1. INTRODUCTION

Rice is the staple food of more than one-half of the world's total population, with an area of nearly 150 million hectares under its cultivation. In India, it is grown over 44.5 million hectares of land with a total production of 84.7 million tonnes food grain (Venkataramani, 2000). The continuing rise in human population coupled with the possible reduction in total cropped area because of increasing industrialization imposes the need to enhance rice production per unit land area in order to meet the future food demand.

As per the recent estimates, India's population is increasing by about 19 million every year and around 121 million tonnes of rice has to be produced by the year 2020. The green revolution of the mid-1960s brought about large increases in rice yield, primarily due to the introduction of high-yielding varieties and increased use of chemical fertilizers. Nitrogen plays a key role in realizing the yield potential of modern rice varieties. Though molecular nitrogen (N₂) constitutes about 80% of the atmosphere, most plant species are unable to assimilate it and have to essentially depend on the supplies from soil. Rice plants absorb 19-21 kg of N from soil to produce one tonne of brown rice (Murayama, 1979). Thus, achieving an yield level of 10 t/ha could require nearly 200 kg of N, of which soil would provide 50-80 kg and the remaining 120-150 kg has tobe supplied externally (Cassman et al., 1993). Assuming 50% N-use efficiency for the applied chemical fertilizer, the N requirement works out at 240-300 kg/ha. The current estimates of the total fertilizer production in the country suggest that the entire N demand of rice crop can not be met through chemical fertilizers alone and their integrated use with organic manures and biofertilizers is the only option for sustaining high yields. Apart from the escalating costs and inadequate supplies of chemical fertilizers, their exclusive and continuous use over the years not only deteriorates soil health but also adversely affects the environmental quality, causing serious concerns to the sustenance of crop productivity and human health. On the other hand, the use of organic manures and biofertilizers is cost-effective as well as eco-friendly.

Certain cyanobacteria blue-green algae and heterotrophic bacteria, in either free-living or symbiotic state, have the ability of fixing atmospheric nitrogen and serve as potential and promising biofertilizers for rice. Water fern *Azolla* with world-wide distribution is commonly found floating on the water surface in lakes, ponds, ditches, canals and rice fields (Singh, 1977 a,b). Its role in nitrogen nutrition of rice has attracted world attention because of its ability to fix atmospheric nitrogen in symbiotic association with the cyanobacteria.
Anabaena azollae, using solar radiation as the source of energy (Moore, 1969; Rains and Talley, 1979; Singh, 1979a, 1989; Lumpkin and Plucknett, 1980; Peters et al., 1982; Watanabe, 1982; Peters and Meeks, 1989; Plazinski, 1990). Its agronomic significance for increasing the growth and yield of rice and soil fertility is well-documented (Watanabe, 1987; Singh and Singh, 1980a; Kulosooriya, 1991; Roger, 1995). The Anabaena is found in the cavities of dorsal leaf lobes of Azolla fronds. The Azolla-Anabaena system is very attractive because both the partners are competent in photosynthesis and nitrogen fixation in the symbiont is highly efficient. Azolla provides protected environment and nutrients to the Anabaena in exchange for the fixed nitrogen for its rapid multiplication. The nitrogen-fixing activity in the symbiont is reported to be 12-30 times higher compared to that in the free-living BGA (Becking, 1976). Azolla can also absorb nitrogen from its aquatic environment, even though the algal symbiont is capable of meeting the association’s total nitrogen requirement.

