

**INFLUENCE OF PROTOZOA ON THE GROWTH AND
ACTIVITIES OF BENEFICIAL SOIL MICRO-ORGANISMS
IN RICE SOILS**

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**DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
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INFLUENCE OF PROTOZOA ON THE GROWTH AND ACTIVITIES OF BENEFICIAL SOIL MICRO-ORGANISMS IN RICE SOILS

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CERTIFICATE

This is to certify that the thesis entitled "INFLUENCE OF PROTOZOA ON THE GROWTH AND ACTIVITIES OF BENEFICIAL SOIL MICRO-ORGANISMS IN RICE SOILS" submitted by Ms. NAGAVENI, N.M. for the degree of MASTER OF SCIENCE in AGRICULTURAL MICROBIOLOGY to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by her under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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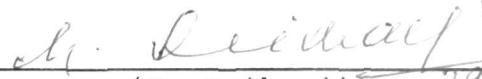

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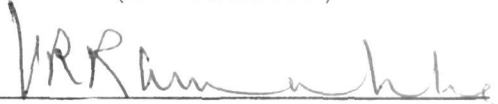
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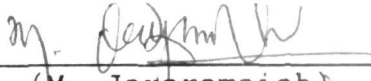
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INTRODUCTION

I INTRODUCTION

Nitrogen which is a very important nutrient limits rice production and it is critical in the tropics, where paddy soils are generally lower in nitrogen. Biological nitrogen fixation is known to amount for much of the nitrogen supply for unfertilized rice crops. Even in the fertilized fields, upto two-thirds of the nitrogen absorbed by rice is derived from the soil. Principle agents in biological nitrogen fixation in flooded rice soils are bluegreen algae, Azolla in association with Anabaena azollae and non-symbiotic nitrogen fixing bacteria like Azotobacter and Azospirillum which can also be inoculated. Paddy is usually rotated with short duration pulses like cowpea, greengram and blackgram in India.

Legumes by virtue of the symbiotic association with rhizobia are known to meet their own nitrogen requirement to a large extent. It is estimated that the process of biological nitrogen fixation yields about 90 to 170 million tonnes of nitrogen annually. Various micro-organisms are involved in the process of biological nitrogen fixation but legumes and associated rhizobia contribute the most.

Responses to Rhizobium inoculation have been demonstrated with principle legumes like limabean, soybean, cowpea, redgram, groundnut etc. However, such responses have been often unpredictable. Such unpredictability to Rhizobium

inoculation is generally attributable to various abiotic and biotic factors. The abiotic factors like pH, nutrients, soil type, temperature, moisture have been recognised. Among biotic factors bacteriophages, Bdellovibrios, protozoa, epiphytic bacteria and indigenous rhizobia are most important.

Among the micro-fauna of soil are protozoa, amoeboid and flagellate unicellular animals. The soil forms are small whereas the water forms are bigger in size. They form cysts which help them to tide over dry conditions. They feed on decaying organic matter or live by digesting soil particles together with bacterial cells, algae, yeasts etc., and a few possess chlorophyll in their body and are capable of synthesising carbohydrates, e.g., Euglena. In 1909, Russell and Hutchinson suggested that the protozoa played a significant role in microbial life of soil and according to them they partially 'sterilized' the soil by eating away bacteria, which reduced organic residues to useful forms of chemicals for plant growth, but this notion has changed, now as there are evidences to show that some bacteria are activated in the presence of protozoa. Protozoa are less sensitive to soil factors such as aeration, moisture and pH, but it is very difficult to enumerate their population in soil. With all these controversies, this study was conducted mainly to know the effect of protozoa like Paramaecium and

Vorticella on the activities of beneficial soil bacteria Rhizobium, Azotobacter and Azospirillum with the following objectives :

1. To determine the protozoan population in rice cultivated zones of Karnataka and to isolate the major types.
2. To know the feeding habit of protozoa on beneficial bacteria.
3. To know the effect of protozoa on nitrogen fixation and also on the growth of paddy and cowpea.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Rhizobium, Azotobacter and Azospirillum are the commonly occurring and important bacteria found in the soil. They are exploited mainly for their ability of nitrogen fixation in legumes and cereals. Many factors affect the growth and activities of these bacteria in the soil. Protozoa is one of the main factors affecting the growth of bacteria in soil. As the literature available on the effect of protozoa on bacteria and crop growth is very less, an attempt was made to review the literature available on nitrogen fixing ability and inoculation response of Rhizobium, Azotobacter and Azospirillum and the influence of protozoa on the growth of some bacteria.

It has been well established by several pioneers that soil fertility could be improved by N fixation which is mediated by micro-organisms. May such species of micro-organisms have since been isolated.

Enumeration of Rhizobia

Several methods and techniques which are both direct and indirect have been found useful in the enumeration of rhizobia in soil as well as in broth cultures (Vincent, 1970). Many workers have enumerated viable number of rhizobia in the aliquots directly by the dilution plate count method (Vincent, 1970; Karen and Wollum, 1980; Hoben and

Somasegaran, 1982). Miles and Misra drop plate count technique was found to be more convenient in estimating viable rhizobia (Vincent, 1970; Hoben and Somasegaran, 1982). Development of immunofluorescence technique further helped in the precise enumeration of rhizobia directly from the soil (Schmidt et al., 1968; Schmidt, 1979; Bohlool and Schmidt, 1973; Rayes and Schmidt, 1979). Enzyme linked immunosorbent assay (ELISA) was also found to be useful to enumerate rhizobia from the soil (Nambiar and Anjaiah, 1985).

Vincent (1970) demonstrated the plate count technique to enumerate viable rhizobia by spreading aliquot on the surface of congo red agar media. The above method gave good results when the population of other bacteria was not more than ten times the population of rhizobia. He also suggested a rough grading for natural rhizobia based on their appropriate number in one gram of soil as follows; absent or sparse (<25), few (25 to 1×10^3), abundant (1×10^3 to 1×10^5) and very abundant ($>1 \times 10^5$). Further the greatest difficulty in plate count method was to distinguish a particular Rhizobium sp. in the presence of other Rhizobium and other similar soil bacteria.

Three methods of dilution plating viz., pour plate, spread plate and Miles and Misra drop plate were compared by Hoben and Somasegaran (1982). They reported that all the three plating methods gave close estimates, but Miles and

Misra drop plate method had advantages over the other methods because of its economy in materials and labour.

Nodulation and nitrogen fixation

With the disclosure that N fixation was possible several workers over the years have identified symbiotic and asymbiotic N fixers which are nature or otherwise.

Tewari (1955) studied the effect of three levels of nitrogen, phosphate and potassium on the formation of nodules. Results indicated that increasing levels of nitrogen depressed the formation of both effective and ineffective nodules, but phosphorus and potash did not produce any significant effects.

Demooy and Pesek (1966) studied nodulation responses of soybeans to added phosphorus, potassium and calcium salts. They reported that maximum nodulation required very high levels of applied phosphorus and potassium salts.

Philpotts (1967) reported that higher the soil temperature at sowing depth of cowpea, the lower was the percentage of plants with nodules and the lower the number of nodules per plant.

Defranco and Decarvalho (1970) studied the effect of nutritional deficiency on the development of legumes like Glycine javanica var. comum, G.javanica var. tinaroo,

Macroptilium atropurpureus, Peuraria javanica and Centrosema pubescens on red latosol called 'Fase cerrado'. They reported that the extensive phosphorus deficiency in the soil decreased the nodule weight.

Ramirez Martinez (1979) studied Rhizobium colonization of spermosphere and rhizosphere of bean (Phaseolus vulgaris). He reported that plants from thiram treated seeds of bean inoculated with Rhizobium phaseoli had 38 per cent increase in dry matter and 50 per cent increase in nodule number compared with plants from inoculated seeds untreated with thiram; and Rhizobium phaseoli counts in the spermosphere and rhizosphere of 5 day old seedlings were 3 orders of magnitude higher. He observed that protozoa appeared to be the cause of the lower R.phaseoli numbers in the spermosphere and rhizosphere of untreated bean seeds. The extent of colonization of soil by R.phaseoli was inversely related to the presence of high numbers of soil bacteria and protozoa.

Zablotowicz et al. (1981) reported that maximum nodulation of 175 mg/plant of California cowpeas was observed at flowering in well watered pots, compared with 15 mg/plant in the plant subjected to drought treatment.

Seripong and Masayna (1984) investigated the growth, nodulation and mineral content of cowpea in a pot trial using four levels of phosphorus and four levels of potassium. Best

plant growth was obtained with 50 to 100 kg P/ha, without K. They reported that applied potassium decreased total and individual weight of nodules. Visual observation on nodulation suggested that the native Rhizobium was effective for cowpea.

Pueppke (1986) studied the nodule distribution on legume roots both in growth pouches and in soil. He reported that the uppermost nodules on the primary roots of Siratro and cowpea were clustered near the position occupied by the root tip at the time of inoculation. This region was maximally susceptible to nodulation. Appreciable numbers of nodules were scattered also down the length of the primary root. He also reported that soil reduced the extent of scattering of nodules along primary roots and shifted the ratios of primary root nodules to lateral root nodules.

Raj et al. (1989) observed nodule initiation within five days after emergence of Vigna mungo, V. radiata, cowpeas, pigeonpeas and chickpeas. Detachable nodules were observed 15 days after sowing in cowpeas and chickpeas, after 20 days in V. radiata and 25 days in pigeonpeas. The number of nodules was negatively correlated with plant age in chickpeas. It was positively correlated with plant dry weight in cowpeas and Vigna radiata but negatively correlated in chickpeas and pigeonpeas.

Krause and Broughton (1992) studied the proteins associated with root-hair deformation and nodule initiation in Vigna unguiculata. The studies revealed the presence of 12 symbiosis specific proteins. Synthesis of three of these proteins was repressed four days after inoculation with Rhizobium. The remaining 9 proteins were induced by Rhizobium 1, 2 or 4 days after inoculation. All the 12 proteins were associated with root hair deformation and nodule development.

Nitrogen fixation

Neves et al. (1982) studied the nitrogen assimilation by nodulated plants of (a) Phaseolus vulgaris L. and (b) Vigna unguiculata (L.) Walp. They found that nodulation and nitrogenase activity were high but nitrate reductase activity lower in (b) than in (a). Since assimilation and redistribution of accumulated N were higher in (b), seed yield was 28 per cent greater than in (a).

Mohamed (1985) studied the effects of pre-plant application of nitrogen fertilizer on symbiotic nitrogen fixation and yield of cowpea [Vigna unguiculata (L.) Walp.]. He observed a linear and quadratic inverse trend between increase in applied N and nodulation. Nitrogenase activity was stimulated by low and inhibited by high rates of ammonium nitrate. Dry weight of vegetative components were greater at 28 kg than at 168 kg N/ha.

Wadiserisuk and Weaver (1985) studied the importance of bacteroid number in nodules and effective nodule mass to dinitrogen fixation by cowpeas. They found that there was a linear relationship between total nodule mass formed by the effective rhizobia and quantity of N_2 fixed. The relationship between total N_2 fixed and total number of bacteroids of the effective strain was not consistent.

Elhassan and Focht (1986) compared the inoculant and indigenous rhizobial dinitrogen fixation in cowpeas. They reported that analysis of N content of plant tissues by direct ^{15}N technique showed that cowpeas were capable of deriving 60 to 90 per cent of shoot N from N_2 fixation. Inoculum density had no effect on yield response of cowpeas but inoculation with strains Pl32, 401 and 22A1 effected greater seed yield, shoot dry matter, total N and percentage of N derived from fixation (86-98%) than other strains and uninoculated control.

