STUDIES ON THE GROWTH OF AZOLLA IN RELATION TO POTASSIUM, CALCIUM, MAGNESIUM AND SULPHUR NUTRITION

BY

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CERTIFICATE

Mr.G. RAVINDRA REDDY has satisfactorily prosecuted the course of research and that the thesis entitled **STUDIES ON THE GROWTH OF AZOLLA IN RELATION TO POTASSIUM, CALCIUM, MAGNESIUM AND SULPHUR NUTRITION** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **STUDIES ON THE GROWTH OF AZOLLA IN RELATION TO POTASSIUM, CALCIUM, MAGNESIUM AND SULPHUR NUTRITION** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Andhra Pradesh Agricultural University, is a record of the bonafide research work carried out by **Mr.G. RAVINDRA REDDY** under my guidance and supervision. The subject of the thesis has been approved by the Student Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigation have been fully acknowledged by the author of the thesis.

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DECLARATION

I, **Mr.G. RAVINDRA REDDY** hereby declare that the thesis entitled **STUDIES ON THE GROWTH OF AZOLLA IN RELATION TO POTASSIUM, CALCIUM, MAGNESIUM AND SULPHUR NUTRITION** is a result of the original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

G. Ravindra Reddy.

Date: 28/12/1990.

(G. RAVINDRA REDDY)

LIST OF ABBREVIATIONS

N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
S	Sulphur
Fe	Iron
Mn	Manganese
Zn	Zinc
Cu	Copper
dsm^{-1}	Deci Siemen per meter
ha^{-1}	per hactare
kg	Kilogram
g	gram
mg	milligram
ppm	Parts per million
$g\ g^{-1}\ day^{-1}$	gram per gram per day
t	tonnes
RGR	Relative growth rate
viz.	namely

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ABSTRACT

Investigations were undertaken to study the response of Azolla to potassium, calcium, magnesium and sulphur nutrition growth in a light-textured Alfisol.

Application of potassium significantly increased the biomass production and N and K uptake upto 150 ppm level of K but, was on par with 200 ppm. At any level of K, potassium sulphate gave higher biomass yields and N and K uptake than potassium chloride. Azolla pinnata (Rajendranagar) was found to respond better than Azolla pinnata (Cuttack) strain with regard to biomass production and N and K uptake.

The biomass production and N and Ca uptake of two strains of Azolla increased significantly with increase in levels of calcium upto 80 ppm but was on par with 120 ppm. Azolla pinnata (Rajendranagar) gave

significantly higher biomass yields and Ca and N uptake than *Azolla pinnata* (Cuttack) strain.

There was no significant increase in biomass production and N and Mg uptake with increase in levels of magnesium. But, *Azolla pinnata* (Rajendranagar) gave significantly higher biomass yields and N and Mg uptake than *Azolla pinnata* (Cuttack) strain.

Application of sulphur significantly increased the biomass production and N and S uptake upto 25 ppm S but was on par with 50 ppm. *Azolla pinnata* (Rajendranagar) gave significantly higher biomass yields than *Azolla pinnata* (Cuttack) strain, but the increase in N and S uptake was non significant.

Availability of K_2O in soil treated with 20 t of *Azolla* on a fresh weight basis was higher due to incorporation of *Azolla* and this availability increased with increase in levels of K applied to *Azolla*. *Azolla* grown at 150 ppm K with potassium sulphate as the source was found to be the best treatment in increasing the K_2O availability of soil. The availability of K_2O in soil increased with the decomposition of *Azolla* upto 80 days but most of the K_2O was available by 60 days of decomposition of *Azolla*.

These studies have brought out the need to maintain balanced nutrition for the cultivation of *Azolla* as a green manure for rice. Potassium, calcium and sulphur was found to positively influence the growth of *Azolla* as well as the yield of nitrogen through the fern.

Azolla application to soil resulted in a significant increase in the status of available potassium in soil.

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INTRODUCTION

CHAPTER I

INTRODUCTION

The main constraints in the use of *Azolla* fertilization to rice are moisture and temperature stress, nutrient status of soil and the incidence of pests and diseases. Since, water is a prerequisite for growth of *Azolla* it can be grown in higher rainfall areas where water is adequate and as such it is not suitable under dryland conditions (Becking, 1979). *Azolla* is reported to grow and multiply well from July to Febraury in India but dies rapidly at higher temperatures (Singh, 1982; Kannaiyan, 1987; and Jayanthi, 1989).

The essentiality of phosphorus, potassium, calcium and magnesium in *Azolla* nutrition has been established. However the research efforts with reference to nutrition and nitrogen fixation in *Azolla* has concentrated mainly on establishing the critical levels of phosphorus at the cost of study of the importance of other nutrients. Since most of the rice soils are deficient in Phosphorus, the importance of phosphorus for establishing *Azolla* in rice paddies has been stressed through research in various types of soils.

Watanabe *et al*. (1977) observed that potassium deficiency decreased the fresh weight production by 32 per cent and the total N content by 24 per cent of the control. Calcium deficient plants had a fresh weight production of only 9 per cent than that of normal plants and total N content was reduced to 5 per cent of that of plants receiving a complete nutrient medium. Magnesium deficiency also likewise had a deleterious effect by reducing the fresh weight by 82 per cent and total N content by 77 per cent of the control. Therefore, it is important to establish the critical concentration of these nutrients in soil for obtaining better establishment, growth and nitrogen fixation by Azolla.

The beneficial effect of Azolla as a green manure has been well documented (Kannaiyan, 1987; Singh, 1978 and Shantaram, 1989). Besides increasing the organic carbon content and N availability in soil, Azolla is a good source of other nutrients.

Azolla is reported to be capable of absorbing and concentrating potassium from the water in which it grows (Liu, 1987). But, there are very few studies on the availability of potassium to rice consequent upon the decomposition of Azolla in soil. Hence a study was conducted to evaluate the efficiency of Azolla as a supplier of potassium to soil.

The economic potential of Azolla is also reported to be considerable. The economic return due to adoption of biofertilizer technology, in terms of saving on cost of chemical fertilizer and weedicides is always more than 10 per cent provided the environmental conditions for growth and multiplication of Azolla are properly met (Ki Kuchi et al., 1984).

Therefore, once Azolla is introduced in a rice field, it can sustain and act as a renewable biofertilizer. Principally there are two methods of using Azolla, as a dual crop with rice and growing in nursery and using prior to transplanting of rice. An increase in paddy yields, due to inoculation of Azolla has been reported by several investigations in India and abroad (Watanabe, 1982; Singh, 1982 and Kannaiyan, 1987).

The studies on Azolla were considered essential because in the Telangana region of Andhra Pradesh, India which is a prominent rice growing area of the state. The role of Azolla in rice cultivation has not been evaluated. Therefore, studies were conducted on aspects of utilization of Azolla in rice cultivation with the following major objectives.

1. Response of Azolla to the application of potassium, calcium, magnesium and sulphur to soil as indicated by the growth and uptake of K, Ca, Mg and S by Azolla.

2. The effect of potassium, calcium, magnesium and sulphur nutrition on the N content of *Azolla*.

3. Effect of application of *Azolla* on the availability of potassium in soil.

These studies were conducted at college of Agriculture, Rajendranagar campus of the Andhra Pradesh Agricultural University during the 1989-90.

The results of these studies are presented and discussed in this thesis.

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REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Azolla Lam., is a genus of small aquatic fern which is native to Asia, Africa and America. Azolla thrives in lakes, swamps, streams and other such water bodies and have been dispersed by man and nature to various parts of the world. Some are strictly tropical or sub-tropical in nature, while others have acclamatized either to temperate or tropical climate. The fern has been extensively exploited as a biofertilizer for rice because of its capacity to fix atmospheric nitrogen symbiotically in association with the cyanobacteria belonging to the genus Anabaena.

2.1 TAXONOMY AND DISTRIBUTION

The generic name, Azolla is a conjugation of two Greek words, Azo (to dry) and ollyo (to kill), suggesting the fern is killed by drought. Some of the fern's vernacular names are : water velvet, mosquito fern (English); Beo gian (vietnamese); Lu P'ing, Ho P'ing (chinese).

The genus Azolla, established by Lamark in 1785 (Svenson, 1944), is rather a coherent group of aquatic ferns which are confined to fresh water, where they float freely. The fern, together with some other aquatic ferns is associated with the family salviniaceae, but more recently some authors have preferred to assign it to a single-genus family of Azollaceae (Moore, 1969).

Species delineation in the genus Azolla is not consistent among the botanists, but usually six existing and about twenty five fossil species are recognised (Hills and Gopal, 1967). Species differentiation is based on the morphology of the sexual organ. Taxonomically the six existing forms are usually divided into two sections or subgenera : Section I : Azolla (or Euazolla) with three floats on the megaspore, and Section II : Rhizosperma with nine floats (Hills and Gopal, 1967).

The four species of the section Euazolla are all originally found in North and South America, i.e. (1) Azolla filiculoides Lam., found throughout America from Southern South America to Alaska; (2) Azolla caroliniana wild., found in eastern parts of United States of America, Mexico and the West Indies; (3) Azolla mexicana Presl., occuring in northern parts of South America to British Columbia and eastward to Illinois: and (4) Azolla microphylla occuring in western and northern South America to Southern North America, and the West Indies (Svenson, 1944). The Section Rhizosperma covers only two species: (1) Azolla pinnata R.Br., widespread in Eastern hemisphere, tropical Africa, South East Asia, Japan and Australia and (2) Azolla nilotica Decaisue., a large species occuring on the Nile in Africa (Moore, 1969).

Only one variety of one species (A. pinnata var. pinnata) is cultivated for agricultural purpose in Northern Vietnam, China and Thailand. However, agronomic studies have been made with A. filiculoides and also A. mexicana in Central California (Talley et al., 1977).

2.2 THE FERN-ALGA RELATIONSHIP

Azolla plants are triangular or polygonal in shape, and float on the water surface individually or in mats. They give the appearance of a dark green to reddish carpet, except A. nilotica which do not produce the red anthocyanin pigment. Plant diameter ranges from 1-2.5 cm for small species such as A. pinnata, to 15 or more cm for A. nilotica (Ridley, 1930).

Anabaena azollae Strasburger is the only species mentioned in symbiotic association with Azolla. Taxonomists place Anabaena azollae within the phylum Cyanophyta, order Nostocales, family Nostocaceae. The species has sinuous trichomes (threads) composed of bead-like or barrel-shaped cells without a sheath (Shen, 1960).

The cavity in which the alga inhabits occurs on the ventral side of the dorsal lobe of the Azolla leaf, near its base (Rao, 1936; Shen, 1960) and consists of a concavity in the leaf overgrown by epidermis possessing only a minute central opening to the exterior (Rao, 1936).

2.3 ENVIRONMENTAL REQUIREMENTS FOR AZOLLA

The overall environmental requirements of Azolla are so interrelated that it is often difficult to single out any one factor as important. The climatic variables that affect Azolla growth, are difficult to manipulate. The ecology of Azolla in nature is still obscure (Becking, 1987).

2.3.1 Water

Water is a fundamental requirement for the growth and multiplication of Azolla. Optimal requirement of relative humidity is reported to be 85-90% (ZAAS, 1975). At relative humidity of less than 60%, Azolla becomes dry and fragile. Although Azolla can grow on a wet mud surface or wettened peat litter, it prefers to grow in a free floating state on the water surface. In shallow water the Azolla plant may touch the soil surface with its roots, but it can also grow in much deeper water where nutrient uptake is completely from the water environment (Becking, 1979). Like rice, *Azolla* grows better during dry (non monsoon) seasons when irrigation water is available (Lumpkin, 1987).

2.3.2 Light

Like all green plants, *Azolla* is dependable on solar energy for photosynthesis. The growth rate of *Azolla* has been reported to saturate at 25-50% of full sunlight (Ashton, 1974), and is not inhibited by full sunlight as long as other factors are not limiting (Gowd *et al*., 1980). Ashton (1974) reported that relative growth rate (RGR) and nitrogenase activity of *A. filiculoides* were maximum at 50% of full sunlight (40-57.5 K lux). Also, nitrogenase activity declined more rapidly when light intensity increased in comparison to when light intensity decreased. Lu *et al*. (1963) reported that 25,000 lux resulted in the highest nitrogen content (4.8%), while 47,000 lux resulted in the highest rates of growth and nitrogen fixation for *A. pinnata*. They also reported that *A. pinnata* survived in a range from 3,500-120,000 lux but 20,000-40,000 lux was preferable since the nitogen content was higher.

Daylength is another important aspect of light. The RGR (g g^{-1} day^{-1}) increases with increasing daylength upto eight hours. The action of light on growth and nitrogen fixation is further complicated by the fact that light intensity and pH of the medium have interacting effects on growth rate.

2.3.3 Temperature

Temperature is probably the most important limiting environmental factor in Azolla cultivation. From traditional agricultural practices with Azolla in Vietnam, where A. pinnata was grown as green manure in rice fields, the most favourable period for vegetative growth of Azolla was found to be August to February, during which period the mean daily temperature was around 16°C-17°C.

The most favourable temperature for growth and nitrogen fixation by A. pinnata is between 20-30°C. Outside of this range, growth decreases until the plant begins to die at temperatures below 5°C and above 45°C (Lu et al., 1963). Temperature affects both nitrogen and water content. Azolla pinnata grown at 5°C contained 1.75% nitrogen (dry wt.) and 84% H_2O (fresh wt.); at 25°C, it contained 4.5% N and 94% H_2O; and at 40°C it contained 2.5% N and 90% H_2O (Anonymous, 1975).

Although Azolla pinnata is widely distributed in tropics, it grows better only in cooler seasons. At Varanasi, A. pinnata grew well from July to December but it disappeared from the ponds in hot summer months

(April-June). Gopal (1967) found *A. pinnata* at Varanasi, to be a typical winter annual plant. Its growth in ponds started from July and reached peak maximum in Nov.-Dec. From April to June, *Azolla* was completely absent. Thus, *Azolla* grows over a wide range of temperatures of 14°C to 40°C. However, the optimum temperature for growth is around 20°-30°C. The correlation studies by Jayanthi (1989) indicate that temperature has a positive relationship with biomass production.

2.3.4 Effect of pH

Azolla can survive within a pH range of 3.5-10, but optimum growth is observed in the range of 4.5-7 (Nickell, 1961; Lu *et al*., 1963). Ashton (1974) found that relative growth rate (RGR) was influenced by direct relationship between light intensity and pH; high light intensity (60,000 lux) with high pH (9-10) and low light intensity (15,000 lux) with low pH (5-6) allowed maximum relative growth rates.

An inverse relationship between pH and temperature influences nitrate reduction and nitrogen fixation. Nitrate reduction was optimal at pH 4.5 and 30°C while nitrogen fixation was optimal at pH 6.0 and 20°C (Ashton, 1974). Both Ashton (1974) and Watanabe *et al*. (1977) reported that nitrogen fixation decreased at neutral pH.

2.4 CHEMICAL COMPOSITION AND N_2 FIXATION IN *AZOLLA*

2.4.1 Chemical composition of *Azolla*

The *Azolla* species vary widely in their gross chemical composition in respect of plant nutrients. Nitrogen content of *Azolla* on a dry weight basis has been reported to be as high as 6.5 per cent for aseptic cultures (Peters *et al*., 1980) but will probably be closer to 3.5 to 4.0 per cent. The dry weight carbon contents of various *Azolla* species ranged from 41.5 to 45.3 per cent (Peters and Calvert, 1982). The optimum nutrient levels in each species may vary as the fern exhibit luxury consumption. The range of elemental composition on a dry weight basis for *Azolla* has been reported as Nitrogen (N) 1.96 to 5.3%, Phosphorus (P) 0.16 to 1.59%, Potassium (K) 0.31 to 5.97%, Calcium (Ca) 0.45 to 1.7%, Magnesium (Mg) 0.22 to 0.66%, Sulphur (S) 0.22 to 0.73%, Silicon (Si) 0.16 to 3.53%, Sodium (Na) 0.16 to 1.31, Chlorine (Cl) 0.62 to 0.90, Aluminium (Al) 0.04 to 0.59%, Iron (Fe) 0.04 to 0.59%, Manganese (Mn) 66 to 2944 ppm, Copper (Cu) 0 to 264 ppm, Zinc (Zn) 26 to 989 ppm (Lumpkin and Plucknett, 1982).

2.4.2 Nitrogen fixation in Azolla

The characteristics of the symbiotic association between Azolla the macrosymbiont and Anabaena the microsymbiont has been widely studied. Heterocyst frequency is one important character directly related to nitrogen fixation. Singh (1979) reported a N_2 fixation rate of 7.5 mg N g^{-1} dry weight day^{-1} in an incubation study. Rates of nitrogen accumulation by Azolla filiculoides, calculated from the results of an experiment conducted in China were found to be as high as 10.5 kg N ha^{-1} day^{-1}. Talley and Rains (1980) reported a rate of 2.7 kg N ha^{-1} day^{-1} under field conditions and Watanabe et al. (1980) calculated a daily average of 1.4 kg N ha^{-1} from a year round field experiment.

Investigations on the application of Azolla as a bio-fertilizer are being conducted in India, Philippines, North America, West Africa, Srilanka and Thailand. Azolla could supply around 25 to 30 kg N ha^{-1} (Kannaiyan, 1987). According to Singh and Singh (1987), Azolla cultivated as a pure stand produced a biomass of 19 to 26 t ha^{-1} contributing 28 to 34 kg N ha^{-1}.

2.5 GROWTH RATE

Peters et al. (1980) obtained under optimum conditions a relative growth rate (RGR) of 0.3355 to

0.390 gg^{-1} day^{-1} using four surface sterilized species. Talley et al. (1977) observed a doubling time of 2.8 days in A. filiculoides. Watanabe et al. (1977) and Watanabe (1978) reported that doubling time in A. pinnata was 3 days under most favourable conditions with an average N content of 3 per cent. They calculated that Azolla may fix 7.8 mg N g^{-1} dry weight day^{-1}.

Singh (1978) conducted an experiment where the ferns were multiplied throughout the year with annual production of 420 t ha^{-1} where average day night mean temperature was 30.5 to 24.5^{o}C. The ferns multiplied mostly vegetatively but reproductive structures were found abundantly from November to December.

