping cough, cold etc. are cured by native tribal and local medicine men of Sagar district by making different remedies from different plant parts of both the species of Calotropis. The medicinal application of both the species varied from root to leaves. A comparative analysis regarding the medicinal properties of different plant parts shows that leaves root and latex are frequently being used for the treatment of various ailments than other parts by the
local tribal inhabitants. It was also observed that only aged persons in the tribal area were familiar with the medicinal uses of this plant. During surveys it was observed that the uses of plant for a particular disease was almost same in different locations but mode of application of drug varied. Certain plant parts are used for a number of ailments and their mode of application is variable. Plant parts are used singly or in combination with other species or natural products like honey, milk, pepper, salt etc. The most common method of preparing remedy is fresh plant material as poultice or pounded and made into paste. Sometimes dried parts ground and used in powdered form. Both the species are depicted with local name, vernacular name, habit, habitat, flowering and fruiting time, morphological description and the native medicinal uses have been described with their usual mode of application.

**Phytochemical studies:**

At present investigation of the plant kingdom for chemical constituents of medicinal importance is a prime activity of pharmaceutical research. The primitive men used the plant parts and their crude extracts without the knowledge of their composition. The objective of the biological screening of plants are to find out sources of the biologically active chemical compounds which can be modified through chemical procedures into useful drug. The phytochemistry has played a dynamic role in the well-being of man kind. The curative value of the plants is due to the presence of active principles in them. After the establishment of the ethnomedicinal importance of both the species, all the plant parts were subjected to phytochemical analysis for detecting the presence of active constituents like
alkaloids, glycosides, carbohydrates, phenols, tannins, saponins, steroids, flavonoids, essential oils etc. In the present study all aerial parts and root of plant were extracted with petroleum ether, ethanol and water solvents and these extracts were subjected to qualitative chemical test to check the presence of different active chemical groups and compounds viz. alkaloids, glycosides, carbohydrates, amino acids, tannins, flavonoids etc. After qualitative analysis, results showed that in both the species there was a little variation in their chemical constituents in different plant parts but there are some chemical constituents which were found same in both the species i.e. flavonoids, carbohydrates, glycosides, alkaloids, saponins and tannins etc.

All the considered plant parts also analysed for amino acids, carbohydrates and phenols, etc. with thin layer chromatography. Thin layer chromatographic analysis of plants on silica gel plates suggested the presence of various components in both petroleum ether and alcoholic extracts. It was observed that Chloroform : Acetone (9:1) solvent system resolves maximum components in petroleum ether extract while Butenol : Acetic acid : Water (4:1:1) solvent system was found to be best phase mixture for resolution of alcoholic extracts.