PREFACE AND SUMMARY

*Cuscuta reflexa* Roxb.,( dodder) of family convolvulaceae (Cuscutaceae) is a troublesome, angiosperm, ‘stem-parasite’. The parasitic weed infest and proliferates over a large number of economically important crops and orchard plants. It abstract organic nutrition from the host plant through haustoria and cause severe losses in yield and productivity, some times leading to even death of host plants. The parasite propagates in nature through seeds and also through vegetative means. Being rootless and leafless total stem parasite it exhibits peculiar physiology and host-parasite relationship. However, Zimmerman (1962) successfully grown dodder (test weed plant) autotrophically on sterile culture medium under laboratory conditions and studied physiology of seed germination and early seedling growth and he observed development of chlorophyll pigment when culture was supplemented with inorganic nitrogen source. Seeds of this parasitic weed (*C. reflexa* Roxb.) have peculiar coiled embryo and exhibits prolonged viability. Much work has been done to investigate its embryology, physiology of seed germination and seedling growth, metabolism and host-parasite relationship of this weed (see details in chapter-2 of thesis). The test plant (*C. reflexa*) has only feasible photosynthesis and ability of chlorophyll biosynthesis when autotrophically grown and supplemented with inorganic nitrogen, under Laboratory conditions. The potential ability of inorganic nitrogen assimilation, physiology of seed germination and autotrophic seedling growth and some aspects of regulation of related assimilatory
enzyme ‘NR’ (nitrate reductase) and nitrogen metabolism for the first time, was investigated from this laboratory (see Chauhan, 1978; Srivastava and Chauhan, 1977; Chauhan and Srivastava, 1979,1980). Seeds of dodder (Cuscuta spp.) have hard seed coat but may germinate easily when chemically scarified with concentrated H₂SO₄ (Gaertner, 1950; Srivastava and Chauhan, 1977; Singh, 1988; Chauhan, et.al.,1989). Seeds have sufficient nutrient reserve for its autotrophic germination and early non-parasitic seedling growth (Rahman and Krishnan, 1977; Nandkumar and Krishnan, 1976). Although Zimmerman (1962), Srivastava and Chauhan (1977) & Chauhan and Srivastava (1979,1980) were able to germinate dodder seeds and to grow the seedlings autotrophically under laboratory conditions, the biochemical parameters involved in obligate requirement of parasitism in the nature, is not yet clear and need further research work.

Efforts have been made to control the test weed under laboratory and field conditions using various different synthetic herbicides (see chapter-2). The earlier findings in this context and particularly in relation to studies on chemical weed control using synthetic herbicides, hazards of indiscriminate application of synthetic herbicides (synthetic chemicals), natural plant growth inhibitors and allelopathic response, possibility of use of natural products as herbicides have been thoroughly reviewed. In view of the marked host range specificity of various species of Cuscuta and the fact that they are not able to parasitize and / or overgrow on some plants in nature was attributed to allelopathic response of such plants on weed infestation and proliferation. A project was therefore, undertaken to investigate possible
role of allelopathic chemicals in weed control management and to explore natural plant products of herbicidal property, active against parasitic test weed. About 100 plant species of 30 different families were screened on which dodder usually does not overgrow in local tarai and assayed for seed germination / seedling growth inhibitory activity against test weed (C. reflexa Roxb.) (see Singh, 1988; Chauhan, et al., 1989). As a result of further biochemical analysis of the most active plant (Azadirachta indica) in the previous study, an active principle ‘Quercetin’ (Falvonoid) was characterized as selective (specific) herbicide active against test weed C. reflexa Roxb. under laboratory conditions (see Singh, 1988; Chauahn et al., 1989).

In view of the above facts the present work was undertaken as advance to previous work mainly to explore more potential, specific, natural herbicidal principle(s). Based on experimental observations, Madhuca indica Gmel was selected for biochemical analysis to isolate and characterize possible allelopathic chemical which showed maximum inhibition of seed germination / seedling growth of the test weed, in the present study. Evaluation of inhibitory effect was based on relative germination rate (RGR), elongation ratio (RER) and dry weight ratio (RDR) respectively. The aqueous ethanolic extract (80 %) from leaf, stem and bark of M. indica Gmel. Was fractionally analyzed with different organic solvents (using standard published methods) and various fractions were tested for inhibitory effect on seed germination and seedling growth. Ethanolic extract from leaf showed maximum inhibition followed by extract in n-butanol and choloform + methanol (3:1). Results were presented in
table-2 and table-3. Fresh leaf material was further analyzed for extraction and isolation of active principle(s). Ethyl-acetate (F-III) and Ethanol (F-IV) fractions showed maximum inhibitory effect on seed germination and seedling growth. Further biochemical analysis of these fractions was made by using TLC, Paper-co-chromatography & U.V. spectroscopy. Two spots were detected from ethyl acetate fraction and three spots from that of ethanol fraction (see table-4). Five flavonoid compounds viz. ‘quercetin’, dihydro-quercetin, myrcetin, quercetin-3-o-rhamnoside, and quercetin-3-o-galactoside were finally characterized. The characterization of isolated flavonoids was based on melting point determination, U.V. spectroscopy and paper co-chromatography of isolated compounds with authentic samples. The active fractions were found to contain a mixture of above five active principles (flavonoids). Last three flavonoids as above were present only in trace amounts.

More experiments were performed to evaluate minimum inhibitory concentration (MIC) and sub-lethal concentration. Higher concentration above 1000 ppm. caused complete inhibition of seed germination. Seedling growth was also considerably inhibited above 500 ppm. And the effect was more pronounced in relation to dry weight (RDR). The concentration of 800 ppm. Proved sub-lethal (see table-5 and table-6 of thesis)

In order to understand the possible mode of action the effect of active principle(s) on some more aspects of physiology of seed germination and seedling growth was studied. The effect of ‘quercetin’ and ‘dihydro-quercetin’ on amylase enzyme activity was studied. 800 ppm of active principle (s) showed marked decline in amylase activity during germination
and early seedling growth. The effect of GA$_3$ treatment and inhibitory response of the active principle(s) on amylase enzyme in test weed seedlings was also examined, which could not overcome inhibitory effect but for at higher concentration (data presented in table-8 & 9).

The effect of active principle(s) on some biochemical parameters of test weed seedlings were also examined to evaluate effectiveness and possible mechanism of action. Total water soluble sugar, starch content, ethanol soluble nitrogen and ethanol insoluble nitrogen and total protein content of seedlings as affected by active principle(s) were determined (data given in table-10). As revealed by general biochemical analysis it became clear that the active principle(s) effected on solublization and mobilization of starch and protein (i.e. reserve food ) in germinating seed s of test weed.

All the observation data were statistically analyzed and found significant. Results were interpreted and discussed in light of known facts and available literature.

The results presented in thesis indicated that active principle(s) (i.e. mixture of flavonoids) can be used as selective herbicide to control test weed (*C. reflexa* Roxb. ). However, the results are based studies under laboratory conditions. More studies are still and essentially required to evaluate possible modelities and effectiveness of inhibitory principle(s) under field conditions, in nature. More research work is also required to understand possible mechanism of action and for exploration and exploitation of such allelopathic compounds as natural herbicides of plant origin which prove to be less toxic, harmless having least residual effect.