Jensen (1986) studied the total amount of nitrogen derived from symbiotic nitrogen fixation in two pea and one field bean cultivar supplied with 50 kg N per hectare at sowing (starter N). The proportion of total N derived from N_2 fixation at maturity was higher in seeds than in vegetative plant parts.

Ofori et al. (1987) reported that field grown cowpea was estimated to have received 53-69 per cent of its N supply

from fixation with N₂ fixation only slightly affected by intercropping or N-fertilizer application in their ¹⁵N dilution studies.

Inoculation response in cowpea

Bagyaraj and Hegde (1978) reported that seed inoculation of cowpea C-152 with UASB 94 strain and composite culture resulted in significant increase in yield over uninoculated control. Although, increased nodulation was seen in Rhizobium inoculated plants over control, the difference was statistically not significant.

Balasubramanian et al. (1980) reported that inoculation of cowpea seeds with a mixture of three Rhizobium strains was superior compared to inoculation with individual strains in increasing nodulation and seed yield.

Rao (1980) reported increase in grain/seed yields resulting from the inoculation of bengalgram (Cicer arietinum), soybean, Arhar (Pigeonpea), Mung (Vigna radiata), cowpea (V. unguiculata), urid (V. mungo) and moth (V. aconitifolia) seeds with Rhizobium strains.

Gunawardena and Vlassak (1986) reported that cowpea [Vigna unguiculata (L.) Walp] inoculated with a combination of Rhizobium and Azospirillum gave the highest values for acetylene reduction, shoot DW, symbiotic effectiveness and nodule FW compared to single inoculation and control.

Kundu (1988) reported that combined inoculation of pigeonpea with Rhizobium and Azospirillum increased nodulation and nitrogen fixation. The establishment of Azospirillum in the pigeonpea rhizosphere and nodules was confirmed by re-isolations.

Yahalom et al. (1988) observed the uppermost nodules on the primary roots of pouch grown burrmedic (Medicago polymorpha), Siratro (Macropilium astropurpureum) and Egyptian clover (Trifolium alerandrinum) appeared significantly earlier following combined inoculation with Rhizobium and Azospirillum compared with Rhizobium alone. The total number of nodules were also increased.

El Mokadem et al. (1989) reported that growth of chickpea was improved by adding Azospirillum and/or Azotobacter with Rhizobium. Cross-sections of chickpea root nodules showed that the two diazotrophs plus Rhizobium improved nodule branching compared to Rhizobium inoculated treatments.

Smith and Porter (1989) studied the response of cowpea inoculated with Rhizobium to variations in planting density and nutrient level. They reported that biomass production was maximum with 0.5 strength nutrient solution and with one plant/pot. Plants were more sensitive to higher planting density than to alterations of nutrient level. Over a 20-

fold range of nutrient supply, whole plant biomass yield varied at most by 44 per cent, whereas increasing planting density from 1 to 5 plants/pot decreased biomass production by as much as 77 per cent.

Nitrogen fixation in rice

About 75 per cent of the 143 million hectares of rice soils are wetlands. Evidence that flooding favour N_2 fixation in soils have been obtained from acetylene reduction assay (Yoshida and Ancajas, 1971), long term field trials (Watanabe et al., 1981) and nitrogen balance studies (Ventura and Watanabe, 1983). Rice soils offer unique conditions for aerobic, microaerophilic and anaerobic nitrogen fixing micro-organisms (Yoshida and Ancajas, 1973; Watanabe, 1978; Rao, 1980).

Burns and Hardy (1975) estimated the biological nitrogen fixation in rice fields at about $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Total N_2 fixed in lowland rice fields was calculated as 3.2 million t $N \text{ yr}^{-1}$. The potential of biological nitrogen fixation due to associative nitrogen fixers was estimated to be 40 kg N ha^{-1} (Watanabe and Roger, 1984). Roger and Ladha (1990) estimated that the biological nitrogen fixation in rice fields by heterotrophic bacteria was about 60 kg N ha^{-1} per crop.

Studies using ^{15}N technique confirmed that bacteria in association with rice roots and submerged portions of shoots can fix nitrogen and provide atleast a part of this fixed nitrogen to the rice plant (Eskew et al., 1981; Ito et al., 1980; Yoshida and Yoneyama, 1980).

Different genera of nitrogen fixing bacteria isolated from wetland rice rhizosphere include Azospirillum (Lakshmikumari et al., 1976), Pseudomonas (Watanabe and Barraquio, 1979), Enterobacter and Klebsiella (Watanabe et al., 1979), Azotobacter and Clostridium (Ishac et al., 1981) and Flavobacterium capsulatum (Bally et al., 1983).

Inoculation response in rice

(a) Response to Azotobacter inoculation

Sims et al. (1980) studied non-symbiotic nitrogen fixation in rice and reported that grain yields were increased by inoculation with Azotobacter vinelandii. They also reported that small amounts of nitrogen stimulated nitrogen fixation.

Bagal and Patil (1984) reported that in field trials with rice, paddy yields with Azotobacter (applied to seeds, seedlings or soil) + 0-50 kg N ha⁻¹ were higher than N alone.

Kundu and Gaur (1984) studied the response of rice to inoculation with N₂ fixing and P-solubilizing micro-

organisms. They reported that inoculation of rice seedlings with Azotobacter chroococcum, Pseudomonas striata and Aspergillus awamori increased grain and straw yields of rice and uptake of N and P, with or without the application of N and P fertilizers. A mixed inoculum comprising all three species was more effective than single species inocula.

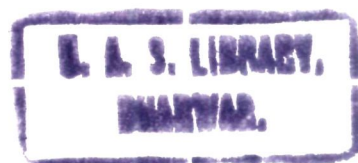
Nandi and Sen (1985) reported that the suspensions of mutant strains of Klebsiella and Azotobacter chroococcum sprayed on the phyllosphere of rice and wheat plants grown in a sandy loam soil increased their dry weight and grain N contents.

Rao (1986) reported that inoculation of rice either with Azotobacter or Bluegreen algae significantly increased the yield. The effect of combined inoculation of these two inocula on grain yield was additive.

Rao et al. (1986) studied heterotrophic nitrogen fixation (C_2H_2 reduction) as influenced by phosphorus application in paddy soils. They reported that addition of phosphorus had little effect on nitrogen fixing micro-organisms in the alluvial soils but increased Azospirillum and Azotobacter in the P-deficient soils.

Rai and Gaur (1988) studied the effect of Azotobacter and Azospirillum as inoculants on the yield and N-uptake of wheat crop. They found that there was significant increases

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in the yield of wheat grain and uptake of nitrogen by the crop over the control when seeds were inoculated either with Azospirillum species or Azotobacter species or the combination of both the inoculants.

(b) Response to Azospirillum inoculation

Spirillum lipoferum was first discovered by Beijerinck (1925) as a possible nitrogen fixer. Later Becking (1963) used ^{15}N and demonstrated the ability of the organism to fix N_2 . Relatively little attention was given to this organism until Dobereiner and Day (1974) isolated it from roots of certain tropical grasses in Brazil. The term Azospirillum was designated by Tarrand *et al.* (1978).

The tropical graminase maize, sorghum, sugarcane, rice and forage grasses are most frequently cited as hosts for Azospirillum (Lakshmikumari *et al.*, 1976; Amer *et al.*, 1977; Hegazi *et al.*, 1979; Wong and Stenberg, 1979).

There is increasing interest in attempts to enhance N_2 - fixation by associated systems through artificial inoculation. Certain experiments have shown the possibility of N_2 fixation associated with Azospirillum inoculation.

Nur *et al.* (1980) obtained both increase in weight and total nitrogen content in Seteria italica and Zea mays when inoculated with Azospirillum in soil under green house conditions.

Dunigan et al. (1981) reported that nitrogen fixation was significantly increased by inoculation of rice with Azospirillum brasilense. They also reported that application of N fertilizers tends to suppress nitrogen fixation.

Hegazi et al. (1981) observed that when wheat was inoculated with Azospirillum brasilense, plant height, tiller number and yield of both grain and straw were increased.

Rinaudo et al. (1981) found that results of inoculation in rice depend on strain of Azospirillum, plant genotype and soil type.

Villasboas and Dobereiner (1981) observed a significant effect of inoculation with Azospirillum on nitrate reductase activity which indicated a specific participation of this bacterium in plant metabolism affecting the reduction and/or translocation of soil NO^{-3} .

Rao and Rao (1983) found that in a field trial during the wet season, Azospirillum inoculation slightly increased the rhizosphere nitrogenase activity for upto 88 days after sowing. After urea treatment of the soil, the effect was greater. The populations of anaerobic nitrogen fixers and Azotobacter were found to increase after inoculation + urea treatment, but no change in the number of Azospirillum was observed.

Rao et al. (1983) studied the response of rice to root inoculation with Azospirillum under different N fertility levels over three consecutive seasons under tropical conditions. They found that the response to Azospirillum was more pronounced at 30 and 45 kg N ha⁻¹ than at 60 kg. Although root inoculation with Azospirillum resulted in a significant increase in mean yields of both grain and straw especially at low N rates, the interaction between the N fertilizer and inoculation was not statistically significant.

Watanabe and Lin (1984) reported that in pot experiments, inoculation of wetland rice cv. IR26 seedlings with nitrogen fixing Pseudomonas sp. (H₈) and Azospirillum lipoferum (34H) promoted tillering and increased the rate of grain filling and grain yield per plant. Inoculation also increased acetylene reduction activity and the number of nitrogen fixing bacteria associated with roots at the early flowering stage.

Rai (1985) reported that inoculation of rice with the mutant strains of Azospirillum brasilense gave higher N contents at the tillering and flowering stages and in grain and straw both with and without butachlor than those of uninoculated rice or rice inoculated with parental strain.

Gopalaswamy and Vidhyasekaran (1987) reported that inoculation of rice with Azospirillum brasilense increased

the grain yield by 0-22 per cent and straw yield by 8-41 per cent. The highest increases were in combination with 75 kg N ha⁻¹.

Omar et al. (1987) reported that inoculation of seeds and seedlings of rice with live inoculum of Azospirillum brasilense gave 16-22 per cent increase in rice grain yield at maximum recommended N fertilizer rate compared with uninoculated controls.

Purushothaman et al. (1987) reported that inoculation of rice seeds with Azospirillum brasilense strains increased germination percentage and vigour index. Inoculation also increased shoot and root lengths and number of primary roots in rice.

Effect of biotic factors on the activities of beneficial soil microorganisms

Among the biotic factors influencing the activities of beneficial soil bacteria and algae, the predators like Bdellovibrios, viruses and protozoa which feed on these organisms are very important.

Bacteriolysis by Bdellovibrios

Starr and Seidler (1971) have reported that a variety of well documented bacteriolytic phenomena of G : Bdellovibrio is known by which they cause the lysis or

dissolution of living or dead bacteria and cell wall preparations. They observed that these organisms have intriguing predatory, endoparasitic and bacteriolytic activities.

Varon and Zeigler (1978) studied the bacterial predator - prey interaction using Bdellovibrio and bioluminescent prey bacteria Photobacterium. The attacking Bdellovibrio caused decay of bioluminescence, which was correlated with the Bdellovibrio penetration into the prey. They reported that the prey density required to give the bdellovibrios a 50 per cent chance of survival was calculated to be at least 3.0×10^6 cells per ml and the density required for population equilibria was about 7×10^5 prey bacteria per ml. These values, not generally characteristic of natural habitats, suggest that the existence of Bdellovibrio in nature is limited to special ecological niches.