Watanabe et al. (1977) reported that phosphorus, potassium, calcium and magnesium deficiencies reduced the fresh weight production to 22, 32, 9 and 82% of the control respectively.

2.6 NUTRIENT REQUIREMENTS OF AZOLLA

Azolla grows in aquatic habitat and absorbs nutrients mainly from water. As Azolla is normally cultivated in rice puddies submerged with a few centimeters of standing water. The nutrient concentration in the standing water appears to be more critical than the nutrient supplying capability of the soil beneath the column of standing water. In shallow waters, the plant roots attach to the soil and can absorb nutrients from the soil also. Azolla requires all the macro and micronutrients required by other green plants. The macronutrients such as P, K, Ca, Mg and micronutrients such as Fe, Mo, Co, Mn, B and Zn have been shown to be essential for the growth and nitrogen fixation of Azolla.

Phosphorus is probably the most common factor limiting the growth of Azolla. Yatazawa et al. (1980) found that both growth and N-fixation declined if nutrient solution P concentration was less than 0.03 m mol/l. Iron and molybdenum have also been reported to limit the growth of Azolla in field culture. Molybdenum is very important for stimulating the nitrogenase activity in the blue green algal symbiont. The threshold levels for Fe and Mo are 50 μg/l and 1 μg/l respectively (Yatazawa et al., 1980). Zinc is required by Azolla in very small amounts. Zinc deficiency treatment gave lower Azolla yield than the complete treatment (Sikander Ali and Watanabe, 1986). Threshold levels for Mn and B were reported to be 10 μg/l and 20 μg/l respectively (Yatazawa et al., 1980). Cobalt is also essential for nitrogen fixation (Johnson et al., 1966). Jayanthi (1989) reported that

20 kg P_2O_5 as SSP was the best source for producing optimum fresh weight of Azolla. This can also be substituted by 30 kg rock phosphate which also gave similar biomass production.

The application of potassium was reported to be essential for the cultivation of Azolla on many light textured soils in both Vietnam and China (Vo and Tran, 1970; Lu et al., 1966). Threshold concentration for K has been reported to be about 0.4 m.mol/l (Yatazawa et al., 1980). Potassium deficiency decreased the fresh weight production and the total N content of the A. pinnata to 32 and 24% of the control (Watanabe et al., 1977). Potassium deficient plants do not develop colour indicated deficiency symptoms but the number of algae inside the leaf cavities were reduced and the algae themselves were less green. The frond size of Azolla is also relatively smaller than that of control (Becking, 1979).

Le and Subochkin (1963) showed that the absence of potassium decreased the yield of Azolla more than the absence of phosphorus. Sikander Ali and Watanabe (1986) reported that Azolla yield was lowest with K deficient treatment compared to complete treatment. They also reported that application of K increased the average K content in Azolla by 1.5 times.

Azolla is not only a biological N source but an extremely promising source of biological K for rice field because of its strong ability to concentrate K from the water in which it grows (Liu, 1987).

Singh (1977) reported that addition of potassium fertililzer (4-10 kg K_2O ha^{-1}) and domestic ash (50 kg ha^{-1}) encouraged growth of *Azolla*. The best rates are 10 kg K_2O or 100 kg of ash ha^{-1} applied every 10 days.

Watanabe *et al*. (1977) reported that calcium deficient plants had a fresh weight production of only 9% of that of normal plants, and total N content was reduced to 5% of that of plants receiving the complete solution. Magnesium deficiency also produced some effects. Fresh weight production was reduced to 82% and total N content to 77% of the Mg fertilized control. Calcium deficient *Azolla* showed more browning than the phosphorus deficient *Azolla* and had the smallest frond size among treatments. Yatazawa *et al*. (1980) reported that inoculated explants of *Azolla* on Ca and Mg deficient solution grew only to a very limited extent as compared to those supplied with Ca and Mg. They also determined the threshold levels of Ca and Mg for *Azolla* growth as 0.4 and 0.5 m.mol./l respectively.

In green house experiments (Singh, 1979) Hoagland's culture medium used at 40 per cent strength was found most suitable for the cultivation of *Azolla*. Le and Subochkin (1963) recommended a N-free nutrient solution of the composition of the Helriegel mixture (1/10 strength) or a Knop's solution (1/10 or 1/5 strength) for the optimal growth of *Azolla*.

2.7 MULTIPLICATION AND UTILIZATION OF *AZOLLA*

2.7.1 Multiplication

A simple method to establish a *Azolla* nursery for large scale multiplication of *Azolla* in the field had been evolved for easy adoption by the farmers (Kannaiyan, 1982). The inoculum of *Azolla* must be healthy and fresh. It should be continuously multiplied in the inoculum preparation plots or ponds. The inoculum density is an important factor in the efficient production of *Azolla*. Singh (1979) recommended 2 tonnes fresh weight of *Azolla* ha^{-1} as the quantum of inoculum. In Vietnam, 5 t ha^{-1} fresh weight of *Azolla* was recommended. Sreenivasan (1977) reported that with an initial inoculum of 500 g m^{-2} of fresh *Azolla* the best growth and multiplication of *Azolla* at 1.158 kg $week^{-1}$ was obtained when depth of water was 20 cm and application of 15 g of superphosphate per 2 m^2 and 7.5 g of Kcl per 2 m^2. Even though water depth gave maximum *Azolla* yield, the difference between 5, 10, 15 and 20 cm water depth was not significant. Kannaiyan *et al*. (1982) obtained better yields of *Azolla* with split application of phosphorus or organic manures like poultry and goat manure, cow dung, pig excreta and gingelly cake. It is imperative that a study of requirements of other nutrients like K, Ca, Mg and S is made to obtain better growth and multiplication of *Azolla* for use as a biofertilizer.

2.7.2 Utilization of *Azolla*

There are three different systems adopted for utilization of *Azolla* as a bio fertilizer for rice. They are (1) Monocropped *Azolla* or basal green manure (2) Inter cropped *Azolla* or top dressed green manure and (3) Dual cropping. The fresh *Azolla* inoculum required for one hactare of transplanted rice field was 800 kg. The inoculated *Azolla* was found to establish well and covered the rice field in 20 to 30 days period. *Azolla* was thus incorporated before planting (Kannaiyan, 1987). Fresh or decomposed *Azolla* can also be top dressed at a later stage of plant growth (Singh, 1978). It is advisable to grow *Azolla* for top dressing in low lying lands water stagnates. In Vietnam, *Azolla* is usually grown on fallows that makes up about 10 per cent of the area reserved for rice crop on co-operative farms (Singh, 1979). *Azolla* can be used effectively in dual cropping with rice to supply nutrients after the formation of a mat, either by decomposition after over growth or by incorporation into the soil (Singh, 1979).

2.8 EFFECT OF APPLICATION OF AZOLLA AS A GREEN MANURE ON THE AVAILABILITY OF POTASSIUM IN SOIL

In addition to nitrogen, Azolla also contributes potassium, phosphorus, zinc and iron to rice crop during its decomposition in rice soil (Kannaiyan, 1987).

Investigations have shown that in addition to its N_2 - fixing activity, Azolla has a strong ability to concentrate K from the water in which it grows. After Azolla decomposition, absorbed K can be utilised by rice plants. Its efficiency is equivalent to that of chemical K fertilizer (Liu, 1987). Nagarajah et al. (1989) reported that incorporation of Azolla increased soil solution K. Azolla is rich in potassium and therefore can provide K to the plants (Pande, 1979). Availability of K_2O was higher due to incorporation of Azolla than mineral N and this effect was also more in red soil than in black soil (Jayanthi, 1989).

The rate of decomposition of organic matter from *Azolla* was higher under upland conditions than in lowland soil. After 40 days, about 35 per cent of Co_2 was evolved from upland soil and only 22 per cent from lowland soils amended with *Azolla* (Ali and Malik, 1985).

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MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The Present investigation on certain aspects of "Studies on the growth of *Azolla* in relation to potassium, calcium, magnesium and sulphur nutrition" was carried out at the College of Agriculture, Andhra Pradesh Agricultural University, Rajendranagar campus during 1989-90. These studies were carried out with the basic objective of obtaining information on the requirement for K, Ca, Mg and S by *Azolla pinnata* strains grown on submerged soil. The studies were conducted on these aspects in separate experiments.

1. Response of *Azolla* to the application of potassium, calcium, magnesium and sulphur to soil as indicated by the growth and uptake of K, Ca, Mg and S by *Azolla*

2. The effect of potassium, calcium, magnesium and sulphur nutrition on the N content of *Azolla*

3. Effect of application of *Azolla* on the availability of potassium in soil.

3.1 EFFECT OF POTASSIUM, CALCIUM, MAGNESIUM AND SULPHUR ON THE GROWTH OF AZOLLA

Four separate experiments were conducted in greenhouse to study the effect of potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) on the growth and uptake of K, Ca, Mg and S by Azolla pinnata strains.

3.1.1 Effect of different sources and levels of potassium application on growth and uptake of potassium by Azolla

This experiment was conducted under green house conditions by inoculating a known quantity of fresh Azolla pinnata strains and following the rate of growth at weekly intervals from 21st May, 1990 to 25th July, 1990 for nine weeks. Two sources of potassium viz., potassium sulphate and potassium chloride were used. The treatments were as follows:

1 Control 70 ppm K in standing water
2 Potassium sulphate, 100 ppm K in standing water.
3 Potassium sulphate, 150 ppm K in standing water
4 Potassium sulphate, 200 ppm K in standing water
5 Potassium chloride, 100 ppm K in standing water
6 Potassium chloride, 150 ppm K in standing water
7 Potassium chloride, 200 ppm K in standing water

This experiment was replicated thrice with two strains of A. pinnata viz., Rajendranagar and Cuttack Strains. Earthern - ware pots of size 20 x 30 cm were used. A polythene sheet was placed in the pot before adding 2 kg of black soil to prevent seepage of water. The soil in every pot was treated with 2.5 g of single superphosphate and 5 mg of carbofuran in addition to 100 g of cowdung. The amounts of potassium sulphate and potassium chloride added to the pots to obtain the required concentration of K in four litres of standing water is given in Table 1. The level of water was maintained constant throughout the study.

Table 1: Amounts of potassium sulphate and potassium chloride added to four litres of standing water to raise the concentration of K from 70 ppm to required levels (mg/4 l).

Source	Concentration (ppm)		
	100	150	200
Potassium sulphate	268.07	714.87	1161.66
Potassium chloride	229.40	611.77	994.13

The ferns were inoculated at the rate of 10 g in each of the pots and they were removed with a sieve at every 7 days interval and fresh weight of the fronds was determined after completely draining the excess water. One gram of sub-sample from the biomass was taken for moisture determination in order to compute the dry weight. From the dry weight at different intervals the relative growth rate (RGR) was calculated as per the formula given by Watson (1952).

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where, W_1 and W_2 represent the phytomass at the time intervals of t_1 and t_2 respectively.

Samples of _Azolla_ were collected after the last sampling at the end of nine weeks. The samples were dried at $60^{o}C$ to constant weight and pulversied in a stainless steel mill (Micro grinder) and analysed for nitrogen and potassium contents. The nitrogen in _Azolla_ was estimated by H_2O_2 digestion followed by steam distillation (A.O.A.C, 1960) whereas for potassium it was extracted by wet ashing with $HNO_3 : H_2SO_4 : HclO_4$ (9:2:1) and was estimated by flame photometer (Muhr _et al_., 1965).

The data were statistically analysed as per the procedure suggested by Rao (1983) for factorial RBD.

3.1.2 Effect of different levels of calcium application on the growth and uptake of calcium by *Azolla*

In order to study the effect of calcium on the growth and uptake of calcium by *Azolla pinnata* strains, an experiment was conducted under green house conditions by inoculating a known quantity of fresh *A. pinnata* strains and following the rate of growth at periodical intervals from 17th July to 11th September 1990. Earthern-ware pots of size 20 x 30 cm were used and 2 kg of black soil was added to each pot. The soil in the pot was treated with 2.5 g single superphosphate and 5 mg carbofuran in addition to 100 g of cow dung. The amount of calcium carbonate added to the pots to obtain the required concentration of Ca in 4 litres of standing water is given in Table 2. The level of water was maintained constant throughout the study. The treatments were as follows:

1 control, 44 ppm Ca in standing water

2 Calcium carbonate, 80 ppm Ca in standing water

3 Calcium carbonate, 120 ppm Ca in standing water.

Table 2: Amounts of Calcium carbonate added to four litres of standing water to raise the concentration of Ca from 44 ppm to required levels (mg/4 l)

Source	Concentration (ppm)	
	80	120
Calcium carbonate	360	760

There were three replications. Two strains of <u>A. pinnata</u> viz., Rajendranagar and Cuttack strains were used. The ferns were inoculated at the rate of 10 g in each of the pots and they were removed with a seive at every 7 days interval and fresh weight of the fronds was determined after completely draining the excess water. One gram of sub-sample from the biomass was taken for moisture determination in order to compute the dry weight. From the dry weight at different intervals the relative growth rate (RGR) was calculated as outlined previously.

Samples of <u>Azolla</u> were collected after final observation at the end of eight weeks and were dried at $60^{o}C$ to constant weight. Later the samples were pulverised in a stainless steel mill (Micro grinder) and analysed for nitrogen and calcium. The nitrogen in <u>Azolla</u> was estimated by H_2O_2 digestion followed by steam distillation (A.O.A.C, 1960). Calcium in <u>Azolla</u>

was extracted by wet ashing with HNO_3 : H_2SO_4 : $HClO_4$ (10:0.5:2) and was later estimated by versanate method.

The data were statistically analysed as per the procedure suggested by Rao (1983) for factorial RBD.

3.1.3 Effect of different levels of sulphur application on growth and uptake of sulphur by Azolla

This experiment was conducted to study the effect of sulphur on the growth and uptake of sulphur by Azolla pinnata strains under green house conditions. A known quantity of fresh Azolla pinnata strains was inoculated and the rate of growth was followed at weekly intervals from 17th July to 11th September, 1990 for eight weeks. Earthern-ware pots of size 20 x 30 cm were used and 2 kg of black soil was added to each pot. The soil in the pot was treated with 2.5 g of single superphosphate and 5 mg carbofuran in addition to 100 g of cowdung. The amount of elemental sulphur added to the pots to obtain the required concentration of S in 4 litres of standing water is given in Table 3. The level of water was maintained constant throughout the study. The treatments were as follows:

1 Control, 12 ppm S in standing water

2 Elemental sulphur, 25 ppm S in standing water

3 Elemental sulphur, 50 ppm S in standing water

Table 3: Amounts of Elemental sulphur added to four litres of standing water to raise the concentration of S from 12 ppm to required levels (mg/4 l)

Source	Concentration (ppm)	
	25	50
Elemental sulphur	62	179

The experiment was replicated thrice. Two strains of *A. pinnata* viz., Rajedranagar and Cuttack strains were used. The procedure followed for recording growth and RGR was same as that followed for calcium experiment.

The sulphur in *Azolla* was estimated by turbidimetric method (Chesnin and Yein, 1951), whereas nitrogen was estimated by H_2O_2 digestion followed by steam distillation (A.O.A.C, 1960).

The data were statistically analysed as per the procedure suggested by Rao (1983) for factorial RBD.

3.1.4 Effect of different levels of magnesium application on growth and uptake of magnesium by *Azolla*

To study the effect of magnesium on the growth and uptake of magnesium by *Azolla pinnata* strains, an experiment was conducted under green house conditions by inoculating a known quantity of fresh <u>Azolla pinnata</u> strains and following the rate of growth at periodical intervals from 17th July to 11th September, 1990 for eight weeks. Earthern-ware pots of size 20 x 30 cm were used and 2 kg of black soil was added to each pot. The soil in the pot was treated with 2.5 g of single superphosphate and 5 mg carbofuran and 100 g of cowdung. The amount of magnesium carbonate added to the pots to obtain the required concentration of mg in 4 litres of standing water is given in Table 4. The level of water was maintained constant throughout the study. The treatments were as follows:

1 control, 48 ppm mg in standing water

2 Magnesium carbonate, 60 ppm mg in standing water

3 Magnesium carbonate, 80 ppm mg in standing water.

Table 4: Amounts of magnesium carbonate added to four litres of standing water to raise the concentration of Mg from 48 ppm to required levels (mg/4 l).

Source	Concentration (ppm)	
	60	80
Magnesium carbonate	126	336

There were three replications. Two strains of <u>A. pinnata</u> viz., Rajendranagar and Cuttack strains were used.

The procedure followed for recording growth and RGR was same as that followed for calcium experiment.

The magnesium in *Azolla* was estimated by versanate method whereas nitrogen was estimated by H_2O_2 digestion followed by steam distillation (A.O.A.C, 1960).

The data were statistically analysed as per the procedure suggested by Rao (1983) for factorial RBD.

3.2 EFFECT OF APPLICATION OF *AZOLLA* AS A GREEN MANURE ON THE AVAILABILITY OF POTASSIUM IN SOIL

This experiment was conducted under greenhouse conditions in pots using 5 kg of red soil (Alfisol) collected from the cultivated field around Rajendranagar.

Table 5: Physico-chemical analysis of soil used in the experiment

S.No	Particulars	
1	Mechanical composition	
	Sand	58.7%
	Silt	24.2%
	Clay	17.0%
2	Texture	Sandy Loam
3	pH	7.98
4	EC	$0.3 dsm^{-1}$
5	Available K_2O (kg ha^{-1})	156
6	Soil type	Alfisol (Red sandy loam)

The source of Azolla for this experiment was taken from the previous potassium experiment which consisted of the following treatments.

1 Control, 70 ppm K in standing water

2 Potassium sulphate, 100 ppm, K in standing water

3 Potassium sulphate, 150 ppm K in standing water

4 Potassium sulphate, 200 ppm K in standing water

5 Potassium chloride, 100 ppm K in standing water

6 Potassium chloride, 150 ppm K in standing water

7 Potassium chloride, 200 ppm K in standing water

This experiment also had seven treatments with three replications for two strains of A. pinnata viz., Rajendranagar and Cuttack.