The agronomic potential of Azolla as a biofertilizer for rice has been recognized in many countries, including India (Singh, 1989; Singh and Singh, 1990b), the Philippines (Watanabe, 1982, 1986), USA (Talley and Rains, 1980a), Sri Lanka (Kulosooriya, 1985) and Thailand (Laddvan, 1985). A 4-year trial under the International Network on Soil Fertility and Fertilizer Evaluation for Rice (INSFER) conducted at 37 sites in 10 countries has further confirmed its biofertilizer potential (Watanabe, 1987). Azolla grown once before or after the planting of rice produced 5-25 t fresh weight/ha (average 15 t/ha) which contains 10-50 kg N (average 30 kg N). The nitrogen in Azolla is available to rice crop mostly after its decomposition. The fern has an excellent ability of self-decomposition after the formation of a thick mat. Though it has a potential of accumulating more than 10 kg N/ha/day (Lumpkin, 1985), the highest annual N-yield is reported at 1000 kg N/ha from Vietnam (Singh and Singh, 1990a). Roger and Watanabe (1986) reviewed the published work on Azolla and reported the daily fixation rate in the range of 0.4-3.6 kg N/ha (average 2.0 kg N/ha). In India (Cuttack), Azolla grown in fallow fields increased its fresh weight by 2-6 folds every week and annually produced 330 t fresh biomass/ha, which was equivalent to 840 kg N (Singh, 1989). When Azolla was grown in planted rice fields, it accumulated 25-30 kg N/ha in about 25 days. These estimates clearly suggest the vast potential of Azolla as an organic source of nitrogen for rice. At least 70% of the total nitrogen accumulated by the fern is derived from the atmosphere (Watanabe and Liu, 1982). Besides supplying nitrogen to the rice crop, Azolla also improves soil fertility (Liu, 1979; Singh et al., 1981, 1982) and checks the growth of aquatic weeds in rice fields (Janiya and Moody, 1981; Singh et al., 1982; Satpathy and Singh, 1985a).
Azolla is either multiplied or incorporated into the soil as a green manure before planting or grown with rice as a dual crop (inter crop). Its combined use and nitrogenous fertilizer increases the efficiency of applied chemical-N and produces the higher rice yield (Singh, 1979a; Singh and Singh, 1986a; Watanabe and Liu, 1992). However, in spite of undoubtedly proven potential, its use by farmers till now is limited, mainly due to the problems associated with production and transport of huge quantities of fresh vegetative inoculum and culture maintenance throughout the year, particularly during the unfavourable dry season. The only option to overcome these difficulties is to use sporocarps for raising Azolla plants, for which the development of suitable and economically viable technology for the production, storage and effective utilization of sporocarps is needed. This would help in the germplasm maintenance and facilitate the storage, transportation and distribution of inoculum.

Azolla is a heterosporous fern and forms both the micro (male) and mega (female) sporocarps under certain specific conditions though some strains are capable of year round sporulation under wide-ranging environmental conditions (Kar et al., 1999). Sporocarps can resist the unfavourable conditions and remain viable for longer periods. Factors causing induction of sporulation in Azolla are largely unknown, but environmental factors are believed to play an important role. A combination of relatively high light intensity, high temperature and short daylength induces sporulation in temperate region (Becking, 1987). On the other hand, relatively low light intensity, low temperature and short day length are congenial for sporulation in tropical regions (Ashton, 1974). The megaspore germinates giving rise to a prothallus, which produces a female gamete (oospre). The oospre is then fertilized by a male gamete (antherozoid) developed from a microspore. Following fertilization and germination the resultant sporeling still temporarily attached to the megasporocarp floats up to the water surface. Growth of the sporelings (young sporophytes) arising from megasporocarps after fertilization and germination is slow during the initial stages of their development, as the heterocyst differentiation and nitrogen fixation occur only after a few leaves are produced (Singh et al., 1990).

The use of sporocarps is presently restricted due to the poor germination and slow initial growth of young sporophytes (Watanabe and Liu, 1992). Information on the factors affecting sporocarp germination and sporeling growth in Azolla is scanty. In view of the foregoing facts, the present investigation on the effects of certain environmental, chemical and soil factors on
germination of sporocarps and growth of sporophytes was undertaken with the following broad objectives:

- To assess the influence of various soil and environmental factors on germination of sporocarps and growth of sporophytes.
- To maximise of germination of sporocarps and growth of sporophytes through nutritional and hormonal amendments.
- To evaluate the adverse effect of heavy metal toxicity on germination of sporocarps and growth of sporophytes.
- To study the influence of various agro-chemicals used for rice cultivation on germination of sporocarp and growth of sporophytes.