Effect of viruses on beneficial activities of bacteria

Hicks et al. (1983) have reported that the infection of soybeans with bean pod mottle virus and soybean mosaic virus decreases the nitrogen fixation in soybeans.

Khadhair et al. (1984) studied the effect of white clover mosaic virus infection on several factors relevant to symbiotic nitrogen fixation in nodulated red clover (Trifolium pratense L.). They found that virus infection

caused a significant reduction of plant growth, nodulation, rhizobial population, nitrogenase activity and leg hemoglobin concentration in the nodules. A significant increase in inducible nitrate reductase activity was associated with virus infection of plants.

Chan et al. (1989) studied the denitrification activity of phage types representative of two populations of indigenous Rhizobium meliloti. They observed that each of the five phage types showed variation in denitrification ability between isolates from different sites, indicating possible adaptation of indigenous R.meliloti to their respective environments.

Protozoan predation

Gray (1952) investigated the association between ciliate protozoa and bacteria. In general, the largest number of Ciliate species and individual species were associated with a growth of green or blue-green filamentous algae. Though there was no correlation between total bacterial numbers and total number of ciliates present at any time, the highest bacterial numbers occurred when diatom eating ciliates were dominant. He also reported that addition of a small quantity of a broth culture of Micrococcus to cultures of soil ciliates in soil extract hay infusion medium led to a rapid multiplication of the ciliates.

Darbyshire and Greaves (1967) reported that the populations of flagellates and amoebae were significantly higher in the rhizosphere soil of Sinapsis alba L. and Trifolium repens L. than in unplanted soil. Dessication of soil initially caused a reduction in both protozoan and bacteria rhizosphere numbers. Subsequently both populations recovered with the flagellates and amoebae again more abundant in the rhizosphere than in unplanted soil.

Drake and Tsuchiya (1977) studied the growth kinetics of Colpoda steinii grown in two stage continuous cultures with Escherichia coli as Prey species. Steady states were reached in the second stage continuous cultures at all growth rates. Although, changes occurred in mean all size of the ciliates and in the number/mm at various growth rates, the yield of protozoan biomass per unit of prey consumed was constant at all growth rates.

Watson et al. (1980) studied the growth kinetics of the ciliate Tetrahymena pyriformis on non-growing Escherichia coli. They reported that viable, non-existed protozoa always stopped feeding before the bacterial density was reduced to zero and non-feeding ciliates tended to swim faster than feeding ciliates.

Beaver et al. (1982) reported that the abundance and biomass of ciliates were positively related to the trophic

state in fresh water lakes. The response is attributed to changes in food availability. Small bodied ($<30\ \mu\text{m}$) bacterivorous taxa were reduced in oligotrophic lakes where bacterial concentrations are limiting and were replaced by larger bodied taxa ($>30\ \mu\text{m}$) able to ingest nanoplanktonic algae in addition to bacteria.

Sambanis and Fredrickson (1987) conducted long term studies of ciliate bacterial interactions using Tetrahymena pyriformis and Escherichia coli. They found that upward drifts of ciliate number density and downward drifts of ciliate mean size and density of residual bacteria occurred for many generations of ciliates before steady states were obtained. Rates of drift were slower and drifts persisted longer both in terms of time and in terms of numbers of generations, in the chemostat operated at the higher dilution rate.

David Glaser (1988) reported that the addition of ciliated protozoa to aquatic microcosms and bench-scale sewage treatment plants increases decomposition rates. One possible mechanism of the increase in decomposition rate was found to be the direct consumption of dissolved organic matter by protozoa that feed primarily on bacteria. Simultaneous consumption of bacteria and dissolved organic matter was proved using Tetrahymena pyriformis and Escherichia coli.

Sambanis and Fredrickson (1988) reported that the interactions between a bacterial population and a population of bacterivorous ciliates consistently showed that the bacteria were able to persist in the presence of viable ciliates. Reproduction of the bacteria, presumably at the expense of substrates produced by death and lysis of the ciliates and/or by their metabolic activity, has been suggested to be a factor involved in the observed bacterial persistence.

Sibbald and Albright (1988) presented laboratory evidence for the selective grazing of aggregated bacteria by the protozoan Bodo sp. and free bacteria by the protozoa Paraphysomonas sp.

Koteswara et al. (1990) noticed that the sizes of the populations of individual bacterial species diminished following their addition to water from lakes with different trophic levels. They concluded that the death of most of the test species was not the result of starvation, abiotic factors, bdellovibrios or bacteriophages but protozoan predation was a significant factor in the fall of bacterial population sizes.

Berninger et al. (1991) reported that ciliates and other grazing microzooplankton probably limit the abundance of bacteria and heterotrophic nanoplankton especially in more

productive water bodies. They also opined that bacterial abundances are regulated by substrate supply and heterotrophic nanoplankton grazing pressure.

Predator-prey relationship

Fenchel and Barkerjorgensen (1977) reported that bacteria constitute an important food source for free living protozoa. Among all major groups of protozoa (flagellates, rhizopods and ciliates), the most complex adaptations for feeding were found among the ciliates, and this biologically diverse group is the best known among the protozoa.

Hunt et al. (1977) developed a simulation model for the effect of predation on bacteria on continuous culture. They reported that in C limiting media predation increased NH_4 uptake inspite of a decrease in bacterial biomass. In N limiting media, predation decreased both biomass and the rate of N uptake. The effect of growth rate on the chemical composition of bacteria was proposed as a possible explanation of the stimulatory effect of predators on bacteria.

Tom Fenchel (1980) studied suspension feeding in ciliated protozoa. He reported that the species specialized on smaller food particles (0.2-1 μm), the size range of most bacteria in natural environments, require a higher concentration of particles. Bacterial population densities

which can sustain ciliate growth are found in sediments, waters rich in organic material and in the early successional stages of decomposing organic material.

Alexander (1981) reported that microbial predators and parasites do not eliminate their prey and hosts completely. He reported that the predators feed on the prey only until the prey density decreases to a certain level and later they stop feeding.

Hatano et al. (1981) studied the seasonal change of protozoa and micrometazoa in a small pond with leaf litter supply. They found that most peaks of bacterivorous protozoa in the leaf litter appeared in the late autumns and spring. This phenomenon was closely related to the litter decomposition because reduction in dissolved oxygen and/or high bacterial density were observed in these two seasons.

Marianne Clarholm (1981) studied the interactions between bacteria and protozoa in soil both in the field and in a pot experiment. He reported that in the field, peaks in numbers and biomass of bacteria appeared after the rain and a peak of naked amoebae quickly followed. The bacterial increase was transient and the amoebae alone were calculated to be able to cause 60 per cent of bacterial decrease. In pot experiment, in the presence of roots, amoebic numbers increased 20 times and caused the whole bacterial decrease

observed but in unplanted soil, consumption by the amoebae caused only one-third of the bacterial decrease.

Mallory et al. (1983) found that in sewage Tetrahymena thermophilus did not significantly reduce the population of Salmonella typhimurium when the density of the bacterium was about 10^4 /ml. However, when more than 10^8 Enterobacter agglomerans cells per ml were added, it reduced the abundance of E. agglomerans and S. typhimurium to 10^4 and 10/ml respectively. He proposed that predators will eliminate a prey spp from a natural environment when an alternative prey is present at concentration above the threshold number for active feeding by the predator and when the rate of growth of the prey is less than the rate of predation.

Baltzis and Fredrickson (1984) reported that for ecosystems involving two predators competing for a growing prey population have shown that the two competitors can co-exist in a state of sustained oscillations for a range of values of the system parameters.

Jackson and Jacques Berger (1984) studied the survival ability of different ciliate protozoa under starvation conditions and at low bacterial levels. They reported that at low bacterial levels, four ciliate species displayed significant differences in survivorship. High survivorship of Euplotes patella relative to that of Paramecium caudatum

and Paraurostyla sp. was attributed to the lower individual energy requirements of this small species. High survivorship of Stentor coeruleus was interpreted as an effect of its large quantity of reserves and low respiratory rate.

At the International Conference on the mechanisms of the relationship between soil plant microorganisms in the rhizosphere (1989) the population dynamics of an introduced bacterial population of the indigenous microflora and of the indigenous amoebal community were compared in a rhizospheric and non-rhizospheric soil and presented. The classical rhizospheric stimulating effect was observed for the indigenous soil bacterial populations and for the amoebal community too.

Muller et al. (1990) found that the abundance peaks of ciliates occurred in early spring and summer/autumn, while ciliate numbers were low in late spring (clear water phase) and winter in lakes. This was related to the availability of potential food organisms and grazers.

Shuichi Shikano et al. (1990) investigated the ecological and morphological characters of bacterial species under predation by a ciliate protozoa Cyclidium sp. The wild type shorter length bacteria appeared to escape predation by elongating cell size, but growth rate and saturation density were declined.

Vaque and Pace (1992) reported that there was not a simple relationship between total abundance of flagellates and total grazing rates. High community grazing by flagellates occurred when attached choanoflagellates were present. These flagellates had higher ingestion rates than free forms.

Effects of protozoa on beneficial soil bacteria

Danso and Alexander (1975) studied the protozoa - Rhizobium relationship and reported that Tetramitus rostratus and strains of Hartmanella, Naegleria and Varlkampfia consumed large numbers of Rhizobium meliloti cells in salt solution, but protozoan multiplication and the bacterial decline stopped when the prey density fell to about 10^6 to 10^7 cells/ml. It is suggested that large populations of bacteria in nature may be reduced in size by predatory protozoa, but many of the prey cells will not be eliminated.

Danso et al. (1975) noticed that a fall in Rhizobium abundance occurred in non-sterile soil inoculated with large numbers of root nodule bacteria, but many of the rhizobia still survived. No such decline was evident in sterile soil. Protozoa feeding on these bacteria were isolated from soil and other environments. As the abundance of Rhizobium meliloti and a cowpea Rhizobium strain in soil decreased, the protozoan density increased. It is suggested that the size of

the prey population diminishes until a density is attained at which the energy used by the predator in hunting for the survivors equals that obtained from the feeding.

Darbyshire (1976) studied the effect of water suction on the growth of the ciliate Colpoda steinii and the bacterium Azotobacter chroococcum in soil. He reported that in the samples maintained at P.F. 2.7, the Colpoda population decreased after inoculation and in other samples it increased after inoculation. Large numbers of Azotobacter cells were present in all samples, although there was a decline in numbers with time. Smaller Azotobacter populations were often present in the mixed compared to the pure cultures at the same P.F. value.

Habte and Alexander (1977) gave further evidence for the regulation of bacterial populations in soil by protozoa. They found that after the addition of large numbers of a cowpea Rhizobium strain to soil, the population declined steadily until the numbers reached about $10^7/\text{g}$ and the protozoa rose to about $10^4/\text{g}$. The addition to actidione treated soil of an antibiotic resistant Paramecium strain led to a rapid decrease in the population of the Rhizobium, the density reaching essentially the same value as in soil receiving neither the drug nor the paramecia. The same changes occurred with Xanthomonas campestris as test prey except that its numbers fell to about $10^5/\text{g}$ of soil.

Sardeshpande et al. (1977) studied the effect of the protozoa Colpoda sp. and Uroleptus sp. on Rhizobium S-12 and Azotobacter chroococcum. In the presence of protozoa, a fall in the population of Rhizobium S-12 and A.chroococcum in soil suggested that these bacteria serve as prey for the protozoa but complete devouring of the prey by the predators never occurred.