Earthern-ware pots of 45 x 20 cm size were taken and filled with sieved and processed red soil. The Azolla from seven treatments of the previous potassium experiment was added to their respective pots at the rate of 50 g/ 5 kg i.e., 20 t ha^{-1}. Azolla was mixed well with the soil and was maintained at puddled conditions. The available potassium in the soil was estimated at 20 days interval for 80 days by flame photometer method (Jackson, 1967).

The data were statistically analysed as per the procedure suggested by Rao (1983) for factorial RBD.

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RESULTS

CHAPTER IV

RESULTS

4.1 EFFECT OF DIFFERENT SOURCES AND LEVELS OF POTASSIUM ON GROWTH AND UPTAKE OF POTASSIUM BY AZOLLA

4.1.1 Growth and dry matter production

The growth and dry matter production of *A. pinnata* (Rajendranagar strain) and *A. pinnata* (Cuttack strain) were studied under green house conditions and RGR calculated at different periods are given in Tables 6 and 7.

There was a sharp increase in the dry weight immediately after inoculation in all the treatments. This was higher in Rajendranagar strain compared to Cuttack strain. The dry matter production in all the treatments increased upto 9th week in both the strains. Among the treatments, potassium sulphate at 200 ppm gave the highest dry matter production in both the strains viz., Rajendranagar strain (17.90 g pot^{-1}) and Cuttack strain (15.49 g pot^{-1}).

The dry matter production was higher in potassium sulphate treatments compared to potassium chloride treatments for both the strains. At any level of potassium, Rajendranagar strain responded better in

Table 6: Effect of potassium on dry matter production (g pot^{-1}) of Azolla

Sampling intervals (days)	Control (70 ppm)	*Azolla pinnata* (Cuttack strain)						Control (70 ppm) K	*Azolla pinnata* (Rajendranagar strain)					
		Potassium sulphate			Potassium chloride				Potassium sulphate			Potassium chloride		
		100 ppm K	150 ppm K	200 ppm K	100 ppm K	150 ppm K	200 ppm K		100 ppm K	150 ppm K	200 ppm K	100 ppm K	150 ppm K	200 ppm K
0	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
7	1.32	1.60	1.98	2.14	1.92	2.22	2.21	1.46	1.84	2.13	2.35	1.70	2.06	2.24
14	2.74	3.12	3.72	4.01	3.50	4.24	4.51	2.89	3.95	4.84	5.12	3.70	4.45	4.98
21	4.24	5.15	6.13	6.48	4.98	6.35	6.49	4.40	6.20	7.39	7.98	5.95	7.19	8.44
28	6.12	7.39	8.74	9.03	6.94	8.44	8.13	6.17	8.98	10.31	10.82	8.14	9.64	11.22
35	7.61	9.19	11.14	11.22	8.59	10.08	9.85	7.99	10.40	12.32	12.32	9.18	11.96	13.46
42	8.90	10.99	13.01	13.00	9.94	11.35	11.22	9.70	12.13	15.64	15.11	11.45	13.69	14.76
49	9.93	12.16	13.99	14.13	10.60	12.33	12.64	10.85	13.24	16.84	16.60	12.50	14.89	15.48
56	10.54	12.94	14.73	15.10	11.44	13.16	13.53	11.74	14.21	17.22	17.45	13.40	15.49	15.91
63	10.76	13.40	15.12	15.49	11.96	13.61	13.82	12.09	14.73	17.43	17.90	13.83	15.78	16.31

Table 7: Effect of potassium on the relative growth rate (RGR) ($g\ g^{-1}\ day^{-1}$) of Azolla

Sampling intervals (days)	Control (70 ppm)	*Azolla pinnata* (Cuttack strain)						Control (70 ppm) K	*Azolla pinnata* (Rajendranagar strain)					
		Potassium sulphate			Potassium chloride				Potassium sulphate			Potassium chloride		
		100 ppm K	150 ppm K	200 ppm K	100 ppm K	150 ppm K	200 ppm K		100 ppm K	150 ppm K	200 ppm K	100 ppm K	150 ppm K	200 ppm K
0-7	0.081	0.108	0.139	0.150	0.134	0.155	0.154	0.098	0.128	0.149	0.163	0.116	0.144	0.156
7-14	0.104	0.095	0.090	0.090	0.086	0.092	0.102	0.098	0.109	0.117	0.111	0.111	0.110	0.114
14-21	0.062	0.072	0.071	0.069	0.050	0.058	0.052	0.060	0.064	0.060	0.063	0.068	0.069	0.175
21-28	0.052	0.052	0.050	0.047	0.047	0.041	0.032	0.048	0.053	0.048	0.043	0.044	0.041	0.040
28-35	0.031	0.031	0.035	0.031	0.030	0.025	0.027	0.037	0.021	0.025	0.018	0.017	0.030	0.026
35-42	0.022	0.025	0.057	0.021	0.021	0.017	0.019	0.028	0.022	0.034	0.029	0.032	0.019	0.013
42-49	0.016	0.014	0.009	0.011	0.009	0.012	0.017	0.016	0.013	0.010	0.013	0.013	0.012	0.007
49-56	0.009	0.009	0.007	0.009	0.011	0.009	0.010	0.011	0.010	0.003	0.007	0.010	0.006	0.004
56-63	0.003	0.005	0.001	0.004	0.006	0.005	0.003	0.004	0.005	0.002	0.004	0.005	0.003	0.004

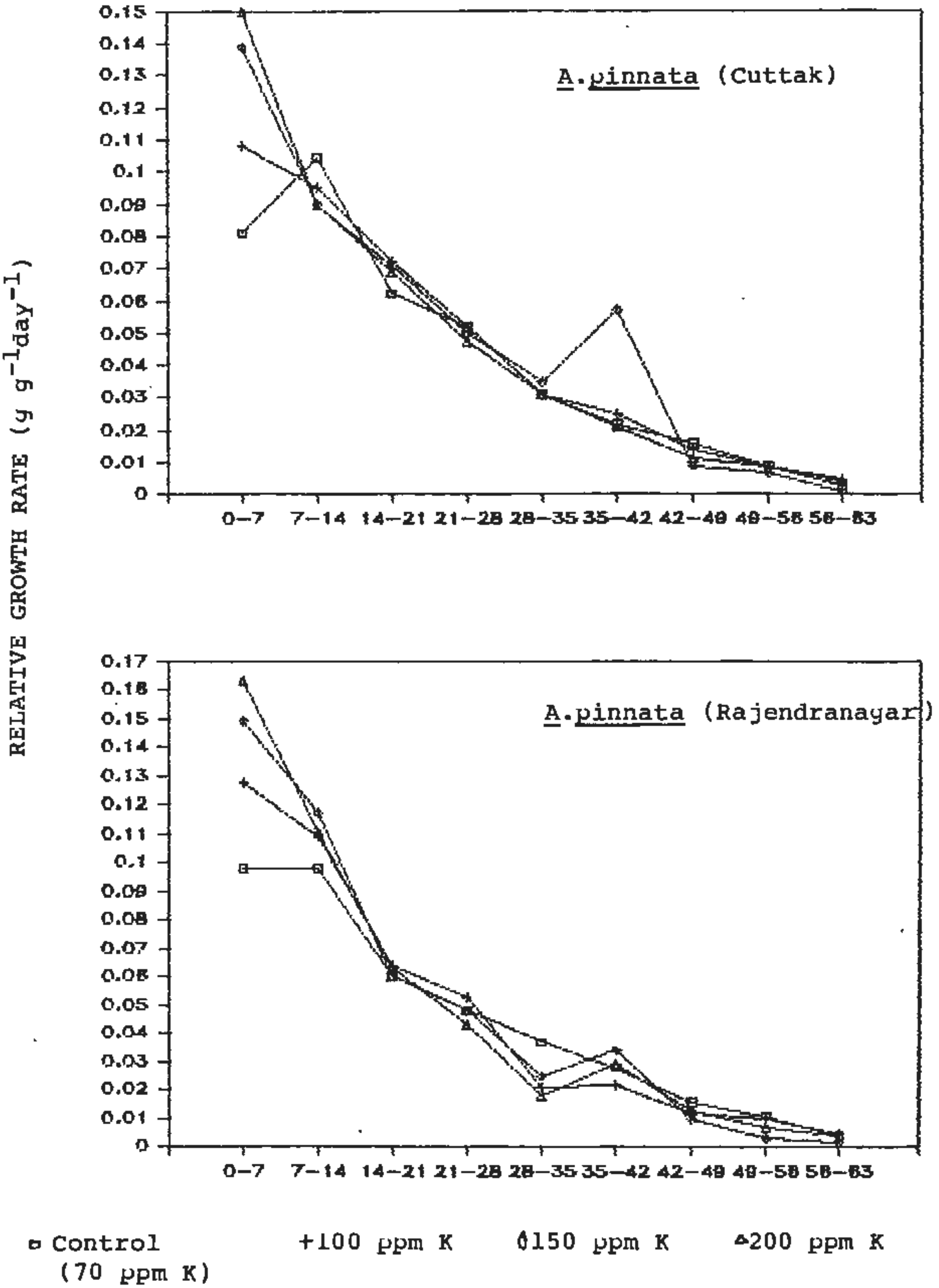
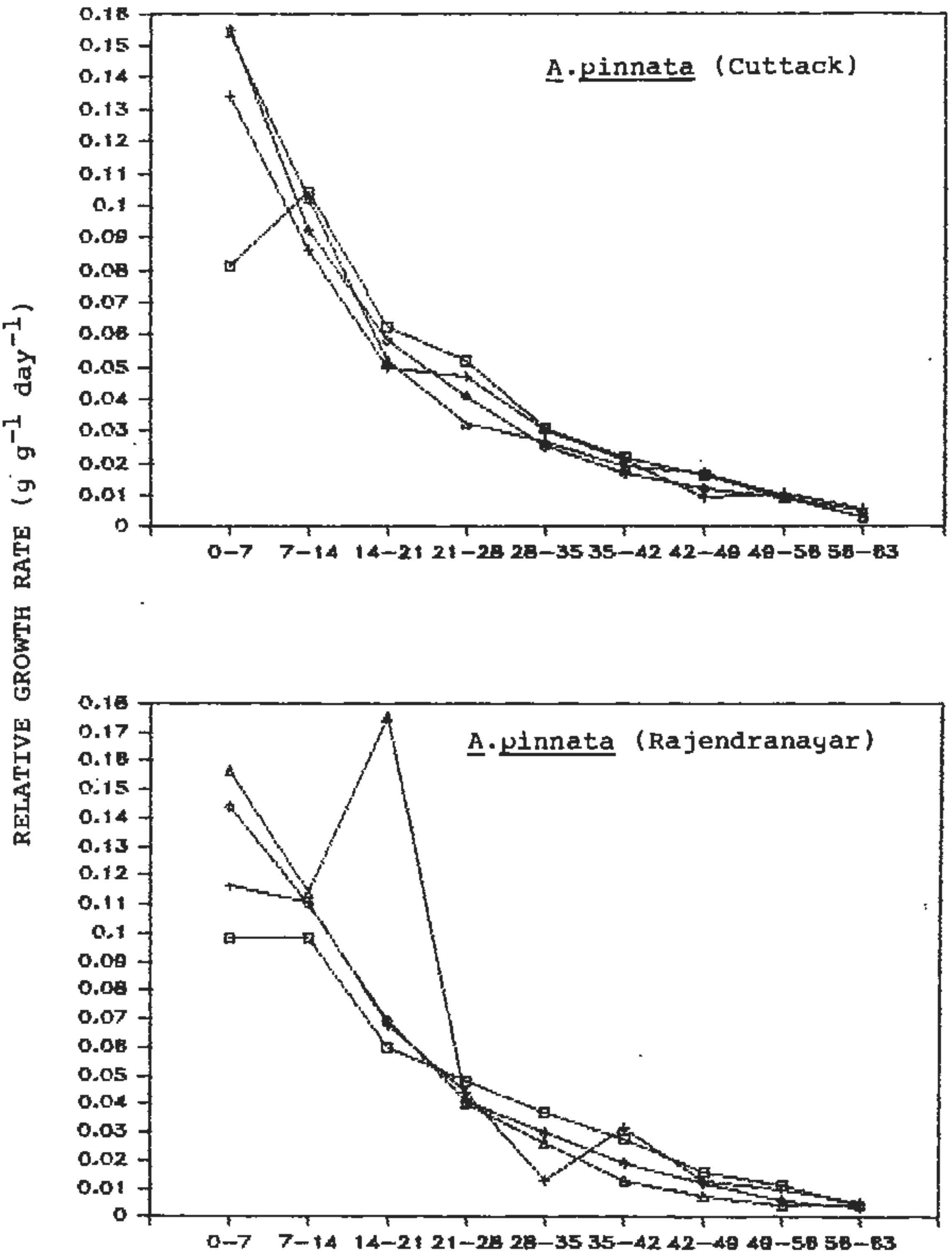


FIG.1 : EFFECT OF POTASSIUM SULPHATE ON THE RELATIVE GROWTH RATE OF AZOLLA.



□ Control (70 ppm K) +100 ppm K ◊150 ppm K △200 ppm K

FIG.2: EFFECT OF POTASSIUM CHLORIDE ON THE RELATIVE GROWTH RATE OF AZOLLA.

terms of dry matter yields than Cuttack strain.

The RGR was also found to be high in Rajendranagar strain compared to Cuttack strain. The highest RGR was recorded after first seven days with Rajendranagar strain (0.163 g g^{-1} day^{-1}) at 200 ppm potassium sulphate. The RGR decreased with time for all the treatments.

There was significant difference between the dry weight of two A. pinnata strains at the end of the experiment as seen in Table 8. Rajendranagar strain recorded higher dry weight (15.42 g pot^{-1}) which was significantly superior to Cuttack strain (13.45 g pot^{-1}). The increase in dry weight of Rajendranagar strain was 12.8 per cent over Cuttack strain. Among the potassium sulphate treatments, there was significant increase in dry weight upto 150 ppm K level while it was on par with 200 ppm K. In potassium chloride treatments also, there was a significant increase in dry weight from 70 to 150 ppm K. The increase in dry matter production with 150 ppm K was 43.2 per cent and 29.3 per cent higher over control for potassium sulphate and potassium chloride respectively. With regard to sources, potassium sulphate gave higher dry matter yields than potassium chloride for all the levels.

Table 8 : Effect of different sources and levels of potassium on dry matter production (g pot^{-1}) of *Azolla*

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (70 ppm K)	11.99	10.76	11.37
T_1 (Potassium sulphate 100 ppm K)	14.73	13.40	14.06
T_2 (Potassium sulphate 150 ppm K)	17.43	15.12	16.28
T_3 (Potassium sulphate 200 ppm K)	17.90	15.50	16.70
T_4 (Potassium chloride 100 ppm K)	13.83	11.96	12.90
T_5 (Potassium chloride 150 ppm K)	15.78	13.61	14.69
T_6 (Potassium chloride 200 ppm K)	16.31	13.82	15.07
Mean	15.42	13.45	

C.D(0.05)

Strains	0.473
Treatments	0.884
Interaction	NS

4.1.2 Uptake of nutrients

Based on per cent composition (Appendix 1) and dry matter yields the uptake of nutrients (N & K) in g pot^{-1} was computed under different treatments. The data are presented in Tables 9 and 10.

4.1.2.1 Uptake of nitrogen: The N uptake was 33.31 g pot^{-1} in control and 68.25 g pot^{-1} in T_3 treatment in Cuttack strain and in Rajendranagar strain it was 39.63 g pot^{-1} in control and 79.16 g pot^{-1} in T_2 treatment. The N uptake increased significantly with increase in levels of potassium upto 150 ppm for both the sources, whereas it was on par with 200 ppm potassium. Between the two sources, potassium sulphate recorded higher N uptake compared to potassium chloride for different levels. Potassium application recorded significantly more N uptake with Rajendranagar strain (64.38 g pot^{-1}) compared to Cuttack strain (54.56 g pot^{-1}). The increase was about 18 per cent. The interaction effects between A. pinnata strains and treatments were found to be non significant.

4.1.2.2 Uptake of potassium: The uptake of K ranged from 23.97 g pot^{-1} in control to 71.14 g pot^{-1} in T_3 treatment in Cuttack strain. In Rajendranagar strain it ranged from 29.67 g pot^{-1} in control to 86.51 g pot^{-1} in T_3 treatment. Uptake of K was significantly

Table 9 : Effect of application of potassium on uptake of nitrogen by *Azolla* (g pot^{-1}).

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (70 ppm K)	39.63	33.31	36.47
T_1 (Potassium sulphate 100 ppm K)	58.69	52.50	55.60
T_2 (Potassium sulphate 150 ppm K)	79.16	66.26	72.71
T_3 (Potassium sulphate 200 ppm K)	79.06	68.25	73.66
T_4 (Potassium chloride 100 ppm K)	53.31	45.13	49.22
T_5 (Potassium chloride 150 ppm K)	69.02	57.31	63.17
T_6 (Potassium chloride 200 ppm K)	71.80	59.16	65.48
Mean	64.38	54.56	

C.D(0.05)

Strains	4.5
Treatments	8.41
Interaction	NS

Table 10: Effect of application of potassium on uptake of potassium by *Azolla* (g pot^{-1}).

