CHAPTER 5
CONCLUSIONS

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Objectives of the present study were accomplished satisfactorily. Significant observations could be recorded in all the aspects of the study. Some new interesting findings were found as the highlights of the study. Outcomes have been concluded briefly and are presented as follow:

- ✓ From the selected **eighteen medicinal plants**, plants such as *Aerva lanata* (Linn.) Juss. ex Schult, *Boerhaavia diffusa* L., *Catharanthus roseus* L (G) Don., *Chlorophytum borivilianum* San. & Fern., *Coleus forskohlii* Briq., *Curculigo orchioides* Gaertn , *Dioscorea alata* L., *Enicostemma littorale* (Blume) and *Urginea indica* (Roxb.) Kunth. have been evaluated for their allelopathic potential for the **first time** in the present study. Out of these medicinal plants, ***Chlorophytum borivilianum* San. & Fern**. and ***Enicostemma littorale* (Blume)** have manifested seemingly promising allelopathic potential.
- ✓ Preliminary allelopathic analysis: The preliminary screening of eighteen medicinal plants performed using aqueous extract bioassay, with *Raphanus sativus* L. as a test plant, resulted in to identification of six medicinal plants viz. ***Asparagus racemosus* Willd.**, ***Artemisia annua* L.**, ***Chlorophytum borivilianum* San. & Fern.**, ***Enicostemma littorale* (Blume)**, ***Solanum nigrum* L. and *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms.** to have high allelopathic potential. For all the six allelopathic medicinal plants, the plant part that confers medicinal importance to the respective plant also manifested promising allelopathic potential, i.e. in case of ***Asparagus racemosus* Willd.** and ***Chlorophytum borivilianum* San. & Fern.** the plant **root** was showing allelopathy, in case of ***Artemisia annua* L.**, ***Enicostemma***

***littorale* (Blume), *Solanum nigrum* L.** the plant **leaves** were allelopathic and in case of ***Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms**., the plant **stem** was allelopathic. *Raphanus sativus* L. as a test plant showed high sensitivity to the aqueous extracts treatments, where in the seed germination and the seedling radicle length were, the parameters that were most affected by plant extract treatments. The **aqueous extract** of the allelopathic medicinal plants were observed to have inhibitory effects on the test plants, at concentrations as low as 2, 1 and even 0.5 %. For aqueous plant extracts to exhibit phytotoxicity at such low concentrations, provides an opportunity for considering these extracts, to their direct use in weed mitigation/ management in agriculture fields. Use of these phytotoxic aqueous plant extracts followed by systematic standardisation, will facilitate weed eradication in a very cost effective and eco-friendly manner.

- ✓ Medicinal plant part toxicity by fractionation guided bioassay: Subsequent to the preliminary screening, the fractionation guided bioassay and phytochemical analysis, performed for each of the six allelopathic medicinal plants resulted in to identification of chemical nature of the plant metabolites that were responsible for its phytotoxicity. For ***Asparagus racemosus* Willd.** roots, **the fraction B** was most inhibitory to the Chloris growth parameters and the chromatographic analysis of the same resulted in to identification of eleven metabolites in the fraction that were alkaloid in nature. Medicinally important metabolites from *Asparagus racemosus* Willd. are steroidal saponins which are a type of terpenoids and they are extracted in the plant's fraction A. In case of ***Artemisia annua* L.** leaves, the **fraction A** was highly inhibitory to the Chloris germination and growth parameters such as radicle

and plumule length and the plant fraction was found to impose allelopathy by means of eleven phenolic compounds and eight terpenoid compounds. Moreover allelopathic nature of ***Artemisia annua* L.** has been attributed to **terpenoids only** which are also the **medicinal important metabolites** from the plant, however our study reports some **phenolic metabolites to additionally contribute** to the same. For *Chlorophytum borivilianum* San. & Fern. roots which are **medicinally important** owing to their **steroidal saponins and alkaloids,** the **fraction A** was most inhibitory and the metabolites responsible for the inhibitory effect of the same were **phenolic** and **terpenoid** in nature. In case of ***Enicostemma littorale* (Blume)** leaf, the fraction B was highly inhibitory fraction affecting the Chloris growth severely. Our study reports the allelopathic effect of *Enicostemma littorale* (Blume) and also attributes its inhibitory effect to the **fourteen alkaloidal metabolites** detected in the **fraction B**. In case of ***Solanum nigrum* L.** leaf, the **fraction A** was most inhibitory fraction and it was observed to confer toxicity to the medicinal plant by means of metabolites that were **phenolic or terpenoids** in nature, contradictory to this the medicinally important metabolites from the plants are different alkaloids. In case of ***Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms.** stem, the **fraction B** was the most allelopathic fraction and it was found to possess **eleven metabolites** that were observed to be **alkaloid** in nature. The test plant i.e. *Chloris barbata* Sw. was highly sensitive to the chemical fraction treatments and it served as an excellent monocot test plant. Again the alcohol dissolved chemical fractions from the allelopathic medicinal plants were exhibiting allelopathy at relatively lower concentrations such as 0.5, 1, and 2%. Thus the results strongly suggest considering the

allelopathic chemical fractions from each of the six studied plants for the purpose of weed management.

- ✓ Appraising the results of the bioassays, **out of the six** plants, **two** plants that were exhibiting **exceptional allelopathic** potential through **leaves** viz. ***Artemisia annua* L. and *Enicostemma littorale* (Blume).** were subjected to the advance analysis such as evaluation of their mechanism of inhibitory action while two of the plants exhibiting allelopathy by their **roots** viz. ***Asparagus racemosus* Willd. and *Chlorophytum borivilianum* San. & Fern.** were subjected to their rhizosphere analysis.
- ✓ Biochemical, physiological and cytotoxic effects:. Respective chemical fractions from both the plants viz. *Artemisia annua* L. and *Enicostemma littorale* (Blume). were exhibiting phytotoxicity by one or more mechanisms.