Habte and Alexander (1978) reported that Colpoda sp. inoculated with Rhizobium sp. into soil sterilized by autoclaving only reduced the prey abundance from 10^9 to 10^8 /g but the protozoa caused the bacterial population to fall to about 100/g in 15 days in presence of antibiotic chloramphenicol. The population of Rhizobium sp. added to non-sterile soil dropped from $>10^9$ to 10^6 /g in 29 days, but it declined to 550/g in the same period with antibiotic. It was concluded that the ability of the bacteria to maintain themselves in solution is governed by their capacity to reproduce and replace the cells consumed by predation.

Ramirez and Alexander (1980) provided evidence suggesting protozoan predation on Rhizobium associated with germinating seeds and in rhizosphere of beans (Phaseolus vulgaris L.). they reported that the extent of colonization of soil by R.phaseoli was inversely related to the presence of large numbers of bacteria and protozoa. Colonization of

R.phaseoli was improved upon suppression of protozoa with thiram and also when the soil was amended with other protozoan inhibitors and mannitol to simulate seed and root exudation.

MATERIAL AND METHODS

III MATERIAL AND METHODS

Experiments were carried out to study the influence of protozoa on the beneficial activities of soil bacteria in cowpea and rice crops. The experiments were conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra Campus, Bangalore.

Collection of soil samples

Soil samples were collected from the rice fields of different agro-climatic zones of Karnataka i.e., from Mandya, Shimoga and Chickamagalur. From Mandya, the soil samples were collected in two different seasons - kharif and summer as the crop was grown continuously in both the seasons. From Shimoga and Chikkamagalore, it was collected in kharif season. Nine inch depth soil along with some water was collected.

Chemical analysis of soil samples

Soil pH : The pH of the soil-water suspension (1 : 2.5 ratio) was measured using pH meter (Toshniwal, Model C₁-43) with combined glass electrode as described by Jackson (1973).

Electrical conductivity (EC) : The suspension used to measure soil pH was also used to measure the electrical conductivity. The electrical conductivity was measured using

an Elico conductivity bridge and was expressed as deci siemens per meter 25°C (Jackson, 1973).

Organic carbon : The organic carbon content was determined by dry combustion method in a muffle furnace as described by Jackson (1973). A known weight of the soil was ignited in silica tubes packed with copper oxide. The organic matter was oxidized by heating at 375°C and estimated by loss on weight basis.

Culturing protozoa

The most favourable media to culture protozoa was found to be hay infusion mixed with horlicks malted milk (Kudo, 1966; Srihari, 1975). Ten g of hay was boiled in 1000 ml distilled water which after cooling and filtering, was stored in the refrigerator as stock solution. 0.4 per cent of the above stock solution mixed with 5 ml of dilute solution (1 g in 100 ml) of horlicks malted milk was used as culture media.

Ten g of the soil sample was diluted in 100 ml distilled water in 250 ml conical flask in replicates and to this the culture media was added every alternate day and incubated at 28°C for one month.

Qualitative and quantitative study of protozoa

Identification of major protozoa

After incubating the soil suspension for one month, about 1-2 ml of the suspension was taken on a watch glass and

observed under low power in a binocular stereomicroscope. The major protozoa present in each soil sample was identified mainly based on the structure and morphology (Kudo, 1966).

Population count of protozoa

The population count of protozoa was done by direct microscopic count using stage and ocular micrometer. The area of a microscopic field was found out by mounting the stage micrometer on the stage of the microscope and ocular micrometer was fixed in the eye piece. After this, the number of microscopic fields present in 1 cm^2 area was found by using the formula.

$$\text{Number of fields} = \frac{1 \text{ cm}^2}{\text{Area of one microscopic field}}$$

One cm^2 area was marked on a clean glass slide and about 0.1 ml of the soil suspension was taken and evenly spread in that area and fixed with very dilute alcohol (10%). This was observed under the microscope and the number of protozoa present in each microscopic field was found out. By multiplying the number of protozoa with the number of microscopic fields, the protozoa present in 0.1 ml of soil suspension was found out. By knowing the moisture per cent, the population per gram of soil was determined.

Isolation and pure culture of predominant types of protozoa

Isolation of the protozoa was done by picking up the individual cells of the predominant types of protozoa present separately by using pasteur pipettes. The individual cells of different protozoa were placed separately in different petridishes containing distilled water. They were cultured separately using hay infusion and malted horlicks media.

Determination of the feeding habit of protozoa on bacteria

The cultures of the beneficial soil bacteria namely Rhizobium, Azotobacter and Azospirillum were obtained from the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore.

Rhizobium was maintained on yeast extract mannitol agar (YEMA) slants, Azotobacter on Waksman-77 (W77) slants and Azospirillum on malate media slants. To know the feeding habit, the bacteria were grown on yeast extract mannitol broth, Waksman 77 broth and malate broth respectively for 3-4 days in a rotary shaker at 28°C. After four days, equal quantities of different broth cultures was mixed with different protozoan suspensions separately and incubated for three days. Control was maintained by mixing the broth cultures with sterile water. Every day the population count of the bacteria and protozoa was done by direct microscopic count as described earlier and also plated.

Pot culture studies

The protozoa which exhibited feeding activity were selected and used in pot culture studies with paddy and cowpea to know their actual effect on the growth and nitrogen fixation in these crops.

Cowpea crop was grown for 45 days and paddy was grown for 120 days to find the effect on the yield of paddy.

Interaction studies of protozoa with bacteria in cowpea

Preparation of pots

The experiment was conducted in earthen pots of 9 inches diameter and 3 kg capacity. The pots were filled with the potting mixture consisting of soil : sand : FYM in 2 : 1 : 1 ratio and watered to reach field capacity.

Sowing

Cowpea [Vigna unguiculata (L.) Walp.] seeds (variety KBC-1) were surface sterilized as described by Vincent (1970). Clean, bold and healthy seeds were taken in a 250 ml sterile beaker and rinsed in 95 per cent alcohol for three minutes with agitation. Then, alcohol was poured off and the seeds were immersed in 0.1 per cent acidified mercuric chloride for three minutes. The mercuric chloride solution was decanted and seeds were washed in several changes of

sterile distilled water. The surface sterilized seeds were then soaked in sterile water and placed in the refrigerator for four hours.

After four hours, the seeds were taken out and sown in the pots at 2 inch depth. Six seeds were sown in each pot and after the seedlings emerged two seedlings were maintained in each pot and the rest were thinned out. Recommended dose of fertilizer (25 : 50 : 25 kg NPK/ha) was applied.

Preparation of inoculum

Preparation of bacterial inoculants

Rhizobium (P 132) and Azotobacter were used as bacterial inoculants in cowpea.

For the preparation of inoculum 500 ml conical flasks were used. 300 ml of sterilized YEM broth was dispensed into one flask and 300 ml of sterilized W-77 broth into the other. They are inoculated with two loops full of the respective cultures of the isolates. The flasks were kept on a rotary shaker for 7 days and then were used as inoculants.

Preparation of protozoan inoculants

The isolated protozoa were taken separately (Paramecium and Vorticella) in 250 ml conical flasks with distilled water and the culture media (Hay infusion and

Horlicks malted milk) was added daily. They were grown for one week and then used as inoculants.

Inoculation

Broth cultures were used in all instances for inoculation. Ten ml of the broth containing 10^8 Rhizobium/Azotobacter or 700 Paramecium/Vorticella was used to inoculate two cowpea seedlings in each pot after thinning out the extra seedlings. Four replications of each treatment was maintained and the pots were arranged in completely randomized design. Recommended dose of fertilizers were added to each pot including control.

Treatment details

Two bacteria Rhizobium, Azotobacter and two protozoa Paramecium, Vorticella were used in single and in combinations to study their effect on the growth and nodulation of cowpea. There were 13 treatments and four replications of each treatment was maintained. The treatment details are as follows :

- T₁ : Uninoculated control (C)
- T₂ : Rhizobium alone (R)
- T₃ : Azotobacter alone (A)
- T₄ : Rhizobium + Azotobacter (R + A)
- T₅ : Paramecium alone (P)

- T₆ : Vorticella alone (V)
 T₇ : Rhizobium + Paramecium (R + P)
 T₈ : Rhizobium + Vorticella (R + V)
 T₉ : Azotobacter + Paramecium (A + P)
 T₁₀ : Azotobacter + Vorticella (A + V)
 T₁₁ : Rhizobium + Azotobacter + Paramecium (R + A + P)
 T₁₂ : Rhizobium + Azotobacter + Vorticella (R + A + V)
 T₁₃ : Rhizobium + Azotobacter + Paramecium + Vorticella
 (R + A + P + V)

Observations

Observations on plant height and stem girth were taken regularly at an interval of 15 days. Soil was collected from the root zone of each pot and analysed for population count of bacteria and protozoa at every 15 days interval. Bacterial count was done by serial dilution plate count method on the respective media and protozan count was done by direct microscopic count.

Plant height was measured in centimeter using scale and stem girth in centimeter using vernier calipers.

Harvesting

The plants were uprooted after 45 days after sowing (DAS) and the following observations were made to know the influence of protozoa on growth, nodulation and nitrogen fixation of cowpea.

Nodule number : Number of root nodules were counted after carefully uprooting cowpea plants from the pots and detaching the nodules from the roots.

Nodule weight : Weight of the nodule was recorded after drying the nodules to constant weight at 75°C and expressed as mg per pot.

Nodule volume : It was determined by using Archimedes principle. The nodules were immersed in a measuring jar containing known quantity of water and the rise in volume was recorded as nodule volume.

Shoot dry weight : Dry weight of the shoot was recorded after separating the roots and dried in a plant drier to constant weight at 75°C and expressed as g per pot.

Root dry weight : Dry weight of the roots was recorded after separating the shoot and nodules and dried in a plant drier to constant weight at 75°C and expressed as g per pot.

Plant colour : Before harvest, the plant colour was scored on a scale 1 to 3 by visual rating with 1 indicating yellow, 2 light green, and 3 dark green as followed by Wynne et al. (1980a).

Determination of total nitrogen content

In order to study the amount of nitrogen harvested by different treatments, nitrogen present in shoot samples were estimated following micro-kjeldahl method (Jackson, 1973).

For this purpose 500 mg powdered oven dried plant samples were digested with 5 ml of concentrated sulphuric acid in the presence of 200 mg of catalyst mixture (K_2SO_4 : $CuSO_4$: Selenium in 100 : 10 : 1 ratio) in digestion tubes. The digestion was done initially keeping at lower temperature ($250^\circ C$) for 30 minutes and then keeping at higher temperature ($400^\circ C$) for 60 minutes in the digestion block. It was sufficient to get a clear solution of the digest, then the digest was cooled and diluted with distilled water and distilled with excess quantity (30 ml) of 40 per cent sodium hydroxide. The liberated ammonia was trapped in four per cent boric acid with mixed indicator and back titrated with against 0.1 N HCl until the colour changed from green to purple. From the volume of acid consumed, the per cent N in the sample was estimated as follows :

$$\text{Percentage N} = \frac{\text{Sample titre} \times \text{N of HCl} \times 0.014 \times 100}{\text{value (ml)}} \div \text{Weight of the sample}$$

Statistical analysis

The replicated data were statistically analysed in a completely randomised design as per the principles and procedures described by Sundararaj et al. (1972).

Interaction studies of protozoa and bacteria in paddy

Seeds and sowing

Seeds of rice (Oryza sativa L.) cv. 'Mangala' were obtained from the Department of Seed Technology, University of Agricultural Sciences, Hebbal and surface sterilized by the method of Dewan and Subba Rao (1979) using concentrated sulphuric acid for three minutes and washed repeatedly with sterilized water containing calcium carbonate till the effervesence development from acid and CaCO_3 reaction stopped. Then they were soaked in sterile water for two hours and bold and healthy seeds were selected.