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (70 ppm K)	29.67	23.97	26.82
T_1 (Potassium sulphate 100 ppm K)	52.06	46.61	49.34
T_2 (Potassium sulphate 150 ppm K)	82.90	65.42	74.16
T_3 (Potassium sulphate 200 ppm K)	86.51	71.14	78.83
T_4 (Potassium chloride 100 ppm K)	47.90	39.76	43.86
T_5 (Potassium chloride 150 ppm K)	69.52	56.32	63.02
T_6 (Potassium chloride 200 ppm K)	73.92	58.68	66.30
Mean	63.22	51.73	

C.D(0.05)

Strains	3.329
Treatments	6.228
Interaction	NS

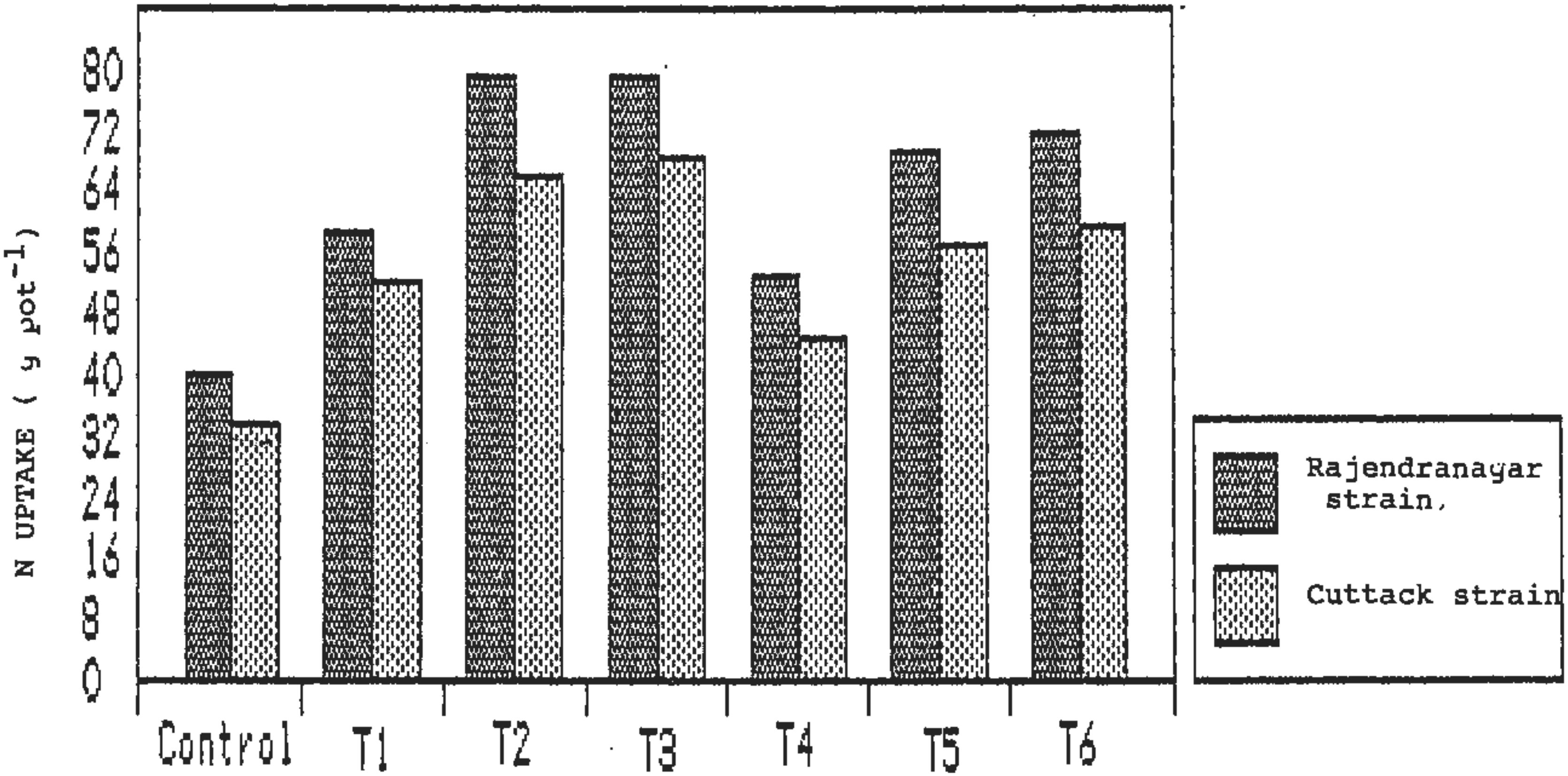


FIG.3 :EFFECT OF POTASSIUM ON UPTAKE OF NITROGEN BY AZOLLA.

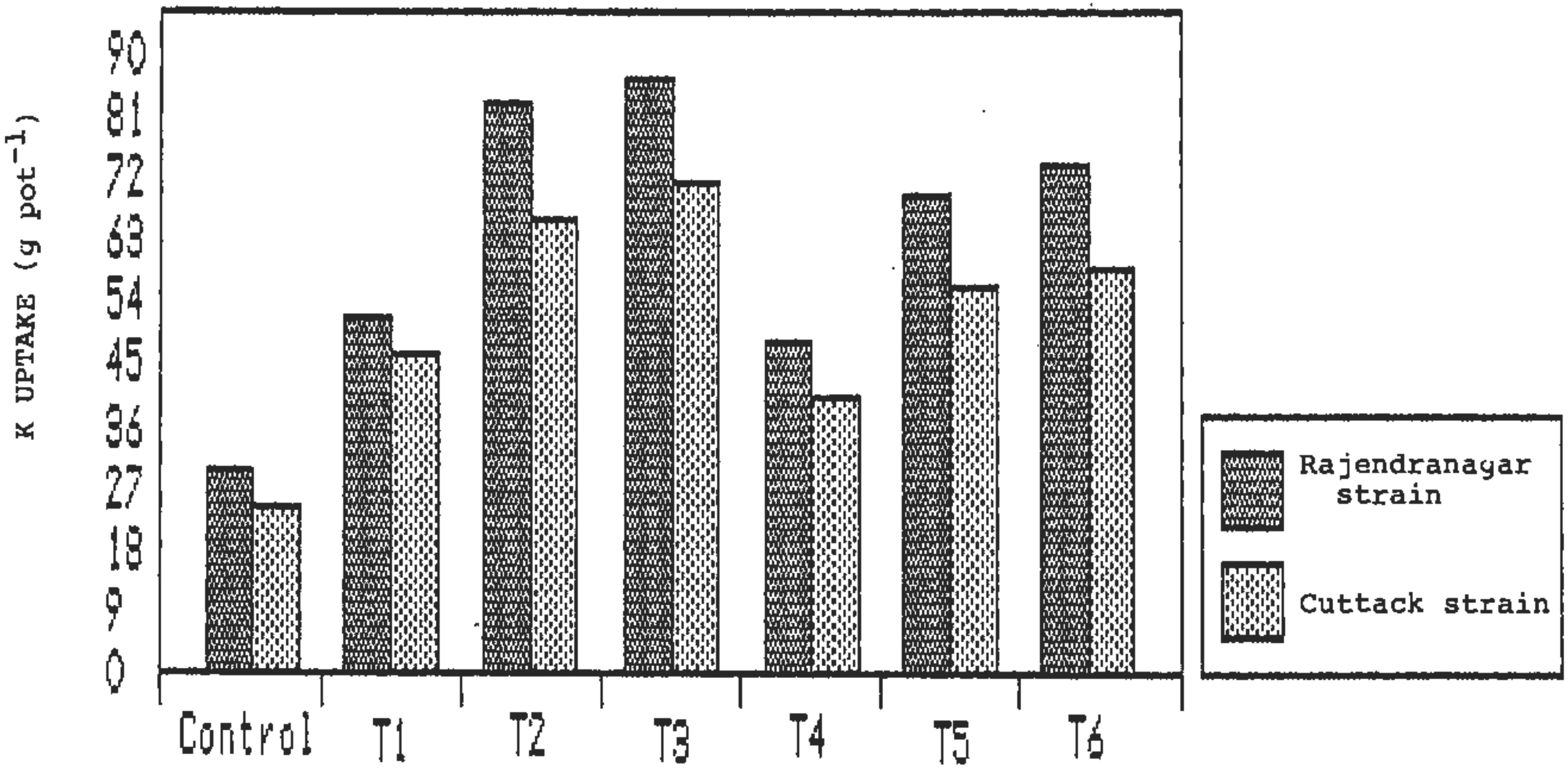


FIG.4: EFFECT OF POTASSIUM ON UPTAKE OF POTASSIUM AZOLLA.

more in Rajendranagar strain (63.22 g pot^{-1}) than in Cuttack strain (51.73 g pot^{-1}. Application of potassium significantly increased the K uptake upto 150 ppm K for both the sources but was on par with 200 ppm K. Uptake of K was higher with potassium sulphate than potassium chloride for all the levels. The interaction effects between the strains of A. pinnata and treatments were found to be non significant.

4.2 EFFECT OF DIFFERENT LEVELS OF CALCIUM APPLICATION ON GROWTH AND UPTAKE OF CALCIUM BY AZOLLA

4.2.1 Growth and dry matter production

The growth and dry matter production of two A. pinnata strains were studied under green house conditions and relative growth rate (RGR) calculated at different periods are given in Tables 11 and 12.

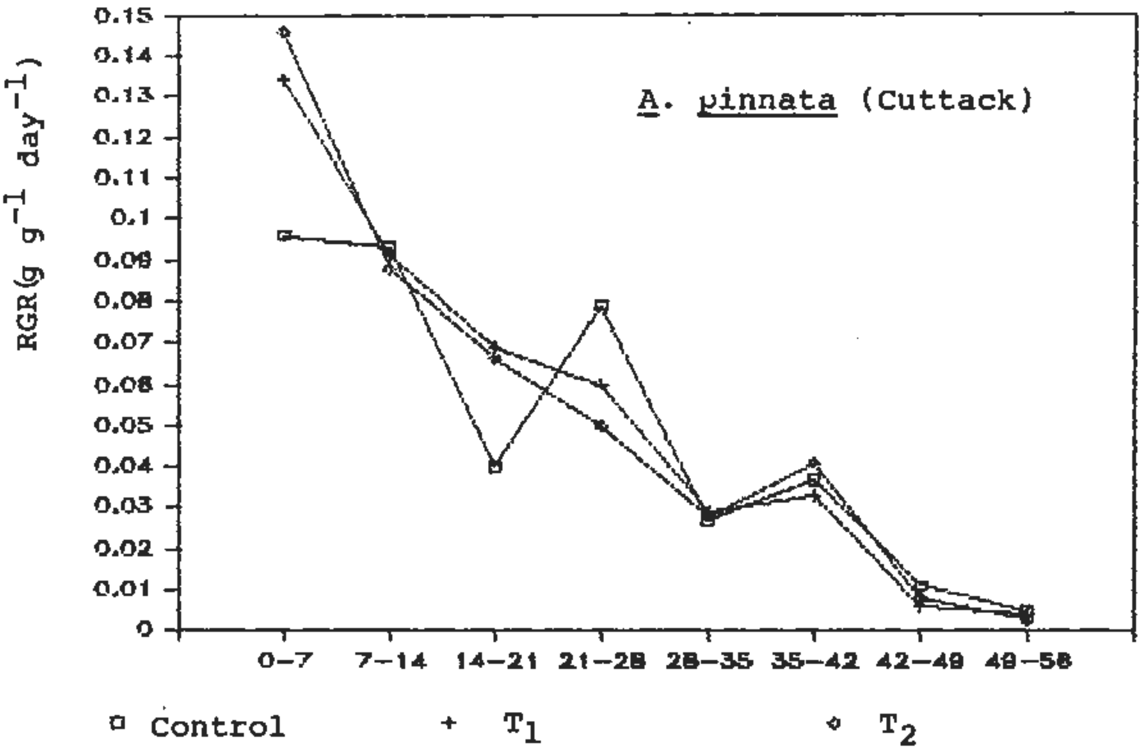
The dry weight increased sharply after inoculation which was higher in Rajendranagar strain compared to Cuttack strain. The dry matter production in all the treatments increased upto 8th week in both the strains. The highest dry matter production was observed with 120 ppm calcium in both the strains of A. pinnata viz., Rajendranagar strain (17.35 g pot^{-1}) and Cuttack strain (15.19 g pot^{-1}). Rajendranagar strain gave higher dry matter yields than Cuttack strain for

Table 11: Effect of calcium on dry matter production (g pot^{-1}) of Azolla

Sampling intervals (days)	*Azolla pinnata* (Cuttack strain)			*Azolla pinnata* (Rajendranagar strain)		
	Control (44 ppm Ca)	T_1 (Calcium carbonate 80 ppm Ca)	T_2 (Calcium carbonate 120 ppm Ca)	Control (44 ppm Ca)	T_1 (Calcium carbonate 80 ppm Ca)	T_2 (Calcium carbonate 120 ppm Ca)
0	0.75	0.75	0.75	0.75	0.75	0.75
7	1.47	1.92	2.09	1.62	2.64	2.29
14	2.82	3.64	3.86	3.33	4.39	4.09
21	3.72	5.88	6.11	6.32	7.38	6.94
28	6.48	8.97	8.68	7.68	8.80	10.10
35	7.84	10.99	10.54	9.63	11.97	12.55
42	10.16	13.83	14.06	10.61	15.50	15.80
49	10.98	14.44	14.89	11.89	16.77	16.98
56	11.36	14.89	15.19	12.33	17.13	17.35

Table 12: Effect of calcium on the relative growth rate (RGR) ($g\ g^{-1}\ day^{-1}$) of Azolla

Sampling intervals (days)	*Azolla pinnata* (Cuttack strain)			*Azolla pinnata* (Rajendranagar strain)		
	Control (44 ppm Ca)	T_1 (Calcium carbonate 80 ppm Ca)	T_2 (Calcium carbonate 120 ppm Ca)	Control (44 ppm Ca)	T_1 (Calcium carbonate 80 ppm Ca)	T_2 (Calcium carbonate 120 ppm Ca)
0–7	0.096	0.134	0.146	0.110	0.150	0.159
7–14	0.093	0.091	0.088	0.102	0.103	0.083
14–21	0.040	0.069	0.066	0.092	0.074	0.076
21–28	0.079	0.060	0.050	0.033	0.025	0.054
28–35	0.027	0.029	0.028	0.032	0.044	0.031
35–42	0.037	0.033	0.041	0.013	0.037	0.033
42–49	0.011	0.006	0.008	0.016	0.011	0.010
49–56	0.005	0.004	0.003	0.005	0.003	0.003



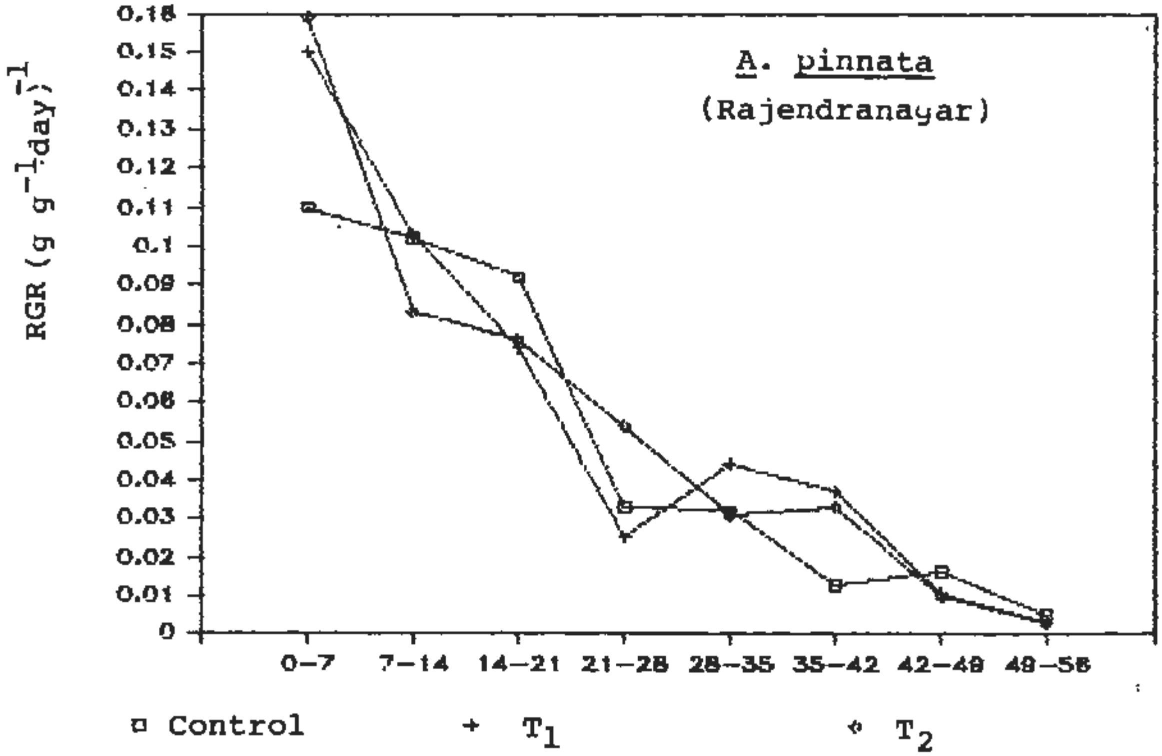


FIG.5 : EFFECT OF CALCIUM ON THE RELATIVE GROWTH RATE OF AZOLLA.

all the levels of calcium.

The highest RGR was recorded after first seven days with Rajendranagar strain (0.159 g g^{-1} day^{-1}) at 120 ppm calcium. Rajendranagar strain recorded higher values than Cuttack strain. The RGR decreased over a period of time for all the treatments.

Table 13 shows the significant difference between the dry weight of two _A. pinnata_ strains at the end of the experiment. Rajendranagar strain recorded higher dry weight production (15.61 g pot^{-1}) which was significantly superior to Cuttack strain (13.81 g pot^{-1}). Among the treatments, there was significant increase in dry weight upto 80 ppm calcium level while it was on par with 120 ppm Ca. The increase in dry weight production with 80 ppm calcium was 37.4 per cent over control.

4.2.2 Uptake of nutrients

The uptake of nutrients (N & Ca) computed based on per cent composition (Appendix 2) and dry matter yield of two _A. pinnata_ strains under different treatments are presented in Tables 14 and 15.