  In case of *Artemisia annua* L., the allelopathic plant fraction A possessing phenolic and terpenoid metabolites was phytotoxic and was found to inhibit the growth in Chloris by affecting a few targeted biochemical and physiological parameters. The allelopathic stress imparted by Aa fraction could significantly **elevate** the activities of certain **antioxidant enzymes** such as **SOD** and **CAT** in the Chloris seedlings. Corresponding to this, **an increase** was also observed in the **content of protein and RNA**, in the treated Chloris seedlings. The Aa plant fraction treatment also **decreased the Chlorophyll** content and this decrease was contributing to the **reduced growth** in Chloris seedling. The **inhibitory** effect of fraction A to the plant growth was also due to its toxic effect on the plant **cell division / mitosis**. It was inhibitory to the onion root growth and the reduced growth of root was due to delayed cell

division wherein the treatment was found to induce the cells to arrest at the interphase.

The allelopathic fraction B from *Enicostemma littorale* (Blume)., was analysed to possess alkaloidal metabolites and the fraction was also found to be phytotoxic to the selected test plant. The plant fraction was found to affect the growth by affecting certain indirect growth parameters. Increase in the lipid peroxidation and subsequent membrane damage in the Chloris seedlings must be contributing to the observed results. The **elevated** level of enzyme activity such as that in **SOD,** suggest the stress imparting behaviour of the plant fraction. Correspondingly relatively **higher content of protein and RNA** in the treated seedlings supports the findings of increased stress experienced by the Chloris seedling. Effect of plant fraction treatment on the Chlorophyll content was in trend with reduced growth, **the decrease in chlorophyll content** was observed to contribute to the observed **decrease in the growth**. The plant fraction was also observed to impose **cytotoxicity.** It affected the onion root growth by affecting the division in the root cell subsequently showing **decreased mitotic index and delayed cell division**.

- ✓ *Artemisia annua* L., artemisinin and the plant metabolite absorbance by *Chloris*: The qualitative study was done recognize the potential allelochemical from an extremely promising plant i.e. *Artemisia annua* L. (highly potential allelopathic plant). The **derivatized and underivatized artemisinin and also underivatized ethanolic leaf extract** from *Artemisia annua* L. showed presence of **Q260 and Q292** metabolites in addition to artemisinin and others, which indicates the natural **occurrence** of these metabolites in the **plant leaf**

extract also and possible **involvement** of these metabolites in the plants allelopathic behaviour.

- ✓ The results of additional bioassay studies conducted for *Artemisia annua* L. suggest that, allelopathic effect of the plant is **more** for the **matrix of compounds** such as ethanolic leaf extract, Harborne's fraction A and leaf mulch as compared to the standard artemisinin and that the inhibitory effect of artemisinin was observed to be **elevated** when it is present in the plant extracts then working alone.
- ✓ In metabolite **absorbance analysis** performed with the selected method, the **artemisinin** was not detected in the seedlings treated with the same, however the seedlings must have absorbed the **other derivatives** of the metabolites such as Q260 and Q292, found in the very minor amount in the standard artemisinin and there exist possibilities of these metabolites to have been absorbed and produce the inhibitory effect or there are possibilities of artemisinin to have converted to some other form and produce the observed inhibitory effect in Chloris.
- ✓ Both the medicinal plants viz. ***Artemisia annua* L. and *Enicostemma littorale* (Blume)** were **strongly allelopathic** to **both the selected test plants** i.e. Radish and Chloris, representing a **dicot and a monocot** plant respectively. The plants exhibited phytoxtoxicity by their **leaves** and were inhibitory in all the studied forms viz. **aqueous leaf extracts, chemical fractions and also the leaf mulch**. For both the plants, the plant extracts and leaf mulch were allelopathic at the **same concentrations**. Also the allelopathic effect of both the plants on the test plants was **consistent** in neutral medium such as agar and in the natural medium such as soil. Considering the outcomes, both the plants

in the studied forms viz. aqueous extracts, chemical fraction or leaf mulch can be **standardised** for their **dose, mode of application and** phytotoxicity to the **main crop** and **extended** for their **application in eco-friendly weed eradication.**

- ✓ Distinguishing feature of the present study is the use of **Harborne's fractionation method** which allowed the detection of phenolics and terpenoids in Harborne's fraction A and alkaloids in fraction B. For the study intended to evaluate the allelopathic metabolite from the plants, this method of metabolite extraction (Harborne 1984) has been adopted for the first time while evaluating allelopathic potential. It is a simple and sequential method through which identification of effective metabolite group and elimination of non effective chemical groups can be done easily wherever needed. Fractionation of crude extract in present study aided identifying the **nature of allelopathic metabolite** followed by evaluation of allelopathic potential of the same.
- ✓ Rhizosphere analysis: Both, *Asparagus racemosus* Willd. and *Chlorophytum borivilianum* San. and Fern, **release metabolites** in their rhizosphere which are **spatially and temporally heterogeneous**. However, the metabolites were found to have dissipated to the distance of 7.5 cm from the plant axis. Owing to the presence of allelopathic metabolites in the plants as well as possible release observed for the same, suggest that both the plants when **incorporated** in the cultivation can **assist effective and ecofriendly** weed mitigation and in addition it will suffice the **medicinal plant's demand through their cultivation**.