Rice (Oryza sativa L.) cv. 'Mangala' was sown in seed beds prepared in battery boxes containing Potting mixture and irrigated twice a day until the seedlings were 20 days old. Recommended dose of fertilizers was added to the nursery beds.

Preparation of pots and transplanting

The experiment was conducted in plastic pots of 2.5 kg capacity. They are filled with the potting mixture as

described earlier. The 20 days old seedlings were carefully uprooted from the nursery beds after irrigating and were transplanted to the pots at the rate of two seedlings per pot. Recommended dose of fertilizers (100 : 50 : 50) were added. N was given in two doses (split dose) and P, K in one dose.

Inoculation

Preparation of bacterial inoculants

Azotobacter and Azospirillum were the bacteria used for study in paddy. Azotobacter was grown in Waksman 77 broth and Azospirillum in malate broth as described earlier.

Preparation of protozoan inoculants

Paramecium and Vorticella were the protozoa used in the study. They were grown using culture media as described earlier.

Each pot was inoculated with 10 ml of bacterial and/or protozoan suspensions. The suspension (inoculum) was poured around the seedlings at the base.

Treatment details

The bacteria Azotobacter, Azospirillum and the protozoa Paramecium and Vorticella were used in single and in combinations to study their effect on the growth and yield of

rice. There were 13 treatments and four replications of each treatment was maintained. The pots were arranged in completely randomized design. The treatment details are as follows.

- T₁ : Uninoculated control (C)
- T₂ : Azotobacter alone (Azt)
- T₃ : Azospirillum alone (Azs)
- T₄ : Azotobacter + Azospirillum (Azt + Azs)
- T₅ : Paramecium alone (P)
- T₆ : Vorticella alone (V)
- T₇ : Azotobacter + Paramecium (Azt + P)
- T₈ : Azotobacter + Vorticella (Azt + V)
- T₉ : Azospirillum + Paramecium (Azs + P)
- T₁₀ : Azospirillum + Vorticella (Azs + V)
- T₁₁ : Azotobacter + Azospirillum + Paramecium (Azt + Azs + P)
- T₁₂ : Azotobacter + Azospirillum + Vorticella (Azt + Azs + V)
- T₁₃ : Azotobacter + Azospirillum + Paramecium + Vorticella
(Azt + Azs + P + V)

Observations

At every 15 days interval, the plant height, number of leaves and number of tillers were recorded regularly. Plant height was measured using a scale in centimeter from the base of the plant to the emergence point of the top most leaf. Number of leaves and number of tillers were counted carefully in each pot.

Harvesting

The plants were harvested after 120 days when the grains were completely filled and dry. Panicles were harvested separately. Before harvest, number of panicles per pot, number of spikes per panicle and length of the panicles was recorded. After harvest, the weight of the panicles per pot was recorded.

The plants were uprooted, shoot and roots were separated and their dry weight were recorded after drying at 80°C for a constant weight in a plant drier.

Microbial analysis of the soil samples from each pot was done regularly at an interval of 30 days for bacterial and protozoan count as described earlier.

Determination of total nitrogen content from shoot

After the shoot samples was dried and powdered the total nitrogen content was found out by micro-kjeldahl method as described earlier.

Statistical analysis

The replicated data was statistically analysed in a completely randomized design as per the principles and procedures described by Sundararaj et al. (1972).

The experiment with cowpea was repeated with the following treatments in order to test the validity of the results obtained in the first experiment. All the conditions remained same in the second experiment also. The observations taken also remained the same. Five treatments and four replications were maintained.

Treatment details :

T₁ : Uninoculated control

T₂ : Rhizobium

T₃ : Rhizobium + Azotobacter

T₄ : Rhizobium + Vorticella

T₅ : Rhizobium + Azotobacter + Paramecium + Vorticella

The replicated data was statistically analysed in a completely randomized design as per the principles and procedures described by Sundaraj et al. (1972).

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The results of the various experiments conducted in order to study the influence of protozoa on the beneficial activities of soil microorganisms are presented below.

Soil chemical analysis

The results of the soil chemical tests are presented in Table 1. Except Shimoga soil, which was acidic in nature, the rest of the soils were almost in the neutral pH range. All the soils had medium organic carbon content. Among them, Shimoga soil showed higher organic carbon content (0.94%). The electrical conductivity of all the soil samples was in normal range.

Identification of the major protozoa

The soil samples along with hay infusion and horlicks malted milk was incubated for 1 month and observed under binocular microscope. Based on the morphology, the major protozoa were identified to be Paramecium, Colpoda and Vorticella. Table 2 shows the major protozoa found in each soil sample. Mandya soil contained Paramecium, Vorticella and Colpoda, Shimoga soil had only Vorticella, Chickmagalore soil had only Paramecium.

Plate 1. Effect of Paramecium on Rhizobium.

Plate 2. Effect of Vorticella on Rhizobium.



PLATE-1

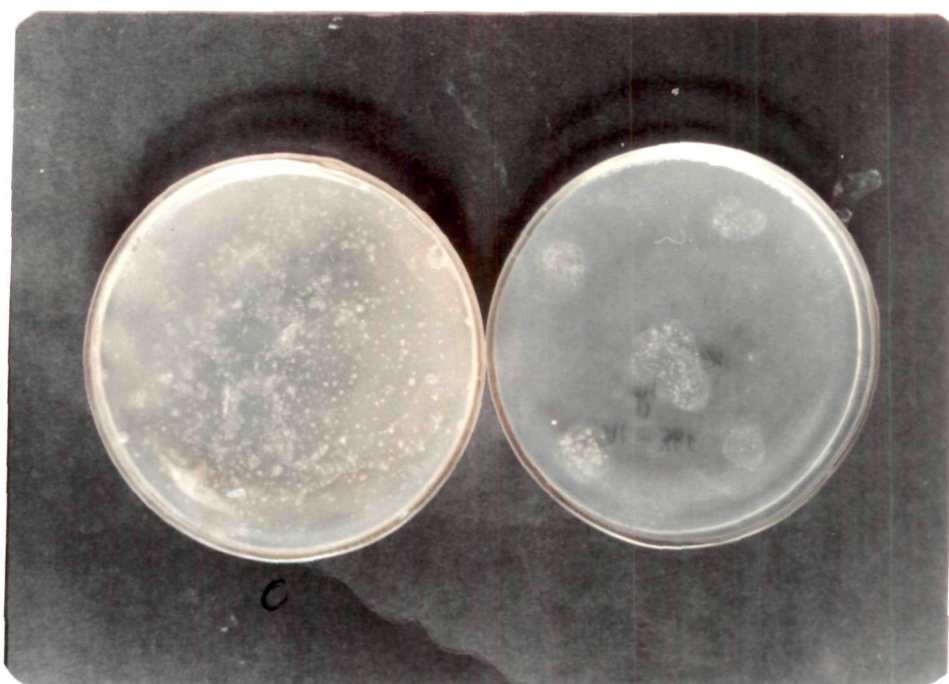


PLATE-2

Enumeration of the protozoa

The protozoa were enumerated by direct microscopic count using a binocular microscope, the protozoan numbers are presented in Table 2. In general, it was observed that Colpoda occurred in higher numbers in Mandya - I (220.00 g^{-1} and 1800 g^{-1} in Mandya - II soil) followed by Vorticella (120.00 g^{-1} in Mandya - II soil and 180.00 g^{-1} in Shimoga soil) and then followed by Paramecium (80.00 g^{-1} in Mandya - I and 120.00 g^{-1} in Chickmagalore soil).

In vitro effect of protozoa on the population of beneficial soil bacteria

The effect of the protozoa on the growth and multiplication of three beneficial soil bacteria Rhizobium, Azotobacter and Azospirillum in broth culture was studied. The experiment was conducted as per the procedure outlined in material and methods and the results are presented in Table 3. The bacterial number was found to be gradually decreasing with the increase in protozoan number. From 0-6 hrs of incubation there was no change in population observed. But from 24 hrs onwards till 96 hrs there was a gradual fall in bacteria with a rise in the protozoan number. Rhizobium was found to decrease more (From 5×10^6 to 2.7×10^4 with Paramecium to 2.3×10^4 with Vorticella and to 40×10^4 with Colpoda after 96 hrs) compared to Azotobacter and Azospirillum. Azotobacter decreased from

Table 1. Some chemical properties of the soil samples.

Soil sample	pH	E.C. (dsm/m)	Organic carbon (%)	Grade
Mandya I	6.35	0.13	0.69	Medium
Mandya II	6.45	0.13	0.67	Medium
Shimoga	5.20	0.10	0.94	High
Chickmagalore	6.60	0.15	0.63	Medium

dsm/m = deci siemens/metre

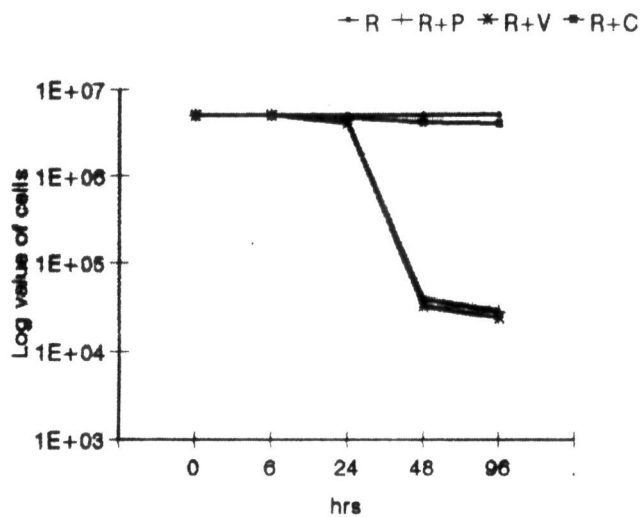
Table 2. Major protozoa found in the soil samples.

Soil sample	Genus of Protozoa	Protozoan number g ⁻¹ soil
Mandya I	<u>Paramecium</u>	80
	<u>Colpoda</u>	220
Mandya II	<u>Vorticella</u>	120
	<u>Colpoda</u>	180
Shimoga	<u>Vorticella</u>	180
Chickmagalur	<u>Paramecium</u>	120

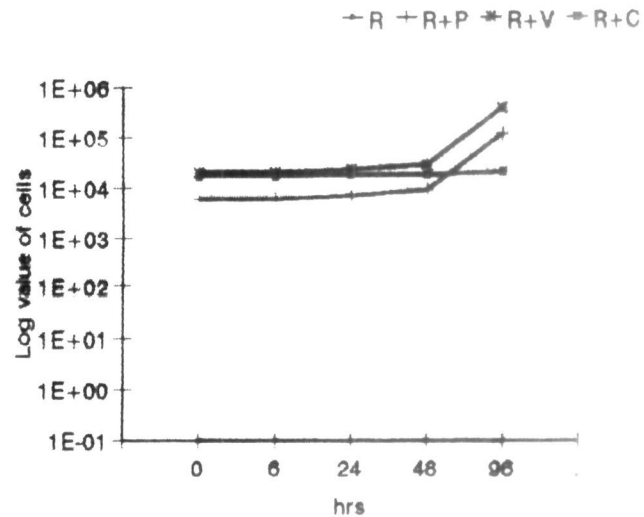
Table 3. Effect of protozoa on the population of bacteria in broth culture (ml^{-1}) in test tubes.