4.2.2.1 Nitrogen uptake: The uptake of N ranged from 35.27 g pot^{-1} in control to 63.55 g pot^{-1} in T_2 treatment in Cuttack strain. In Rajendranagar strain it

Table 13: Effect of different levels of calcium on dry matter production (g pot^{-1}) of *Azolla*

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (44 ppm Ca)	12.33	11.35	11.84
T_1 (Calcium corbonate 80 ppm Ca).	17.14	14.89	16.01
T_2 (Calcium corbonate 120 ppm Ca)	17.35	15.19	16.27
Mean	15.61	13.81	

C.D(0.05)

Strains	1.630
Treatments	1.996
Interaction	NS

Table 14 : Effect of application of Calcium on uptake of nitrogen by *Azolla* (g pot^{-1})

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (44 ppm Ca)	38.64	35.27	36.96
T_1 (Calcium corbonate 80 ppm Ca).	71.15	61.44	66.30
T_2 (Calcium corbonate 120 ppm Ca)	74.28	63.55	68.92
Mean	61.36	53.42	

C.D(0.05)

Strains	6.81
Treatments	8.337
Interaction	NS

Table 15 : Effect of application of Calcium on uptake of calcium by *Azolla* (g pot^{-1})

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (44 ppm Ca)	6.38	5.44	5.91
T_1 (Calcium corbonate 80 ppm Ca).	16.87	13.84	15.36
T_2 (Calcium corbonate 120 ppm Ca)	17.80	14.94	16.37
Mean	13.68	11.41	

C.D(0.05)

Strains	1.65
Treatments	2.02
Interaction	NS

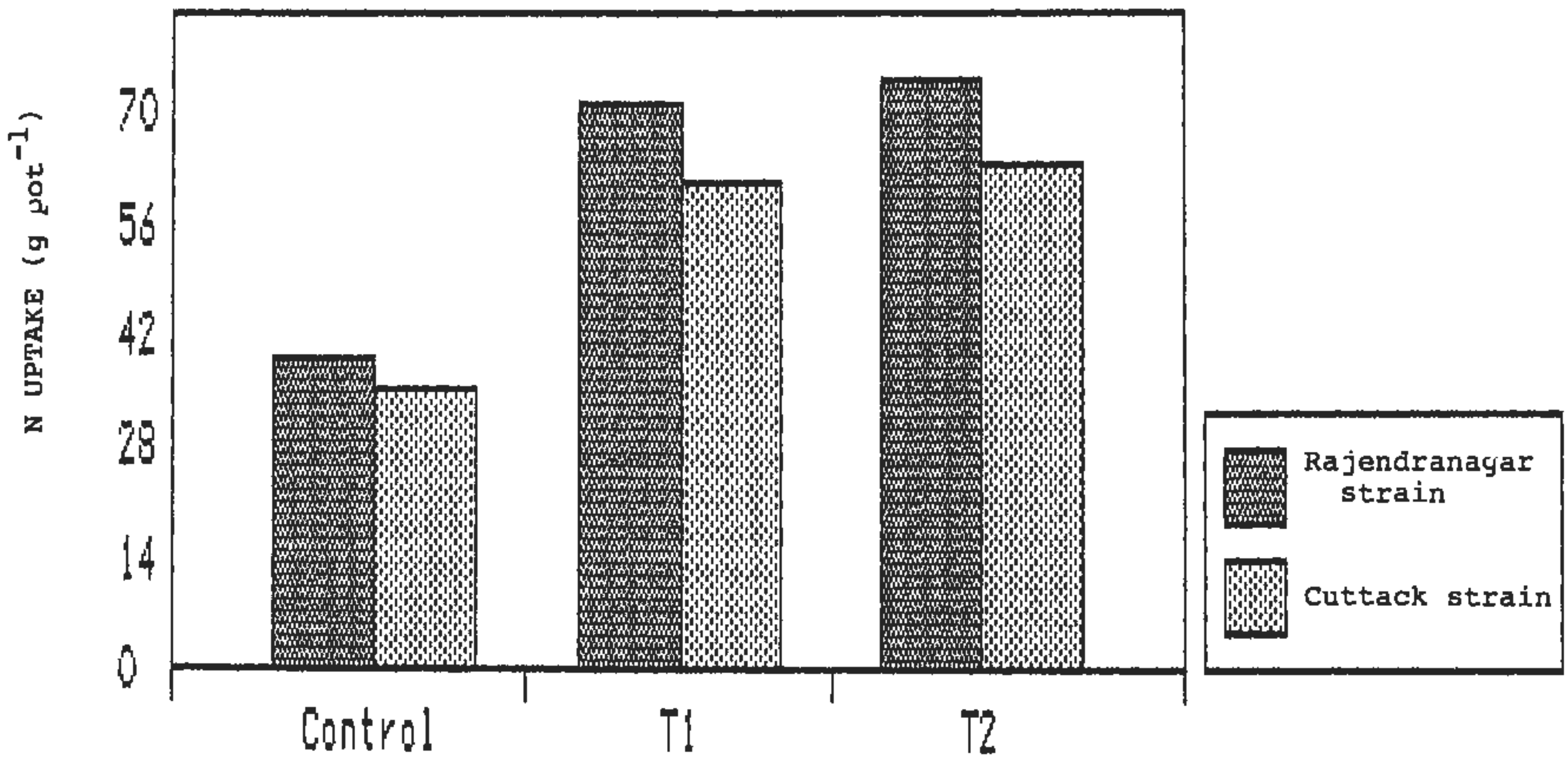


FIG.6: EFFECT OF CALCIUM ON UPTAKE OF NITROGEN BY AZOLLA.

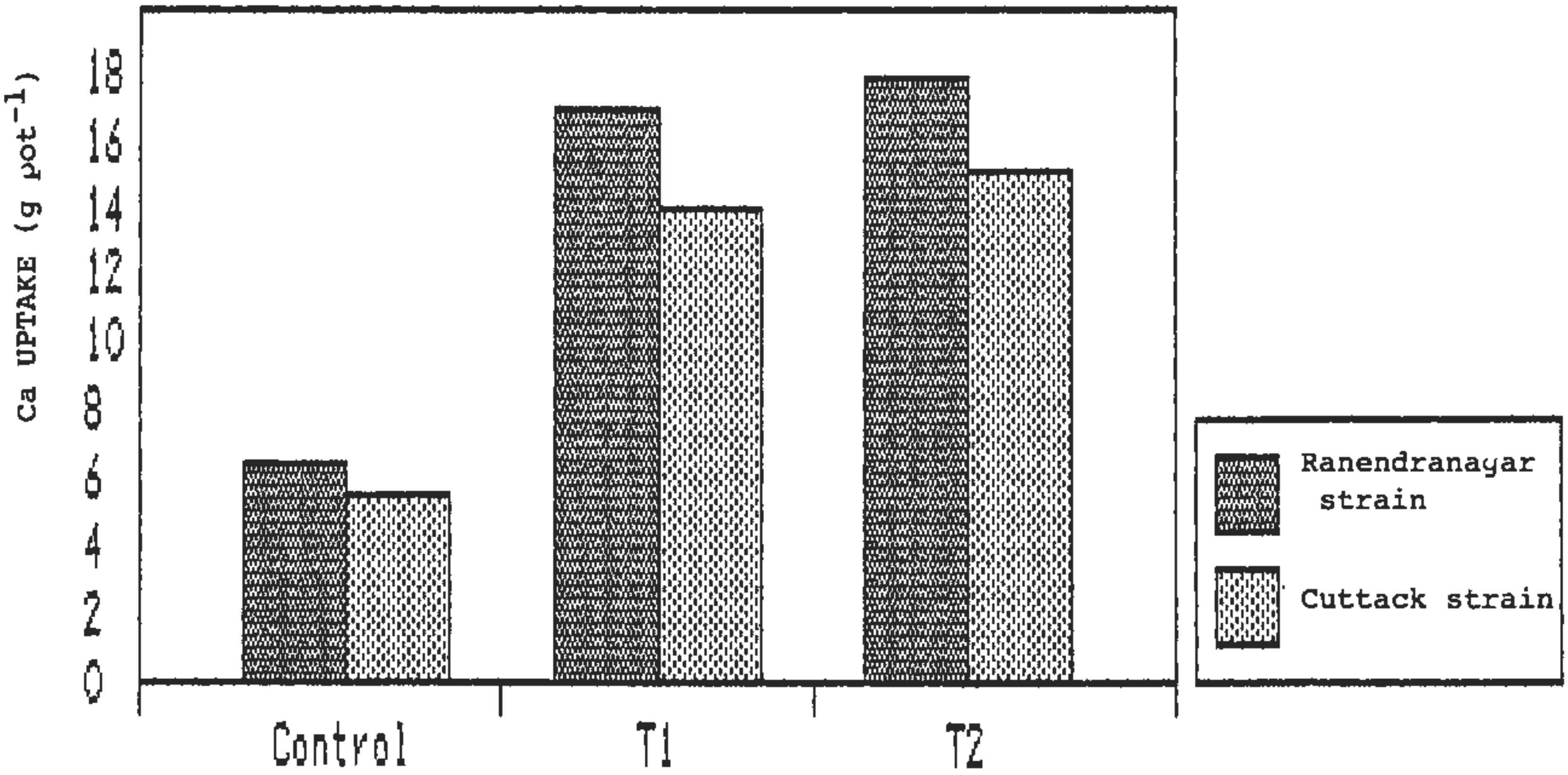


FIG.7 : EFFECT OF CALCIUM ON UPTAKE OF CALCIUM BY AZOLLA.

ranged from 38.64 g pot^{-1} in control to 74.28 g pot^{-1} in T_2 treatment. Uptake of N was significantly more in Rajendranagar strain (61.36 g pot^{-1}) than in Cuttack strain (53.42 g pot^{-1}). Application of calcium significantly increased the N uptake upto 80 ppm calcium but was on par with 120 ppm calcium. The interaction effects between two strains *A. pinnata* and treatments were non significant.

4.2.2.2 Calcium uptake: The calcium uptake was 5.54 g pot^{-1} in control and 14.94 g pot^{-1} in T_2 treatment in Cuttack strain and in Rajendranagar strain it was 6.38 g pot^{-1} in control and 17.80 g pot^{-1} in T_2 treatment. The calcium uptake increased significantly with increase in levels of calcium upto 80 ppm, but was on par with 120 ppm of calcium. Calcium application significantly increased the calcium uptake of Rajendranagar strain than in Cuttack strain. The interaction effects between the strains of *A. pinnata* and treatments were found to be non significant.

4.3 EFFECT OF DIFFERENT LEVELS OF MAGNESIUM APPLICATION ON GROWTH AND UPTAKE OF MAGNESIUM BY *AZOLLA*

4.3.1 Growth and dry matter production

The growth and dry matter production of Rajendranagar and Cuttack strains of *A. pinnata* were studied under green house conditions and RGR calculated at different periods are given in Tables 16 and 17.

The dry matter production in all the treatments increased upto 8th week in both the strains of *A. pinnata*. Rajendranagar strain recorded higher dry matter yields compared to Cuttack strain. Both the strains recorded highest dry matter production at 80 ppm magnesium viz., 12.71 g pot^{-1} and 11.36 g pot^{-1} for Rajendranagar and Cuttack strains, respectively.

Rajendranagar strain showed higher RGR values compared to Cuttack strain. The highest RGR was recorded with Rajendranagar strain at 80 ppm magnesium (0.127 g g^{-1} day^{-1}). The RGR decreased with time for all the treatments.

There was significant difference between the dry weights of two *A. pinnata* strains at the end of the experiment as seen in Table 18. Rajendranagar strain recorded higher dry matter production (12.41 g pot^{-1}) which was significantly superior to Cuttack strain (11.04 g pot^{-1}). Among the magnesium treatments there was non significant increase in dry matter production for all the levels.

Table 16: Effect of magnesium on dry matter production (g pot^{-1}) of Azolla

Sampling intervals (days)	*Azolla pinnata* (Cuttack strain)			*Azolla pinnata* (Rajendranagar strain)		
	Control (48 ppm Mg)	T_1 (Magnesium carbonate 60 ppm Mg)	T_2 (Magnesium carbonate 80 ppm Mg)	Control (48 ppm Mg)	T_1 (Magnesium carbonate 60 ppm Ca)	T_2 (Magnesium carbonate 80 ppm Ca)
0	0.75	0.75	0.75	0.75	0.75	0.75
7	1.47	1.56	1.71	1.33	1.48	1.83
14	3.11	3.27	3.11	3.20	2.96	3.45
21	5.62	5.73	5.44	4.84	4.68	6.88
28	6.94	7.17	7.61	7.09	7.25	8.49
35	8.45	8.67	8.98	9.19	9.33	10.39
42	9.50	10.09	10.46	11.05	11.38	11.58
49	10.30	10.77	11.06	11.87	11.96	12.27
56	10.69	11.08	11.36	12.11	12.42	12.71

Table 17: Effect of magnesium on the relative growth rate (RGR) (g g^{-1} day^{-1}) of Azolla

Sampling intervals (days)	*Azolla pinnata* (Cuttack strain)			Azolla pinnata (Rajendranagar strain)		
	Control (48 ppm Mg)	T_1 (Magnesium carbonate 60 ppm Mg)	T_2 (Magnesium carbonate 80 ppm Mg)	Control (48 ppm Mg)	T_1 (Magnesium carbonate 60 ppm Ca)	T_2 (Magnesium carbonate 80 ppm Ca)
0-7	0.096	0.105	0.118	0.082	0.097	0.127
7-14	0.107	0.106	0.085	0.125	0.099	0.092
14-21	0.084	0.080	0.080	0.059	0.065	0.097
21-28	0.031	0.032	0.048	0.056	0.063	0.030
28-35	0.028	0.027	0.024	0.037	0.036	0.029
35-42	0.016	0.022	0.022	0.026	0.028	0.015
42-49	0.012	0.009	0.008	0.010	0.007	0.008
49-56	0.005	0.004	0.004	0.003	0.005	0.005

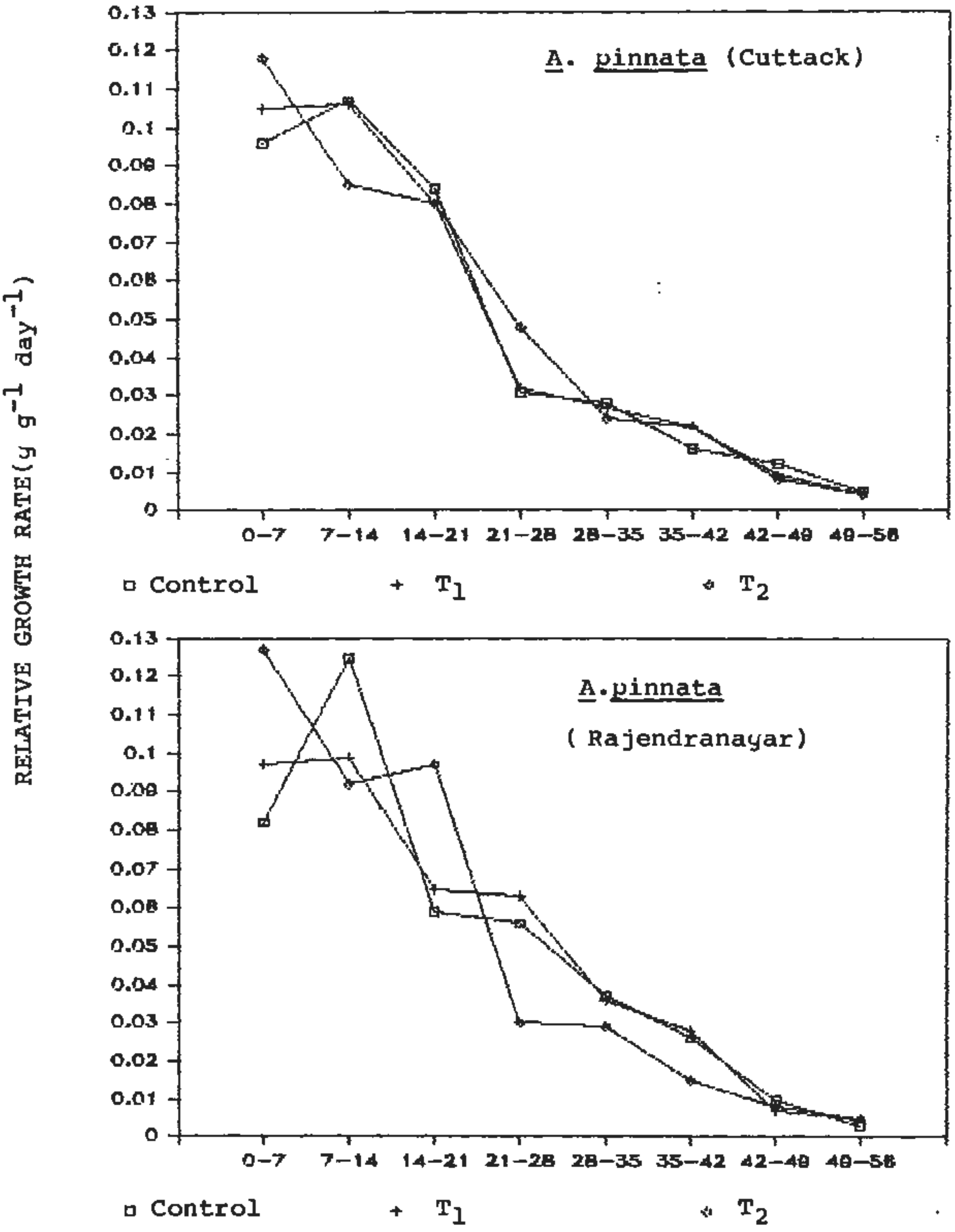


FIG.8 :EFFECT OF MAGNESIUM ON THE RELATIVE GROWTH RATE OF AZOLLA.

Table 18 : Effect of different levels of magnesium on dry matter production (g pot^{-1}) of *Azolla*

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (48 ppm Mg)	12.11	10.69	11.40
T_1 (Magnesium carbonate 60 ppm Mg).	12.42	11.09	11.75
T_2 (Magnesium carbonate 120 ppm Mg)	12.71	11.36	12.06
Mean	12.41	11.04	

C.D(0.05)

Strains	0.915
Treatments	NS
Interaction	NS

4.3.2 Uptake of nutrients

Based on per cent composition (Appendix 3) and dry matter yield, the uptake of nutrients (N & Mg) in g pot^{-1} was computed under different treatments are presented in Tables 19 and 20.

4.3.2.1 Nitrogen uptake: There was no significant increase in N uptake for all the treatments and also between the strains of <u>A. pinnata</u>. None of the interaction effects between strains of <u>A. pinnata</u> and treatments were significant.

