Summary
6. SUMMARY

The present investigation deals with the phenology of *Neptunia natans*; various aspects of nodulation such as morphology, distribution, ontogeny, development, structure and classification; isolation, identification and characterization of the nodulating bacterium and the possible utilization of the bacterium as biofertilizer.

Studies on the phenological aspects of *N. natans* revealed that it was a perennial herb and perpetuated its life through perennating organs. Thus, it survived both under aquatic and terrestrial conditions. Hence it was categorized as an amphibious hydrophyte.

The plant produced both root and stem nodules. The production of nodules in root and stem varied depending on the environmental conditions. It produced both stem and root nodules when grown under aquatic conditions but it produced only root nodules under terrestrial conditions. The plant did not produce root nodules under extremely dry condition, but it produced lot of root knots caused by *Meloidogyne* spp.

The nodules were formed on stems and roots when plants grown in aquatic environment. The stem-located root nodules were also found on the stem covered by spongy tissue.

Both root and stem nodules are categorized into five distinct morphological types such as spherical, short rod, long rod, dichotomously branched and nodules with multiple branches. All the five types of nodules were produced on the roots and stems of terrestrial plants, but only four types of nodules excluding nodules with multiple branches were produced in the aquatic condition.
Ontogenetically, three types of nodules were developed in *N. natans*, which include root nodules, stem nodules and stem-located root nodules. The root nodules were distributed on the adventitious roots of terrestrial and aquatic forms, the stem nodules were strictly confined to the nodes of submerged stems and the stem-located root nodules were found on the stems and spongy internodes of aquatic forms and terrestrial stems. The root and stem-located root nodules showed an endogenous origin, where the stem nodule showed an exogenous origin.

Based on the position of the nodule meristem, the nodules were grouped into spherical and apical types. In case of nodules formed at the spongy internodes, hemispherical meristem, a modified apical meristem with lateral extensions was observed. Spherical types were determinate in growth, while the apical and hemispherical types were indeterminate and referred as cylindrical nodules.

Three zones of differentiation were found in cylindrical nodules during the development. They were (i) zone of cell division, (ii) zone of cell enlargement and (iii) zone of cell differentiation. The zone of cell enlargement was not distinct in spherical nodules.

The bacteroid zone was found in the mature nodules. This zone was centrally located and contained hypertrophied large cells filled with bacteroids, intermingled with small, isodiametric and uninfected cells. The bacteroid zone was surrounded by nodule cortex. It was either homogeneous or heterogeneous in nature.

The bacterium was isolated from the nodules, the cultural and biochemical characteristics were studied; the cross-inoculation tests were carried out and the bacterium was identified as *Rhizobium* spp. belonging to 'cowpea group'.
The nodulating response of *Rhizobium* spp. (*N. natans*) was studied in three legumes, *V. radiata*, *V. mungo* and *A. hypogaea*, belonging to the same cross-inoculation group of *N. natans*. The response of the legume to *Rhizobium* was estimated in terms of nodule number, nodule biomass, root and shoot biomass, seed number and seed biomass. *Rhizobium* spp. (*N. natans*) inoculated plants showed better response than uninoculated. Among the inoculated, the response of *V. radiata* was greater and that of *A. hypogaea* was less. The data were statistically analysed and the results were highly significant.