Treatments	0 hr		6 hr		24 hr		48 hr		96 hr	
	B ($\times 10^6$)	P ($\times 10^4$)	B ($\times 10^6$)	P ($\times 10^4$)	B ($\times 10^6$)	P ($\times 10^4$)	B ($\times 10^6$)	P ($\times 10^4$)	B ($\times 10^6$)	P ($\times 10^4$)
<u>Rhizobium</u> (Control)	5.0	-	5.0	-	5.0 (0.0)	-	500.0 (0.0)	-	500.0 (0.0)	-
<u>Rhizobium</u> + <u>Paramecium</u>	5.0	0.6	5.0	0.6	4.4 (-12.0)	0.7 (16.66)	3.8 (-99.24)	0.9 (+50.00)	2.7 (-99.46)	1.1 (+1733.3)
<u>Rhizobium</u> + <u>Vorticella</u>	5.0	2.0	5.0	2.0	4.0 (-20.0)	2.3 (15.00)	3.1 (-99.38)	2.8 (+40.00)	2.3 (-99.54)	3.6 (+1800.0)
<u>Rhizobium</u> + <u>Colpoda</u>	5.0	1.7	5.0	1.7	4.7 (-6.0)	1.8 (+5.88)	411.5 (-9.17)	1.8 (+5.88)	400.0 (-9.20)	0.2 (+17.64)
<u>Azotobacter</u> (Control)	3.0	-	3.0	-	3.0 (0.0)	-	300.0 (0.0)	-	300.0 (0.0)	-
<u>Azotobacter</u> + <u>Paramecium</u>	3.0	0.6	3.0	0.6	2.8 (-6.66)	0.7 (+16.66)	2.3 (-99.23)	0.8 (+33.33)	1.8 (-99.4)	1.0 (+1566.6)
<u>Azotobacter</u> + <u>Vorticella</u>	3.0	2.0	3.0	2.0	2.6 (-13.33)	2.2 (10.00)	2.1 (-99.30)	2.8 (+40.00)	1.4 (-99.53)	3.2 (+1500.0)
<u>Azotobacter</u> + <u>Colpoda</u>	3.0	1.7	3.0	1.7	3.0 (-0.0)	1.7 (+0.0)	290.9 (-9.00)	1.8 (+5.88)	290.8 (-9.06)	0.18 (+5.88)
<u>Azospirillum</u> (Control)	3.0	-	3.0	-	3.0 (0.0)	-	300.0 (0.0)	-	300.0 (0.0)	-
<u>Azospirillum</u> + <u>Paramecium</u>	3.0	0.6	3.0	0.6	2.8 (-6.66)	0.7 (+16.66)	2.3 (-99.23)	0.8 (+33.33)	2.0 (-99.53)	1.0 (+1560.0)
<u>Azospirillum</u> + <u>Vorticella</u>	3.0	2.0	3.0	2.0	2.5 (-16.66)	2.3 (15.00)	2.0 (-99.33)	2.8 (+40.00)	1.8 (-99.4)	3.1 (+1450.0)
<u>Azospirillum</u> + <u>Colpoda</u>	3.0	1.7	3.0	1.7	3.0 (-0.0)	1.7 (+0.0)	290.9 (-9.00)	1.7 (0.00)	290.9 (-9.00)	0.17 (+0.00)

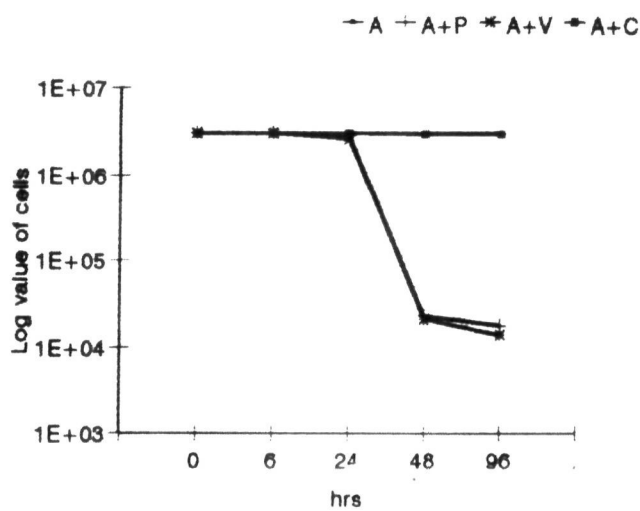
Note : Each value is a mean of four replications : Values in the parentheses indicate per cent increase or decrease (-) over 1 control
 B = Bacteria : Rhizobium / Azotobacter / Azospirillum as per the treatment
 P = Protozoa : paramecium / Vorticella / Colpoda as per the treatment



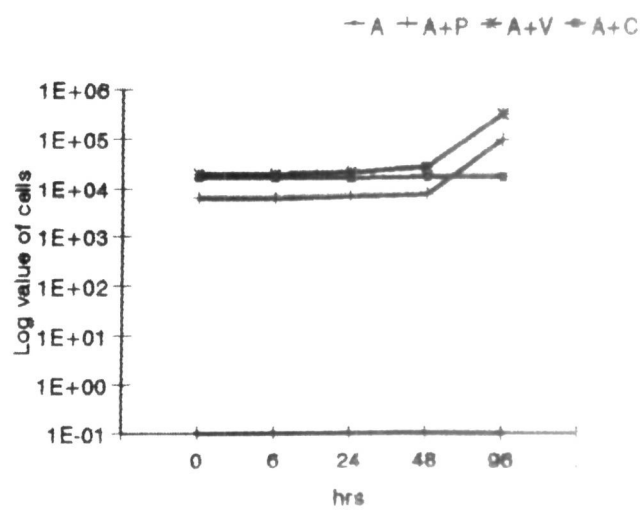
Ia. Effect of protozoa on *Rhizobium*



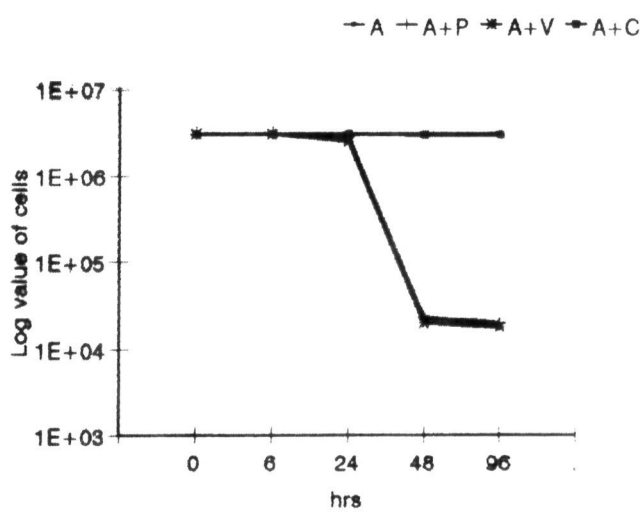
Ib. Effect on protozoa



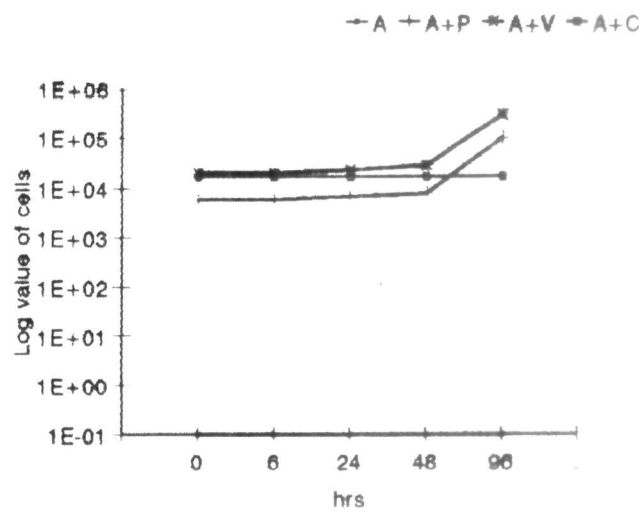
IIa. Effect of protozoa on *Azotobacter*



IIb. Effect on protozoa



IIIa. Effect of protozoa on *Azospirillum*



IIIb. Effect on protozoa

Fig.1: Effect of protozoa on the population of bacteria in broth culture

3×10^6 to 1.8×10^4 with Paramecium, to 1.4×10^4 with Vorticella and to 29.8×10^4 with Colpoda. Azospirillum decreased from 3×10^6 to 2×10^4 with Paramecium, 1.8×10^4 with Vorticella and to 29.9×10^4 with Colpoda after 96 hrs. In general, Vorticella reduced the bacterial count to a larger extent compared to Paramecium and Colpoda. Colpoda did not much affect the bacterial number, the reduction in bacterial number was only meagre (5×10^6 to 400×10^4 in Rhizobium, 3×10^6 to 290.8×10^4 in Azotobacter and 3×10^6 to 290.9×10^4 in Azospirillum) (Fig. 1). So only Paramecium and Vorticella were used for further pot culture studies.

Pot culture experiments

Soil for the pot culture studies was collected from the Gandhi Krishi Vignana Kendra farm. Soil sample from this collection was used for the analysis of physico-chemical properties and also to enumerate initial population of Rhizobium, Azotobacter, Azospirillum and the protozoa Paramecium and Vorticella. The study was conducted using cowpea (KBC-1) and rice (Mangala) varieties. Cowpea was grown for 45 days and rice crop was grown for 120 days. The results of these experiments are presented below.

Effect of protozoa on the plant height of cowpea

Plant height of cowpea plants ranged from 20.75 cm in R + A + P + V to 37.00 cm in R + A at harvest. The maximum

plant heights were recorded in the treatments without protozoa and the minimum in the treatments with protozoa. R + A showed maximum plant heights compared R and A alone. A similar trend was noticed even the observations recorded at 15 DAS and 30 DAS. Table 4 gives the details of the results of this experiment.

Effect of protozoa on the stem girth of cowpea

The stem girth of cowpea plants was recorded in centimeter using vernier calipers. The results of the effect of protozoa on the stem girth of cowpea is presented in Table 5. The treatments did not show any significant difference between them regarding stem girth statistically. But maximum stem girth was noticed in Rhizobium + Paramecium (0.5 cm) and minimum in Rhizobium + Azotobacter + Vorticella and Rhizobium + Azotobacter + Paramecium + Vorticella inoculated plants (0.4 cm) after 45 DAS. The stem girth ranged from 0.4 cm to 0.5 cm in other treatments.

Effect of protozoa on the colour of leaves in cowpea

The colour of the leaves was graded based on the visual observation. The results are presented in Table 6. The leaf colour ranged from 1.5 to 2.75. Maximum colour grade (2.75) was noticed in Rhizobium + Azotobacter inoculated plants and minimum (1.5) in plants inoculated with Vorticella, Rhizobium + Vorticella, Azotobacter + Paramecium, Azotobacter +

Plate 3. Effect of protozoa on Rhizobium in cowpea.

- 1 = Rhizobium alone
- 2 = Rhizobium + Vorticella
- 3 = Rhizobium + Paramecium
- 4 = Rhizobium + Azotobacter + Paramecium + Vorticella

Plate 4. Effect of protozoa on Azotobacter in cowpea.

- 1 = Azotobacter alone
- 2 = Azotobacter + Vorticella
- 3 = Azotobacter + Paramecium
- 4 = Azotobacter + Rhizobium + Paramecium + Vorticella



PLATE-3



PLATE-4

Table 4. Effect of protozoa on the plant height of cowpea.