4.3.2.2 Magnesium uptake: Uptake of magnesium was significantly more in Rajendranagar strain (3.91 g pot^{-1}) than in Cuttack strain (2.93 g pot^{-1}). The increase in magnesium uptake of Rajendranagar strain was 12.4 per cent higher than Cuttack strain. There was no significant increase among the treatments. All interaction effects between <u>A. pinnata</u> strains and treatments were found to be non significant.

4.4 EFFECT OF DIFFERENT LEVELS OF SULPHUR APPLICATION ON GROWTH AND UPTAKE OF SULPHUR BY <u>AZOLLA</u>

4.4.1 Growth and dry matter production

The growth and dry matter production of two <u>A. pinnata</u> strains were studied under green house condi-

Table 19 : Effect of application of magnesium on uptake of nitrogen by *Azolla* (g pot^{-1}).

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (48 ppm Mg)	37.01	33.53	35.27
T_1 (Magnesium carbonate 60 ppm Mg).	44.82	38.96	41.89
T_2 (Magnesium carbonate 120 ppm Mg)	45.90	40.82	43.36
Mean	42.58	37.77	

C.D(0.05)

Strains	NS
Treatments	NS
Interaction	NS

Table 20 : Effect of application of magnesium on uptake of magnesium by *Azolla* (g pot^{-1}).

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (48 ppm Mg)	3.42	2.58	3.00
T_1 (Magnesium carbonate 60 ppm Mg).	3.99	2.99	3.49
T_2 (Magnesium carbonate 120 ppm Mg)	4.32	4.39	3.77
Mean	3.91	2.93	

C.D(0.05)

Strains	0.50
Treatments	NS
Interaction	NS

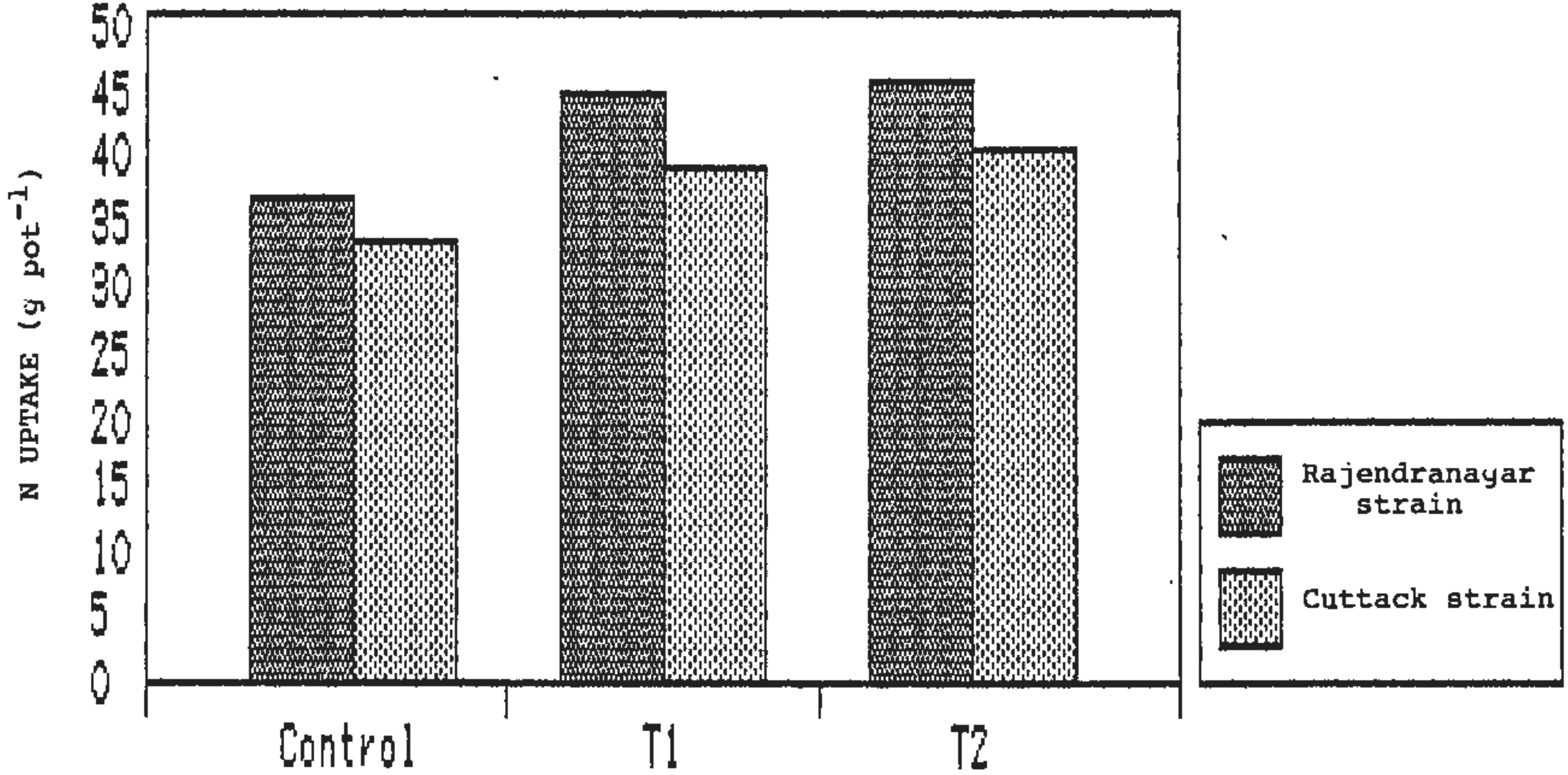


FIG.9 : EFFECT OF MAGNESIUM ON UPTAKE OF NITROGEN BY <u>AZOLLA</u>.

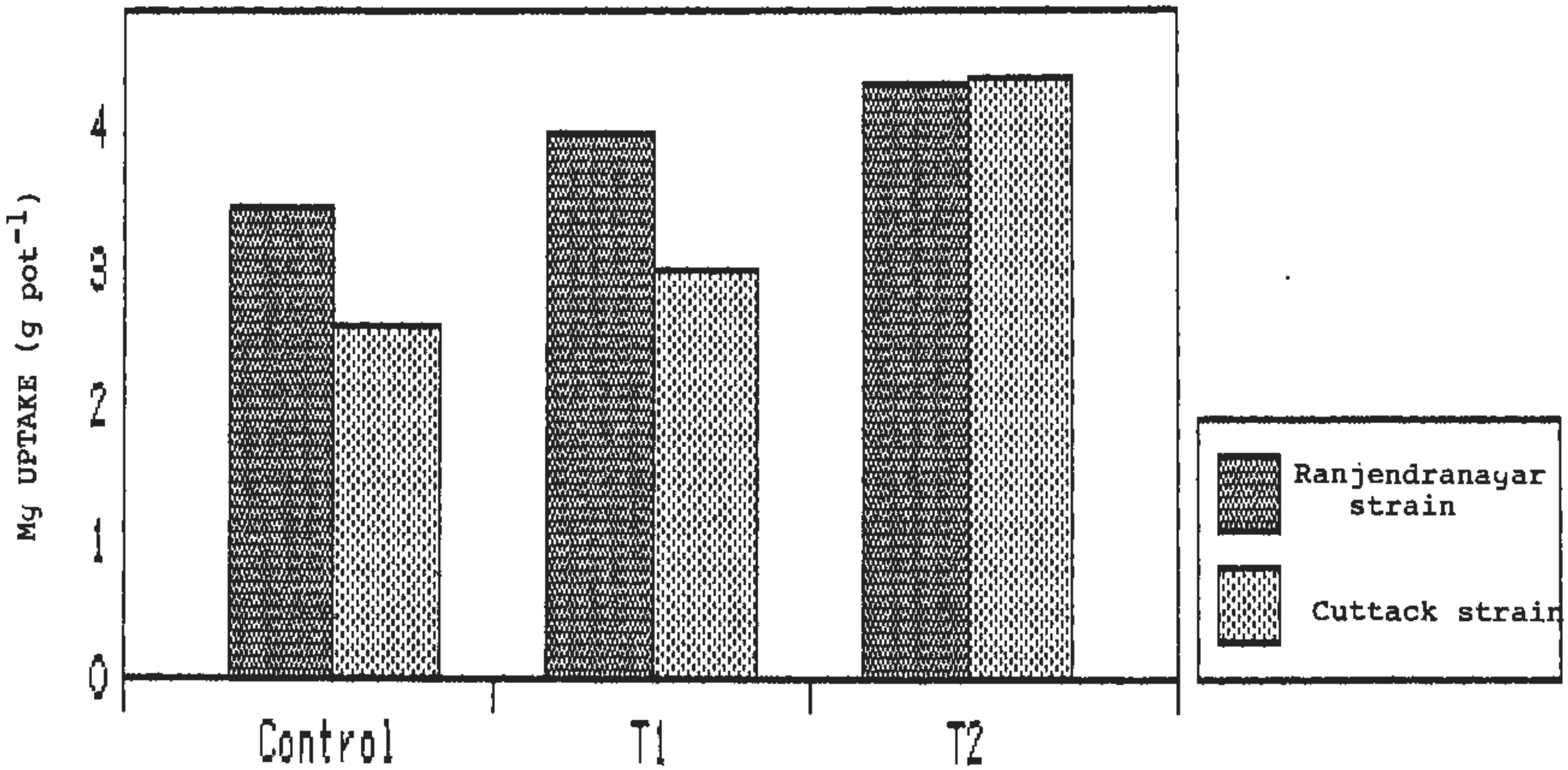


FIG.10 : EFFECT OF MAGNESIUM ON UPTAKE OF MAGNESIUM BY AZOLLA.

tions and RGR calculated at different periods are given in Tables 21 and 22.

The dry weight increased sharply after inoculation. Dry matter production in all the treatments increased upto 8th week in both the strains of *A. pinnata*. The highest dry matter production was observed with 50 ppm sulphur in both the strains of *A. pinnata* viz., Rajendranagar strain (15.27 g pot^{-1}) and Cuttack strain (13.61 g pot^{-1}). Rajendranagar strain gave higher dry matter yield than Cuttack strain for all the levels of sulphur.

The highest RGR was recorded after first seven days interval with Cuttack strain (0.150 gg^{-1} day^{-1}) at 50 ppm S. The RGR decreased with increase in time for all the treatments.

Table 23 shows the significant difference between the dry weight of two strains of *A. pinnata* at the end of 8th week. Rajendranagar strain recorded higher dry weight production (14.16 g pot^{-1}) which was significantly superior to Cuttack strain (12.64 g m pot^{-1}). Among the treatments, there was significant increase in dry weight upto 25 ppm S level while it was on par with 50 ppm S.

Table 21: Effect of sulphur on dry matter production (g pot^{-1}) of Azolla

Sampling intervals (days)	*Azolla pinnata* (Cuttack strain)			*Azolla pinnata* (Rajendranagar strain		
	Control (12 ppm S)	T_1 (Elemental sulphur 25 ppm S)	T_2 (Elemental sulphur 50 ppm S)	Control (12 ppm S)	T_1 (Elemental sulphur 25 ppm S)	T_2 (Elemental sulphur 50 ppm S)
0	0.75	0.75	0.75	0.75	0.75	0.75
7	1.39	1.83	2.14	1.35	1.84	2.00
14	2.96	3.56	4.39	2.74	3.94	4.23
21	5.81	6.93	7.83	4.48	6.91	7.99
28	7.38	8.35	10.61	7.37	10.39	10.83
35	8.96	10.68	11.89	8.75	13.19	12.35
42	9.91	12.32	12.70	10.39	14.10	13.68
49	10.61	12.93	13.38	11.89	14.56	14.81
56	10.97	13.35	13.61	12.26	14.96	15.27

Table 22: Effect of sulphur on the relative growth rate (RGR) (g g^{-1} day^{-1}) of Azolla

Sampling intervals (days)	*Azolla pinnata* (Cuttack strain)			*Azolla pinnata* (Rajendranagar strain)		
	Control (12 ppm S)	T_1 (Elemental sulphur 25 ppm S)	T_2 (Elemental sulphur 50 ppm S)	Control (12 ppm S)	T_1 (Elemental sulphur 25 ppm S)	T_2 (Elemental sulphur 50 ppm S)
0-7	0.088	0.127	0.150	0.084	0.128	0.140
7-14	0.108	0.095	0.103	0.101	0.108	0.107
14-21	0.096	0.095	0.083	0.070	0.080	0.090
21-28	0.034	0.027	0.043	0.071	0.058	0.043
28-35	0.028	0.046	0.016	0.025	0.034	0.019
35-42	0.014	0.020	0.009	0.031	0.010	0.015
42-49	0.010	0.007	0.007	0.019	0.005	0.011
49-56	0.005	0.005	0.002	0.004	0.004	0.004

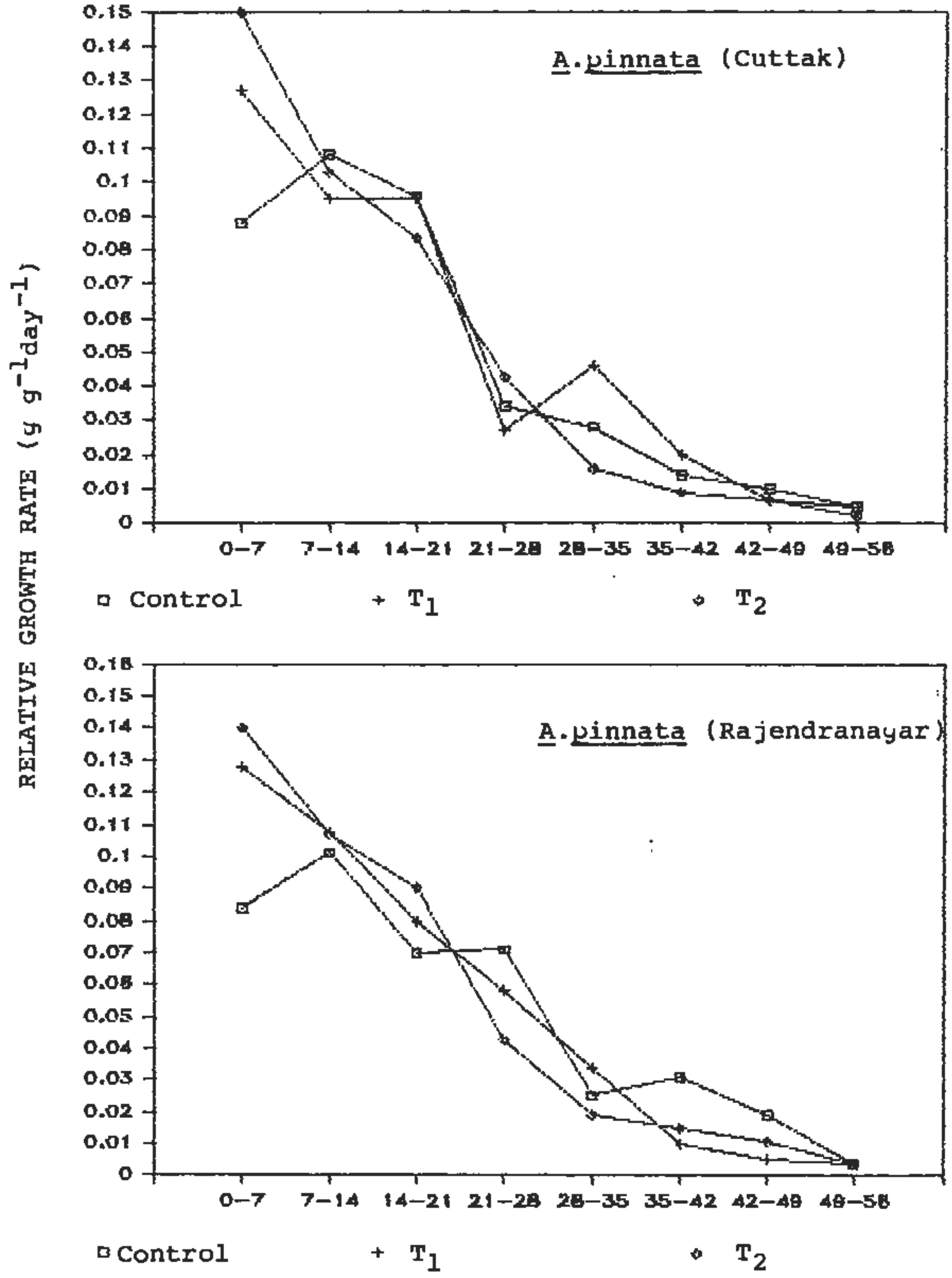


FIG.11 :EFFECT OF SULPHUR ON THE RELATIVE GROWTH RATE OF AZOLLA

Table 23: Effect of different levels of Sulphur on dry matter production (g pot^{-1}) of *Azolla*

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (12 ppm S)	12.24	10.97	11.62
T_1 (Elemental sulphur 25 ppm S)	14.96	13.34	14.15
T_2 (Elemental sulphur 50 ppm S)	15.26	13.61	14.44
Mean	14.16	12.64	

C.D(0.05)

Strains	1.17
Treatments	1.44
Interaction	NS

4.4.2 Uptake of nutrients

The uptake of nutrients (N & S) computed based on per cent composition (Appendix 4) and dry matter yields of two A. pinnata strains under different treatments are presented in Tables 24 and 25.

4.4.2.1 Nitrogen uptake: The uptake of N ranged from 4.73 g pot^{-1} in control to 8.75 g pot^{-1} in T_2 treatment in Cuttack strain. In Rajendranagar strain it ranged from 5.02 g pot^{-1} in control to 10.28 g pot^{-1} in T_2 treatment. The application of sulphur significantly increased the N uptake upto 25 ppm S whereas there was non significant increase from 25 ppm to 50 ppm S. And there was no significant difference in N uptake between Rajendranagar strain and Cuttack strain. The interaction effects between the A. pinnata strains and treatments were found to be non significant.

4.4.2.2 Sulphur uptake: The S uptake was 34.76 g pot^{-1} in control and 54.5 g pot^{-1} in T_2 treatment in Cuttack strain and in Rajendranagar strain it was 39.25 g pot^{-1} in control and 63.18 g pot^{-1} in T_2 treatment. The S uptake increased upto 25 ppm for both the strains of A. pinnata, but was on par with 50 ppm of S. There was non significant increase in S uptake between Rajendranagar and Cuttack strains. The interaction effects between two A. pinnata strains and treatments were found to be non significant.