Treatments	Plant height (cm)		
	15 DAS	30 DAS	45 DAS
Uninoculated (control)	11.63	21.25	30.00
<u>Rhizobium</u> (R)	14.13 (21.49)	23.00 (8.23)	34.38 (14.60)
<u>Azotobacter</u> (A)	13.00 (11.77)	22.88 (7.67)	31.88 (6.26)
R + A	16.03 (37.83)	23.25 (9.41)	37.00 (23.33)
<u>Paramecium</u> (P)	12.13 (4.29)	21.13 (-0.56)	29.13 (-2.9)
<u>Vorticella</u> (V)	10.63 (-8.59)	18.38 (-13.5)	25.50 (-15.0)
R + P	11.50 (-1.11)	21.80 (+2.58)	28.75 (-4.16)
R + V	11.38 (-2.14)	21.00 (-1.17)	27.00 (-10.0)
A + P	11.88 (+2.14)	22.88 (7.67)	27.25 (-9.16)
A + V	14.63 (25.79)	21.88 (2.96)	25.50 (-15.00)
R + A + P	15.50 (33.27)	21.88 (2.96)	29.50 (-1.66)
R + A + V	14.63 (25.79)	21.63 (1.78)	26.00 (-13.33)
R + A + P + V	8.44 (-27.42)	15.88 (-25.27)	20.75 (-30.83)

F test	*	*	*
C.D. at 5%	4.06	3.29	7.73

Note : Each value is a mean of four replications

The values in the parentheses indicate per cent increase or decrease (-) over uninoculated control.

Table 5. Effect of protozoa on the stem girth of cowpea.

Treatments	Stem girth (cm)	
	30 DAS	45 DAS
Uninoculated (control)	0.39	0.42
<u>Rhizobium</u> (R)	0.42 (7.69)	0.44 (2.27)
<u>Azotobacter</u> (A)	0.43 (10.25)	0.43 (0.00)
R + A	0.47 (20.51)	0.49 (13.95)
<u>Paramecium</u> (P)	0.49 (25.64)	0.46 (6.97)
<u>Vorticella</u> (V)	0.40 (2.56)	0.43 (0.00)
R + P	0.45 (15.38)	0.50 (16.27)
R + V	0.44 (12.82)	0.44 (2.27)
A + P	0.41 (5.12)	0.41 (-4.65)
A + V	0.41 (5.12)	0.46 (6.97)
R + A + P	0.45 (15.38)	0.48 (11.62)
R + A + V	0.43 (10.25)	0.40 (-6.97)
R + A + P + V	0.46 (17.94)	0.40 (-6.97)

F test	NS	NS
C.D. at 5%	0.06	0.08

Note : Each value is a mean of four replications.

Values in the parenthesis indicate per cent increase or decrease over the control.

Table 6. Effect of protozoa on the leaf colour of cowpea.

Treatments	Colour grade
Uninoculated (control)	1.75
<u>Rhizobium</u> (R)	2.25 (28.57)
<u>Azotobacter</u> (A)	2.00 (14.28)
R + A	2.75 (57.14)
<u>Paramecium</u> (P)	2.00 (14.28)
<u>Vorticella</u> (V)	1.50 (-14.28)
R + P	1.75 (0.00)
R + V	1.50 (-14.28)
A + P	1.50 (-14.28)
A + V	1.50 (-14.28)
R + A + P	1.50 (-14.28)
R + A + V	2.00 (14.28)
R + A + P + V	1.75 (0.00)

F test	*
C.D. at 5%	0.64

Note : Each value is a mean of four replications.

Values in the parenthesis indicate per cent increase or decrease over the control.

Vorticella and Rhizobium + Azotobacter + Paramecium. A significant difference between the treatments was noticed with respect to the colour grade.

Effect of protozoa on the plant biomass of cowpea

Shoot dry weight : The shoot dry weight ranged from 0.73 g to 3.78 g/pot. The results on shoot dry weight is presented in Table 8. The maximum shoot dry weight of 3.78 g was recorded in plants inoculated with Rhizobium + Azotobacter and minimum of 0.73 g was recorded in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella.

Root dry weight : The root dry weight ranged from 0.63 g to 3.43 g/pot. The results are presented in Table 7. Maximum root dry weight was observed (2.43 g) in Rhizobium + Azotobacter inoculated plants. Minimum root dry weight (0.63 g) was noticed in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella.

Total biomass : The results of total biomass are presented in Table 8. The total biomass of cowpea ranged from 1.36 to 6.21 g/pot. The maximum biomass of 6.21 g was recorded in the plants inoculated with Rhizobium + Azotobacter and minimum of 1.36 g in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella (Fig. 2).

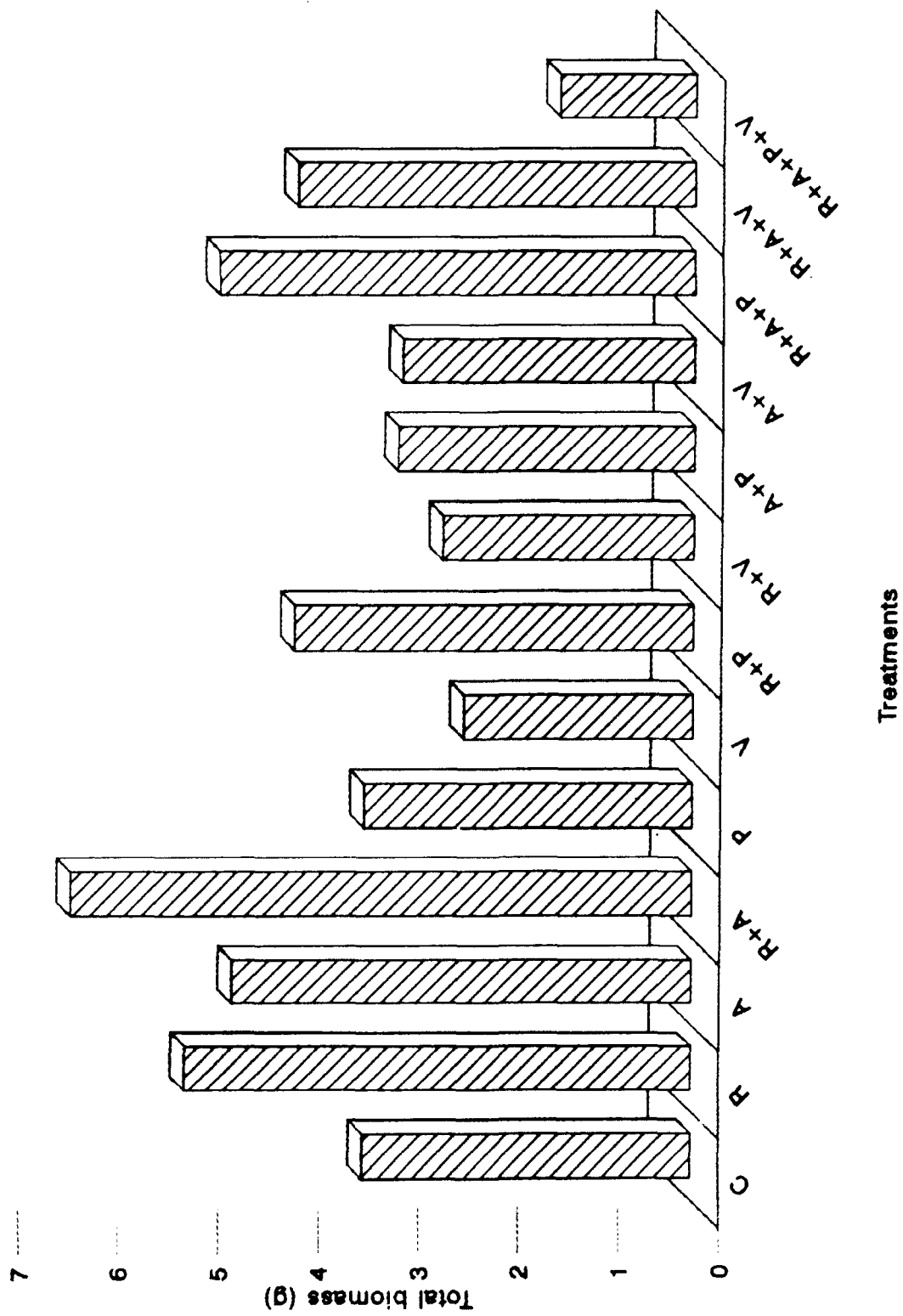
Table 7. Effect of protozoa on the plant biomass of cowpea.

Treatments	Shoot dry weight (g/pot)	Root dry weight (g/pot)	Total biomass (g/pot)
Uninoculated (control)	2.05	1.23	3.28
<u>Rhizobium</u> (R)	3.08 (50.24)	1.98 (60.97)	5.06 (54.26)
<u>Azotobacter</u> (A)	2.85 (39.02)	1.74 (44.46)	4.59 (39.93)
R + A	3.78 (84.39)	2.43 (97.50)	6.21 (89.32)
<u>Paramecium</u> (P)	2.05 (0.00)	1.23 (0.00)	3.28 (0.00)
<u>Vorticella</u> (V)	1.23 (-40.00)	1.06 (-13.82)	2.29 (-30.18)
R + P	2.90 (41.46)	1.09 (-11.38)	3.99 (21.64)
R + V	1.38 (-32.68)	1.13 (-8.13)	2.51 (-23.47)
A + P	1.98 (-3.41)	0.98 (-20.32)	2.96 (-9.75)
A + V	1.90 (-7.31)	1.02 (-17.07)	2.92 (-10.97)
R + A + P	3.20 (56.09)	1.55 (26.06)	4.75 (44.81)
R + A + V	2.67 (30.24)	1.30 (5.69)	3.97 (21.03)
R + A + P + V	0.73 (-64.39)	0.63 (-48.78)	1.36 (-58.53)

F test	*	*	*
C.D. at 5%	1.40	1.27	2.67

Note : Each value is a mean of four replications.

Values in the parenthesis indicate per cent increase or decrease over the control.



(N.B: C-Control; R-Rhizobium; A-Azotobacter; P-Paramecium; V-Vorticella)

Fig.2: Effect of protozoa on the total plant biomass of cowpea

Effect of protozoa on the nodulation in cowpea

Effect of nodule number : The nodule number in cowpea plants ranged from 47 to 97.50 per pot. The results on nodule number are presented in Table 8. The maximum nodule number 97.50 was recorded in the plants inoculated with Rhizobium + Azotobacter and minimum of 47.00 in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella followed by 49.75 in the plants inoculated with Rhizobium + Vorticella (Fig. 3).

Effect of nodule weight : The nodule weight ranged from 0.41 to 1.3 g/pot. The results on the effect of protozoa on nodule weight are presented in Table 9. Maximum nodule weight of 1.3 g was recorded in the plants inoculated with Rhizobium and minimum of 0.41 g in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella followed by 0.47 g in Vorticella inoculated plants..

Effect on nodule volume : The nodule volume ranged from 1.25 ml to 0.63 ml/pot. The results are presented in Table 9. The maximum nodule volume (1.25 ml) was observed in Rhizobium + Azotobacter + Vorticella inoculated plants. Minimum nodule volume (0.63 ml) was observed in the plants inoculated with Vorticella and Rhizobium + Azotobacter + Paramecium + Vorticella.