Table 24 : Effect of application of Sulphur on uptake of nitrogen by *Azolla* (g pot^{-1}).

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (12 ppm S)	5.02	4.73	4.88
T_1 (Elemental sulphur 25 ppm S)	9.66	8.06	8.86
T_2 (Elemental sulphur 50 ppm S)	10.28	8.75	9.52
Mean	8.32	7.18	

C.D(0.05)

Strains	NS
Treatments	1.281
Interaction	NS

Table 25 : Effect of application of sulphur on uptake of sulphur by *Azolla* (g pot^{-1}).

Treatments	*Azolla pinnata* (Rajendranagar strain)	*Azolla pinnata* (Cuttack strain)	Mean
Control (12 ppm S)	39.25	34.76	37.01
T_1 (Elemental sulphur 25 ppm S)	61.53	53.15	57.34
T_2 (Elemental sulphur 50 ppm S)	63.18	54.59	58.89
Mean	54.65	47.50	

C.D(0.05)

Strains	NS
Treatments	10.43
Interaction	NS

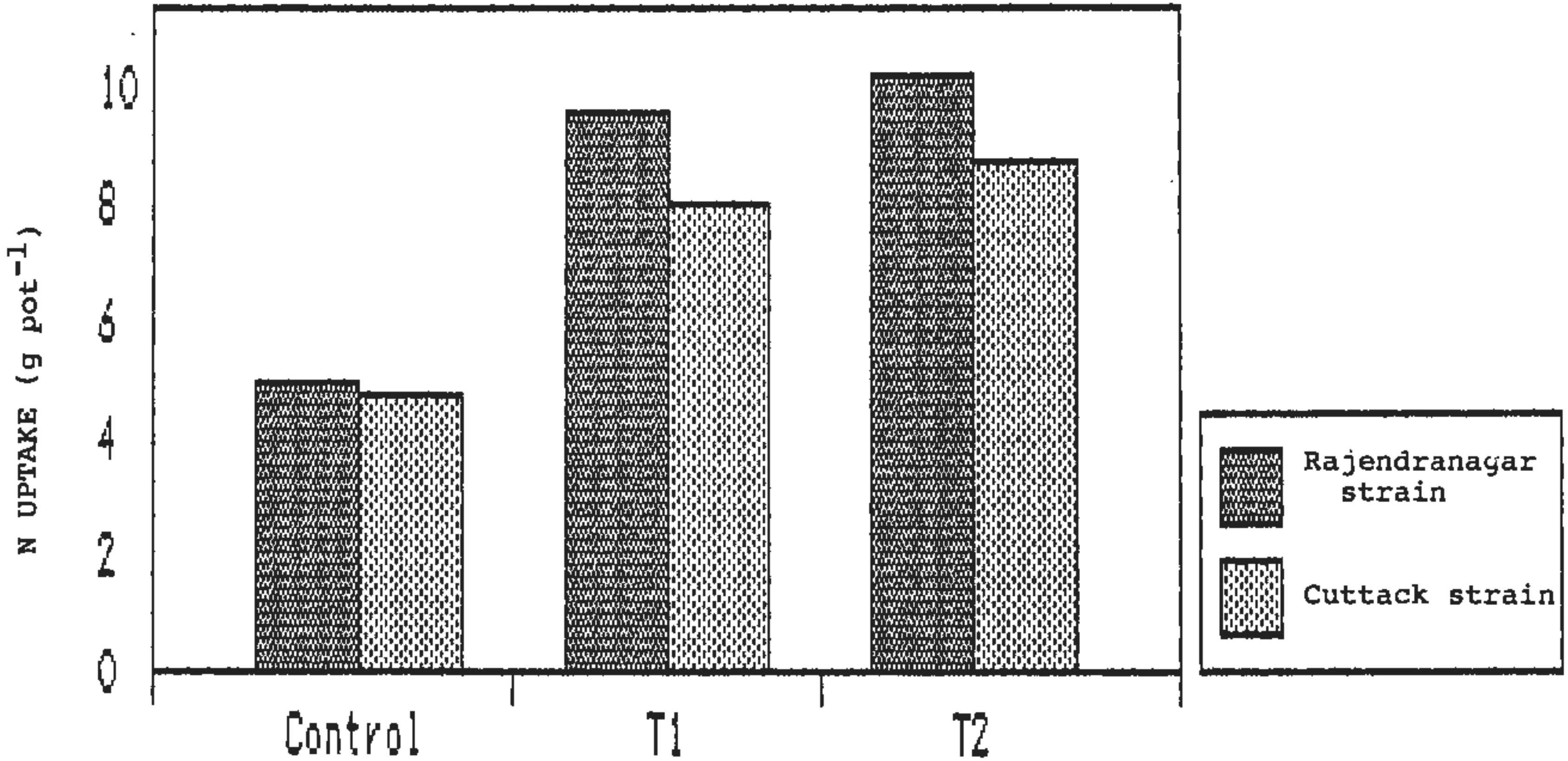


FIG.12 : EFFECT OF SULPHUR ON UPTAKE OF NITROGEN BY AZOLLA.

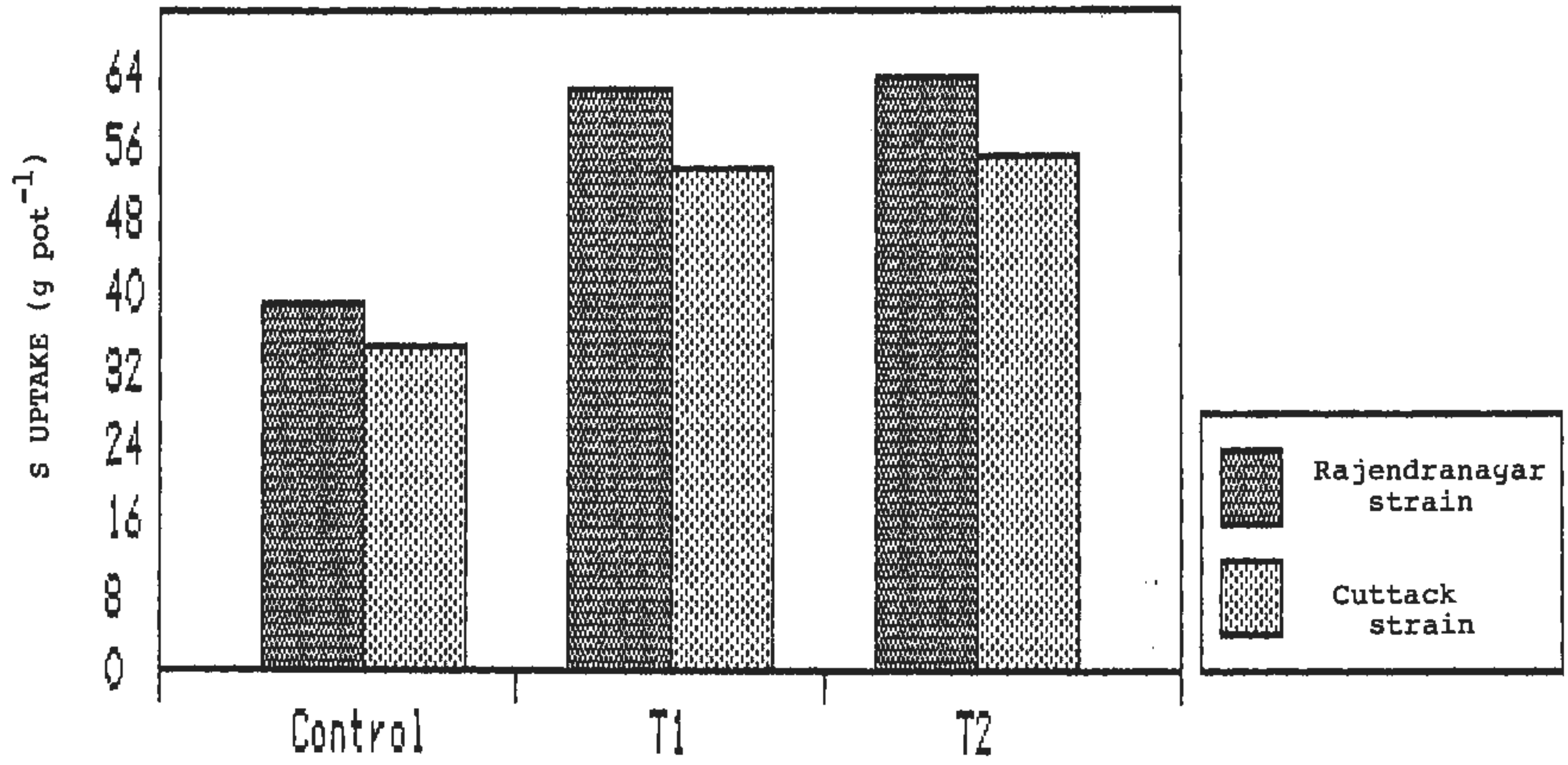


FIG.13 : EFFECT OF SULPHUR ON UPTAKE OF SULPHUR BY AZOLLA.

4.5 EFFECT OF APPLICATION OF AZOLLA AS A GREEN MANURE ON THE AVAILABILITY OF POTASSIUM IN SOIL

The availability of K_2O in soil (kg ha^{-1}) at 20, 40, 60 and 80 days under different treatments are presented in tables 26 to 29.

20 days : The availability of potassium (K_2O) in soil at 20 days varied non significantly with levels and sources of K. In Azolla pinnata (Cuttack) strain the available K_2O ranged from 158 kg ha^{-1} in control to 169 kg ha^{-1} in T_3 treatment. Similarly, in Azolla pinnata (Rajendranagar) strain the available K_2O was lower in control (159 kg ha^{-1})and highest in T_3 treatment (171 kg ha^{-1}). The interaction effects of Azolla pinnata strains with treatments were found to be non significant.

40 days : There was an increase in availability of K_2O in all treatments at 40 days when compared to 20 days. The K_2O availability varied from 161 kg ha^{-1} in control to 191 kg ha^{-1} in T_3 treatment for Azolla pinnata (Cuttack) strain whereas for Azolla pinnata (Rajendranagar) strain, it varied from 165 kg ha^{-1} in control to 194 kg ha^{-1} in T_3 treatment. The availability of K_2O in soil increased with increase in levels of K applied to Azolla. Treatments T_2, T_3, T_5 and T_6

Table 26: Effect of application of *Azolla* grown on two sources and at three levels of K on the availability of K_2O in soil at 20 days (kg ha^{-1})

Treatments	*A. pinnata* (Rajendranagar)	*A. pinnata* (Cuttack)	Mean
Control (70 ppm K)	159	158	158.5
T_1 (Potassium sulphate 100 ppm K)	163	162	162.5
T_2 (Potassium sulphate 150 ppm K)	168	165	166.5
T_3 (Potassium sulphate 200 ppm K)	171	169	170.0
T_4 (Potassium chloride 100 ppm K)	163	161	162.0
T_5 (Potassium chloride 150 ppm K)	165	163	164.0
T_6 (Potassium chloride 200 ppm K)	170	167	168.7
Mean	165.62	163.57	

CD (0.05)	
Strains	NS
Treatments	NS
Interaction	NS

Table 27: Effect of application of *Azolla* grown on two sources and at three levels of K on the availability of K_2O in soil at 40 days (kg ha^{-1})

Treatments	*A. pinnata* (Rajendranagar)	*A. pinnata* (Cuttack)	Mean
Control (70 ppm K)	165	161	163.0
T_1 (Potassium sulphate 100 ppm K)	176	175	175.5
T_2 (Potassium sulphate 150 ppm K)	188	183	185.7
T_3 (Potassium sulphate 200 ppm K)	194	191	192.5
T_4 (Potassium chloride 100 ppm K)	177	174	175.5
T_5 (Potassium chloride 150 ppm K)	184	181	182.5
T_6 (Potassium chloride 200 ppm K)	191	187	189.0
Mean	182.19	178.86	

CD (0.05)	
Strains	NS
Treatments	17.32
Interaction	NS

were the treatments in which potassium was applied for growing *Azolla* which, when added to soil resulted in a significant increase in the concentration of potassium in soil as compared to the control where *Azolla* was fertilized with potassium. Between the sources, potassium sulphate proved better than potassium chloride. The K_2O availability in soil was not significant between the two *Azolla pinnata* strains. The interaction effects of *Azolla pinnata* strains with treatments were found to be non significant.

60 days : Compared to 40 days, there was an increase in availability of K_2O in all the treatments at 60 days. In *Azolla pinnata* (Cuttack) strain the available K_2O ranged from 168 kg ha^{-1} in control to 206 kg ha^{-1} in T_3 treatment. Similarly, in *Azolla pinnata* (Rajendranagar) strain the available K_2O was lower in control (171 kg ha^{-1}) and was highest in T_3 treatment (211 kg ha^{-1}). Though there was an increase in the availability of K_2O in soil with increase in the levels of K applied to *Azolla*, only treatments T_2, T_3, T_5 and T_6 were found to be significant over control. Between the strains, *Azolla pinnata* (Rajendranagar) strain was found to be better than *Azolla pinnata* (Cuttack) strain whereas potassium sulphate was better than potassium chloride. The interaction effects of *Azolla pinnata*

Table 26: Effect of application of Azolla grown on two sources and at three levels of K on the availability of K_2O in soil at 60 days (kg ha^{-1}) .

Treatments	*A. pinnata* (Rajendranagar)	*A. pinnata* (Cuttack)	Mean
Control (70 ppm K)	171	168	169.5
T_1 (Potassium sulphate 100 ppm K)	183	181	182.0
T_2 (Potassium sulphate 150 ppm K)	206	199	202.5
T_3 (Potassium sulphate 200 ppm K)	211	206	208.5
T_4 (Potassium chloride 100 ppm K)	184	179	181.5
T_5 (Potassium chloride 150 ppm K)	201	196	198.5
T_6 (Potassium chloride 200 ppm K)	207	203	205
Mean	194.71	190.29	

CD (0.05)	
Strains	NS
Treatments	19.76
Interaction	NS

strains with treatments were found to be non significant.

80 days : There was slight increase in availability of K_2O in all treatments at 80 days when compared to 60 days. The K_2O availability varied from 168 kg ha^{-1} in control to 206 kg ha^{-1} in T_3 treatment for _Azolla pinnata_ (Cuttack) strain whereas for _Azolla pinnata_ (Rajendranagar) strain it varied from 171 kg ha^{-1} in control to 211 kg ha^{-1} in T_3 treatment. The availability of K_2O in soil increased with increase in levels of K applied to _Azolla_. Treatments T_2, T_3, T_5 and T_6 were found to increase significantly over control. The K_2O availability in soil was found non significant between the _Azolla pinnata_ strains. Between the sources, _Azolla_ applied with potassium sulphate was found to be better than potassium chloride in K_2O availability. The interaction effects of _Azolla pinnata_ strains with treatments were found to be non significant.

...

Table 29: Effect of application of Azolla grown on two sources and at three levels of K on the availability of K_2O in soil at 80 days (kg ha^{-1})

Treatments	*A. pinnata* (Rajendranagar)	*A. pinnata* (Cuttack)	Mean
Control (70 ppm K)	173	169	171.0
T_1 (Potassium sulphate 100 ppm K)	185	183	184.0
T_2 (Potassium sulphate 150 ppm K)	207	201	204.0
T_3 (Potassium sulphate 200 ppm K)	214	209	211.5
T_4 (Potassium chloride 100 ppm K)	187	182	184.5
T_5 (Potassium chloride 150 ppm K)	202	198	200.0
T_6 (Potassium chloride 200 ppm K)	210	205	207.5
Mean	196.86	192.43	

CD (0.05)	
Strains	NS
Treatments	19.35
Interaction	NS

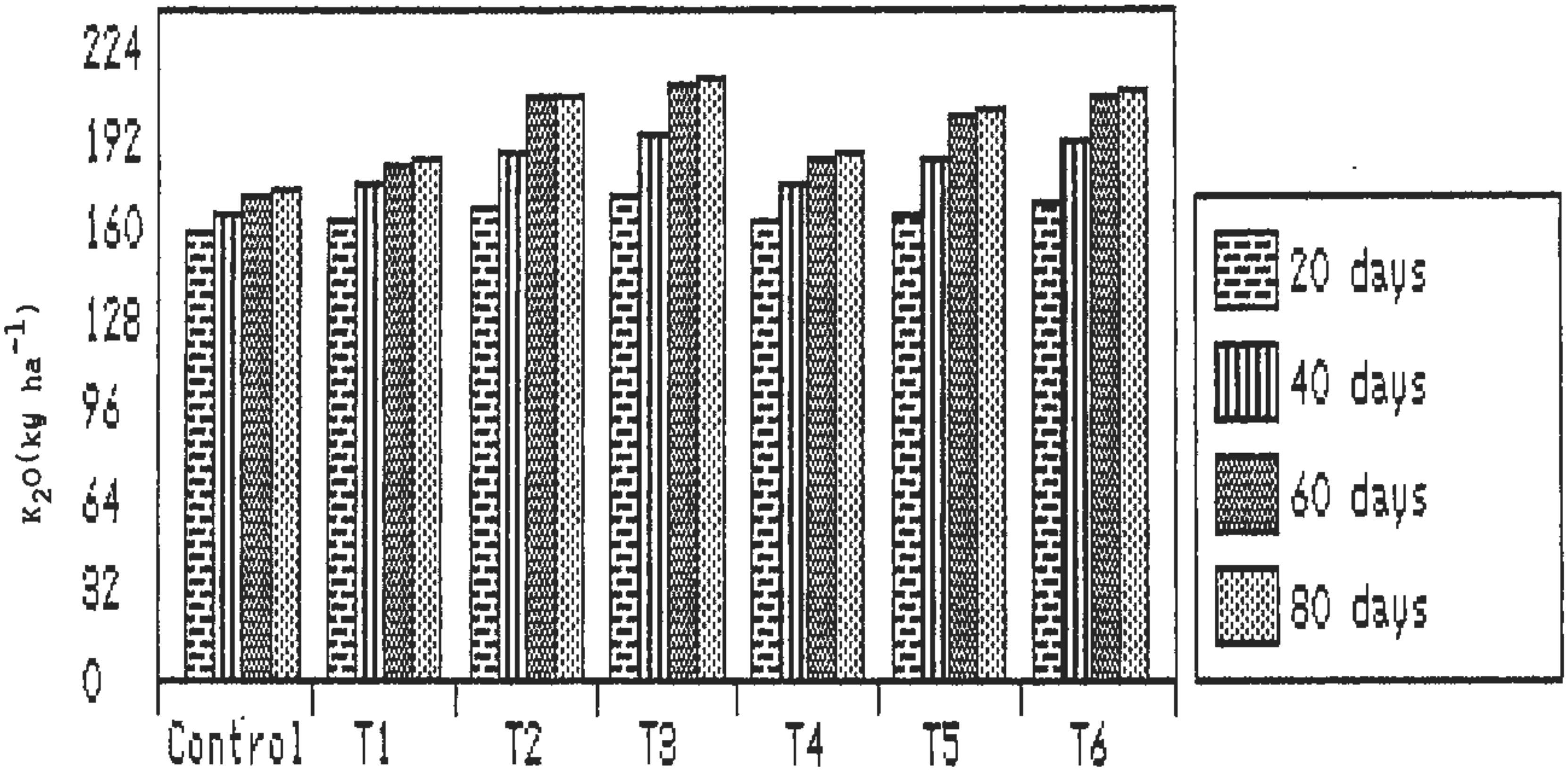


FIG.14 : EFFECT OF APPLICATION OF A.PINNATA(RAJENDRANAGAR STRAIN) ON THE AVAILABILITY OF K_2O IN SOIL AT VARIOUS STAGES.

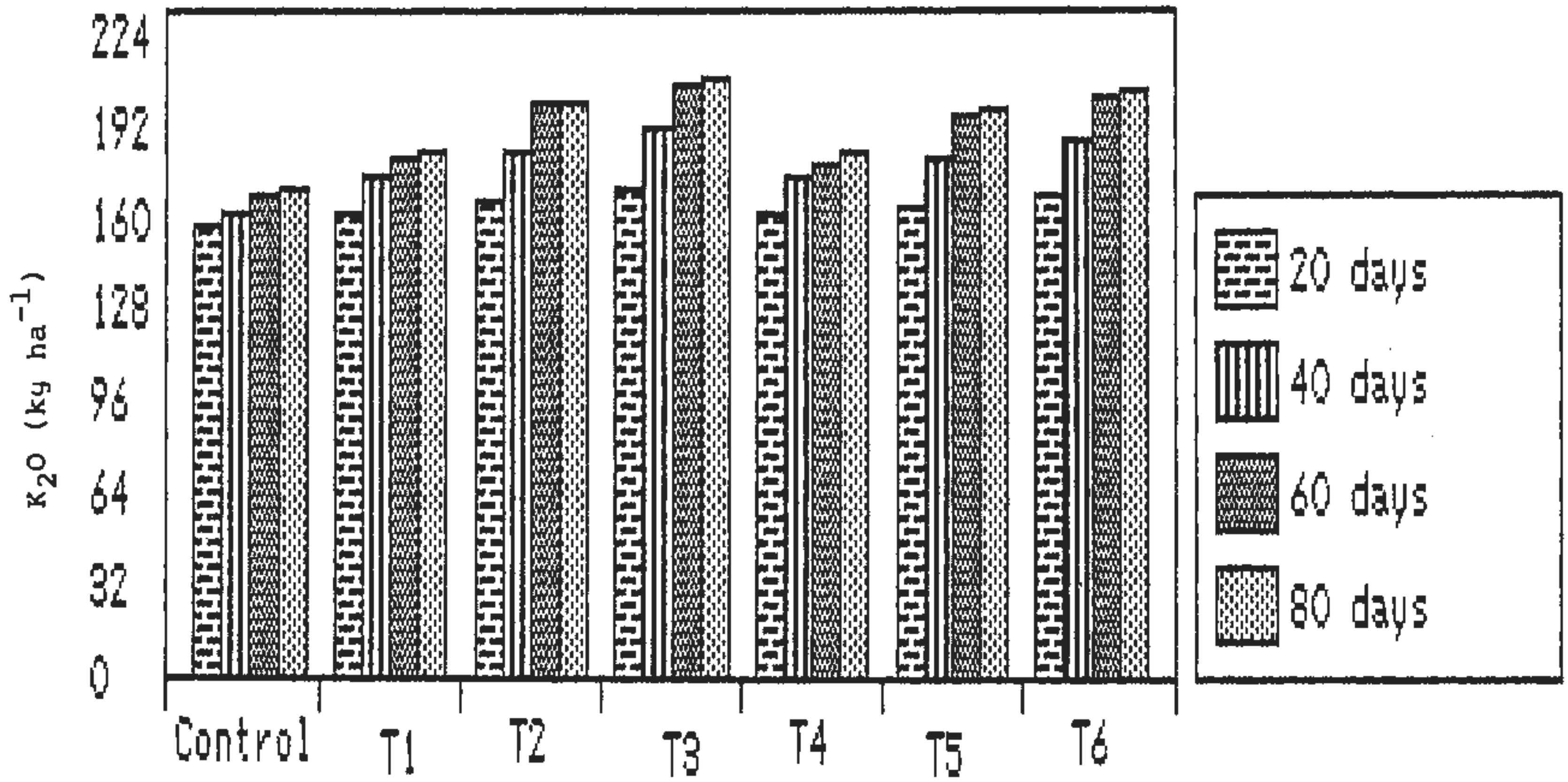


FIG.15 : EFFECT OF APPLICATION OF <u>A.PINNATA</u> (CUTTACK STRAIN) ON THE AVAILABILITY OF K_2O IN SOIL AT VARIOUS STAGES.

DISCUSSION

CHAPTER V

DISCUSSION

Azolla is a water fern that assimilates atmospheric nitrogen in association with nitrogen fixing blue green algae *Anabaena azollae* (Becking, 1979). It is a potential biological system that can be used as biofertilizer in rice production, because of the comparable conditions required for growth of both rice and *Azolla*. Once introducted into rice paddies, it can sustain and act as a renewable biofertilizer. It has a high relative growth rate and can produce a large biomass in a short period unlike the conventional green manure crops. *Azolla* can multiply and produce biomass ranging from 15 to 20 t ha^{-1} within 20 to 30 days under favourable conditions. *Azolla* has a high N content ranging from 3 to 5 per cent on dry weight basis which is relatively higher than any other green manure plant. It therefore has the potential to be used as a biofertilizer for rice and is reported to contribute 30 to 40 kg ha^{-1} in one growing season for rice. Because of these characteristics, *Azolla* is being widely used as green manure in rice cultivation in some countries like China, Vietnam and Philippines.

In recent years, the beneficial effects of *Azolla* in increasing the rice yields are well recognised and research on *Azolla* has attracted the attention of the agricultural scientists. Besides supplying nitrogen, Azolla is also a good source of other nutrients and adds large amounts of organic matter to soil. It can also establish well even in N deficient soils since it has the capacity for biological fixation of atmospheric nitrogen (Becking, 1979). Hence, Azolla is considered as an ideal biological system for rice yeilds under low cost rice production technology. Azolla used for wet land culture is increasing in several rice growing areas in India. There are two methods of Azolla application viz., dual culture along with rice followed by periodic incorporation in soil or growing Azolla in separate nurseries and incorporating the biomass in soil prior to transplanting of crop.

There are certain constraints in the use of Azolla as a biofertilizer for rice. It is a farm oriented, labour intensive technology suitable only for adoption in countries facing severe resource constraints to supply fertilizer inputs and where the farm labour is cheap. There are several environmental constraints in the growth and multiplication and use of Azolla in tropical rice production. Growth of Azolla is frequently reported to be responsive to the macro-nutrient status of the soil. As such it becomes imperative to study the effect of potassium, calcium, magnesium and sulphur on the growth and their uptake by *Azolla*. Further, the effect of application of *Azolla* as a green manure on the availability of potassium in soil was also studied. All the studies were conducted under green house conditions of Rajendranagar, Hyderabad.

The application of potassium is reported to be essential for the cultivation of *Azolla* on light textured soils. Le and Subochkin (1963) showed that absence of potassium decreased the yield of *Azolla* more than the absence of phosphorus. Potassium deficient plants do not develop colour indicated deficiency symptoms. The threshold concentration for this element has been reported to be about 0.4 m mol l^{-1} (Yatazawa *et al*., 1980). Potassium is supplemented by the application of potassium sulphate or potassium chloride. The two strains studied belonged to the same species but were obtained from different sources. Considering the total biomass production of two strains of *Azolla*, 150 ppm potassium was found to be optimum while there was no significant increase at 200 ppm potassium. In terms of dry matter production potassium sulphate proved better than potassium chloride. This is because potassium sulphate supplies sulphur, an essential element in plant nutrition in addition to potassium. The effect of potassium levels on nutrient uptake (N and K) of Azolla followed the same trend as in case of dry matter yeild. Increasing the uptake of N and K significantly upto 150 ppm but was on par with 200 ppm potassium application. With regard to sources, potassium sulphate was found to increase the N and K uptake more than that of potassium chloride. The increase in N and K uptake is due to higher dry matter yields as well as increased K and N content with increase in levels of potassium. Thus, 150 ppm of potassium sulphate was found to be the best level and source of potassium for maximum growth and N and K uptake by Azolla.

The importance of calcium in supporting the growth of Azolla has been demonstrated (Watanabe et al., 1977). Calcium deficient Azolla showed more browning than the phosphorous deficient Azolla and had smaller frond size. The thershold concentration for this element has been reported to be about 0.4 m mol l^{-1} (Yatazawa et al., 1980). Calcium carbnonate was used as the source of calcium. The two strains studied were Azolla pinnata (Rajendranagar) and Azolla pinnata (Cuttack). The biomass production of two Azolla pinnata strains increased significantly upto 80 ppm of calcium but was on par with 120 ppm of calcium. Azolla pinnata (Rajendranagar) strain gave significantly higher yields than Azolla pinnata (Cuttack). The N and

Ca uptake has also increased significantly upto 80 ppm level of calcium. The increase in N and Ca uptake was mainly due to higher dry matter yields and N and Ca content at 80 ppm level of calcium. Thus, 80 ppm calcium was found to be best level for growth and N and Ca uptake by Azolla.

The biomass production of two Azolla pinnata strains increased significantly with increase in levels of sulphur upto 25 ppm level but, was on par with 50 ppm sulphur. Azolla pinnata (Rajendranagar) strain gave significantly higher yields than Azolla pinnata (Cuttack)strain. There was significant increase in N and S uptake with increase in levels of sulphur upto 25 ppm because of increase in S content and higher dry matter yields at that level of sulphur.

Watanabe et al. (1977) demonstrated the importance of magnesium in supporting the growth of Azolla. The threshold concentration for this element has been reported to be about 0.5 m mol l^{-1} (Yatazawa et al., 1980). Magnesium carbonate was used as a source of magnesium. There was no significant increase in biomass production of Azolla with increase in level of magnesium. But, Azolla pinnata (Rajendranagar) strain gave significantly higher biomass yields than Azolla pinnata (Cuttack) strain. The Mg uptake was also significantly higher in Azolla pinnata (Rajendranagar) which was more due to higher dry matter yields.

In all these four experiments, Azolla pinnata (Rajendranagar) gave significantly higher yields than Azolla pinnata (Cuttack). This is mainly because Azolla pinnata (Rajendranagar) is more adaptable to Rajendranagar conditions than Azolla pinnata (Cuttack) (Jayanthi, 1989).

The effect of incorparation of Azolla on nutrient availability was shown to be more in soils of low fertility than those of higher fertility (Becking, 1979). Since the alfisol (red soil) used in this study had only medium level of potassium, the beneficial effect of Azolla application was more on K_2O availability.

Azolla is a good accumilator of K from the nutrient medium and when Azolla is incorporated into soil as green manure, it decomposes and K is released in the form of available or slowly available K (Liu, 1987) resulting in increase of the available status of the soil.

Availability of K_2O was higher due to incorporation of Azolla and the availability increased with increase in levels of K applied to Azolla grown at 150 ppm of K with potassium sulphate as the source was found to be the best treatment when compared with potassium chloride. The availability of K_2O in soil was higher with potassium sulphate because of higher K content in Azolla grown with potassium sulphate than potassium chloride. The availability of K_2O in soil increased with the decomposition of Azolla upto 80 days, but most of the K_2O was available by 60 days of decomposition of Azolla.

The studies revealed that there was positive response to the increase in the status of soil K, Ca, S and Mg levels in terms of both dry matter production and uptake of the individual nutrients added as well as N_2 fixation by Azolla.

These studies have indicated the importance of balanced nutrition of Azolla to obtain the better yeild of the fern for use as green manure in rice cultivation. The previous studies have emphasised mostly the role of phosphorus in the nutrition and biomass production of Azolla which however appears to be overstressed at the cost of the importance of the other nutrient elements.

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SUMMARY

CHAPTER VI

SUMMARY

Investigations were undertaken to study the response of *Azolla* to potassium, calcium, magnesium and sulphur nutrition in a light textured alfisol. These studies include the effect of application of potassium, calcium, magnesium and sulphur on the growth and uptake of K, Ca, Mg and S by *Azolla*, effect of K, Ca, Mg and S nutrition on N content of *Azolla* and effect on K availability in soil due to incorporation of *Azolla*.

The total biomass production and N and K uptake of two *Azolla pinnata* strains increased significantly with increase in levels of potassium upto 150 ppm but was on par with 200 ppm. At any level of K, potassium sulphate gave higher biomass yields and N and K uptake than potassium chloride. *Azolla pinnata* (Rajendranagar) strain was found to respond better than *Azolla pinnata* (Cuttack) strain with regard to biomass production and N and K uptake.

Application of calcium significantly increased the biomass production and N and Ca uptake upto 80 ppm level but was on par with 120 ppm. *Azolla pinnata* (Rajendranagar) strain gave significantly higher biomass yields and Ca and N uptake than *Azolla pinnata* (Cuttack) strain.

The biomass production and N and S uptake of two *Azolla pinnata* strains increased significantly with increase in levels of sulphur upto 25 ppm but were on par with 50 ppm sulphur. *Azolla pinnata* (Rajendranagar) strain gave significantly higher biomass yields than *Azolla pinnata* (Cuttack) strain but with regard to N and S uptake the increase was non significant.

There was no significant increase in biomass production and N and Mg uptake with increase in levels of magnesium. But, *Azolla pinnata* (Rajendranagar) strain gave significantly higher biomass yields and N and Mg uptake than *Azolla pinnata* (Cuttack) strain.

Availability of K was higher due to incorporation of *Azolla* which was better with increase in levels of K applied to *Azolla*. *Azolla* grown at 150 ppm K with potassium sulphate as the source was found to be the best treatment in increasing the K availability of the soil. The availability of K in soil increased with the decomposition of *Azolla* upto 80 days but, most of the K was available by 60 days of decomposition of *Azolla*.

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* Original not seen

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APPENDICES

Appendix 1: Nitrogen and potassium content of Azolla in potassium experiment

Treatments	*Azolla pinnata* (Rajendranagar strain)		*Azolla pinnata* (Cuttack strain)		Mean	
	N %	K %	N %	K %	N %	K %
Control (70 ppm K)	3.31	2.47	3.10	2.24	3.21	2.36
T_1 (Potassium sulphate 100 ppm K)	3.98	3.56	3.91	3.47	3.95	3.52
T_2 (Potassium sulphate 150 ppm K)	4.54	4.76	4.39	4.34	4.47	4.55
T_3 (Potassium sulphate 200 ppm K)	4.59	4.82	4.42	4.58	4.51	4.70
T_4 (Potassium chloride 100 ppm K)	3.84	3.48	3.76	3.32	3.80	3.40
T_5 (Potassium chloride 150 ppm K)	4.37	4.43	4.21	4.16	4.29	4.30
T_6 (Potassium chloride 200 ppm K)	4.40	4.54	4.28	4.25	4.34	4.40
Mean	4.15	4.01	4.01	3.77		

Appendix II: Nitrogen and calcium content of Azolla in calcium experiment

Treatments	*Azolla pinnata* (Rajendranagar atrain)		*Azolla pinnata* (Cuttack strain)		Mean	
	N %	Ca%	N %	Ca%	N %	Ca%
Control (44 ppm Ca)	3.12	0.52	3.09	0.48	3.11	0.50
T_1 (Calcium carbonate 80 ppm Ca)	4.24	0.99	4.13	0.93	4.19	0.96
T_2 (Calcium carbonate 120 ppm Ca)	4.29	1.03	4.21	0.98	4.25	1.01
Mean	3.88	0.85	3.81	0.80		

Appendix III: Nitrogen and magnesium content of Azolla in magnesium experiment

Treatments	*Azolla pinnata* (Rajendranagar strain)		*Azolla pinnata* (Cuttack strain)		Mean	
	N %	Mg%	N %	Mg%	N %	Mg%
Control (48 ppm Mg)	3.07	0.21	3.12	0.17	3.10	0.19
T_1 (Magnesium carbonate 60 ppm Mg)	3.59	0.38	3.53	0.34	3.56	0.36
T_2 (Magnesium carbonate 80 ppm Mg)	3.63	0.41	3.61	0.39	3.62	0.40
Mean	3.43	0.33	3.42	0.30		

Appendix IV: Nitrogen and sulphur content of Azolla in sulphur experiment

Treatments	*Azolla pinnata* (Rajendranagar strain)		*Azolla pinnata* (Cuttack strain)		Mean	
	N %	S %	N %	S %	N %	S %
Control (12 ppm S)	3.19	0.41	3.16	0.43	3.18	0.42
T_1 (Elemental sulphur 25 ppm S)	4.09	0.65	3.96	0.61	4.04	0.63
T_2 (Elemental sulphur 50 ppm S)	4.13	0.67	4.02	0.64	4.08	0.66
Mean	3.80	0.58	3.72	0.58		