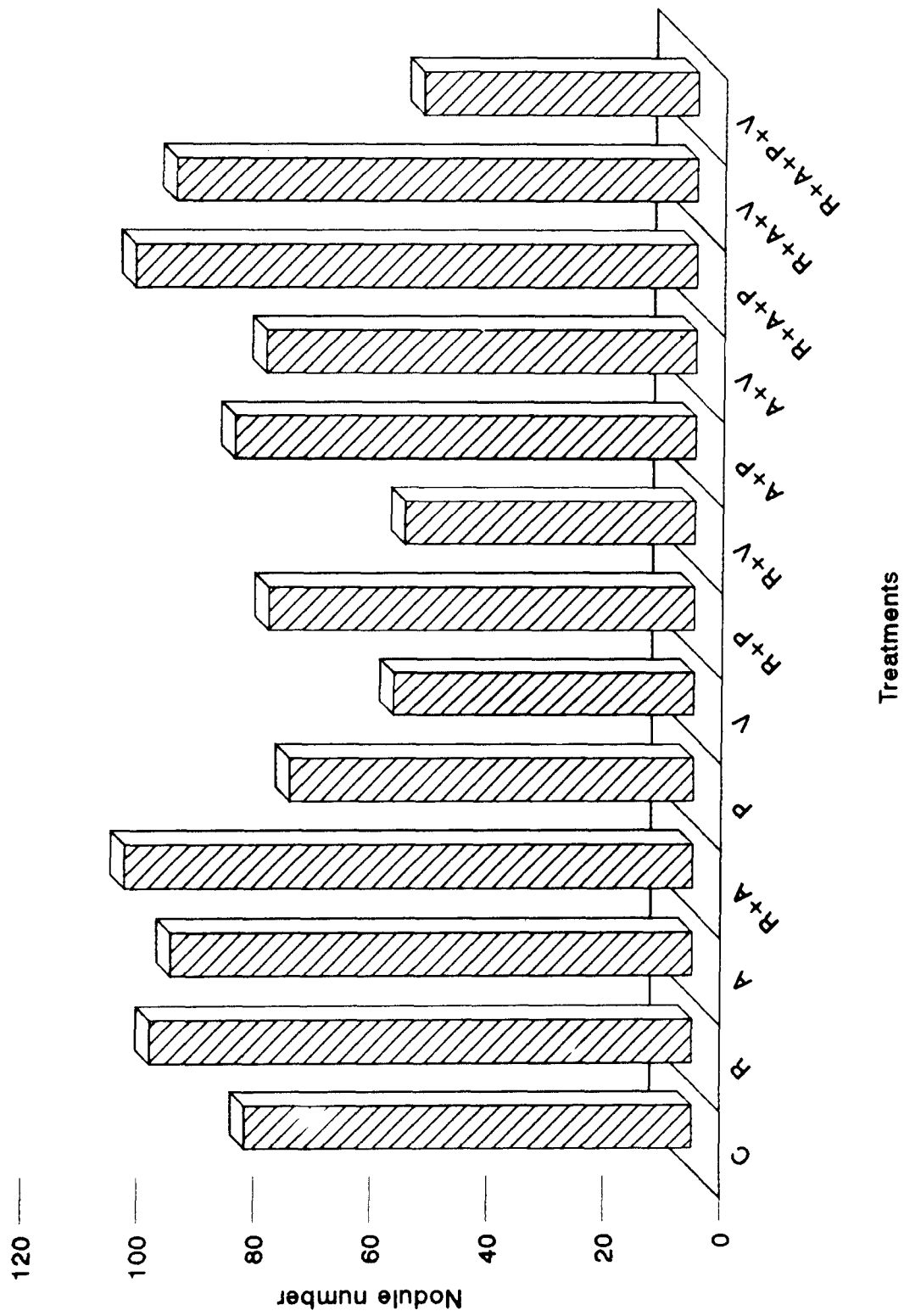
Table 8. Effect of protozoa on the nodulation in cowpea.

Treatments	Nodule number	Nodule weight (g/pot)	Nodule volume (ml/pot)
Uninoculated (control)	76.75	0.84	0.88
<u>Rhizobium</u> (R)	93.00 (21.17)	1.30 (54.76)	1.25 (42.04)
<u>Azotobacter</u> (A)	89.50 (16.61)	1.00 (19.04)	1.13 (28.40)
R + A	97.50 (27.03)	1.05 (25.00)	1.05 (42.04)
<u>Paramecium</u> (P)	69.25 (-9.77)	0.61 (-27.38)	0.81 (-7.95)
<u>Vorticella</u> (V)	51.50 (-32.89)	0.47 (-44.04)	0.63 (-28.40)
R + P	73.00 (-4.88)	1.00 (19.04)	0.88 (0.00)
R + V	49.75 (-35.17)	0.55 (-34.52)	0.69 (-21.59)
A + P	79.00 (2.93)	0.88 (4.76)	0.88 (0.00)
A + V	73.75 (-3.90)	0.77 (-8.33)	1.19 (35.22)
R + A + P	96.25 (25.40)	1.01 (20.23)	1.19 (35.22)
R + A + V	89.25 (16.28)	0.96 (14.28)	1.25 (42.04)
R + A + P + V	47.00 (-38.76)	0.41 (-51.19)	0.63 (-28.40)

F test	*	*	*
C.D. at 5%	6.28	0.50	0.41

Note : Each value is a mean of four replications.

Values in the parenthesis indicate per cent increase or decrease over the control.



(N.B.: C-Control; R-Rhizobium; A-Azotobacter; P-Paramecium; V-Vorticella)

Fig.3: Effect of protozoa on the nodulation in cowpea

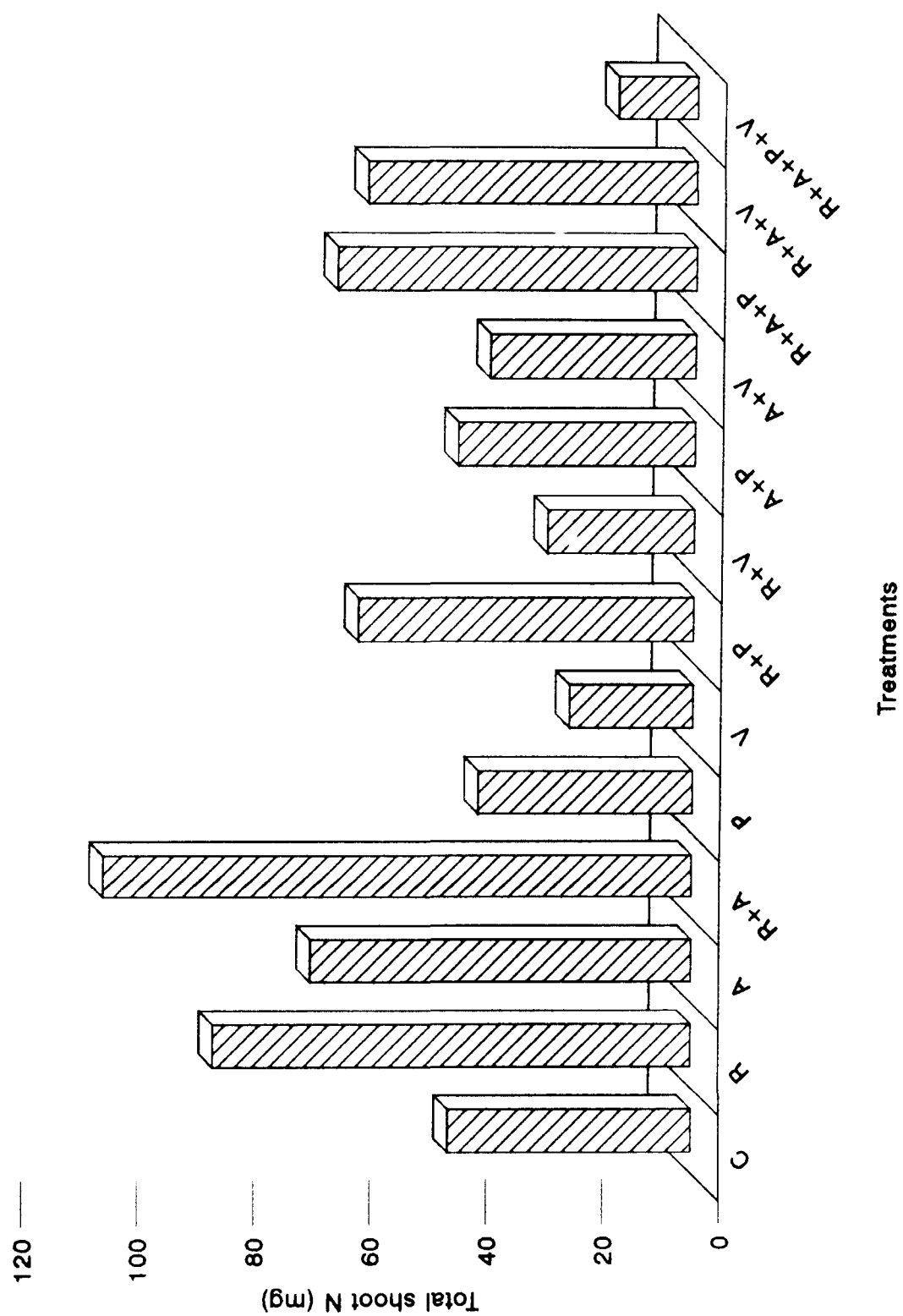
Table 9. Influence of protozoa on the shoot N content in cow pea.

Treatments	Per cent N	Total shoot N (mg/pot)
Uninoculated (control)	2.04	41.82
<u>Rhizobium</u> (R)	2.67 (30.88)	82.23 (96.62)
<u>Azotobacter</u> (A)	2.30 (12.74)	65.55 (56.74)
R + A	2.68 (31.37)	101.30 (142.20)
<u>Paramecium</u> (P)	1.80 (-11.76)	36.90 (-11.76)
<u>Vorticella</u> (V)	1.73 (-15.19)	21.28 (-49.11)
R + P	1.99 (-2.45)	57.71 (37.99)
R + V	1.83 (-10.29)	25.25 (-39.62)
A + P	2.06 (0.98)	40.78 (-2.48)
A + V	1.86 (-8.82)	35.34 (-15.49)
R + A + P	1.93 (-5.39)	61.76 (47.68)
R + A + V	2.12 (3.92)	56.60 (35.34)
R + A + P + V	1.87 (-8.33)	13.65 (-67.36)

F test	*	*
C.D. at 5%	0.075	1.05

Note : Each value is a mean of four replications.

Values in the parenthesis indicate per cent increase or decrease over the control.



(N.B.: C-Control; R-Rhizobium; A-Azotobacter; P-Paramecium; V-Vorticella)

Fig.4: Influence of protozoa on the shoot N content in cowpea

Influence of protozoa on the shoot N content in cowpea

Effect on per cent N : The per cent N values ranged from 1.73 per cent to 2.68 per cent. The results on per cent N are presented in Table 9. The maximum of 2.68 per cent was noticed in the plants inoculated with Rhizobium + Azotobacter and minimum of 1.73 per cent was noticed in the plants inoculated with Vorticella.

Effect on total shoot N content : The total shoot N content varied from as low as 13.65 mg to as high as 101.30 mg/pot. The results are presented in Table 10. The maximum shoot N of 101.30 mg was seen in the plants inoculated with Rhizobium + Azotobacter and minimum of 13.65 mg in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella (Fig. 1).

Effect of protozoa on the population of bacteria in cowpea

The effect of protozoa on the growth and multiplication of Rhizobium and Azotobacter was studied in the rhizosphere soil of cowpea. The results are presented in Table 10. The bacterial number was found to be gradually decreasing with the increase in protozoan number. About 10^8 cells ml^{-1} of Rhizobium and Azotobacter were inoculated at sowing time with or without 800 ml^{-1} and 600 ml^{-1} of Paramecium and Vorticella respectively. By the end of 45 days, Rhizobium decreased to about 1.5×10^6 with Paramecium and to 1.2×10^6 with

Table 10. Effect of protozoa on the population of bacteria in cowpea.

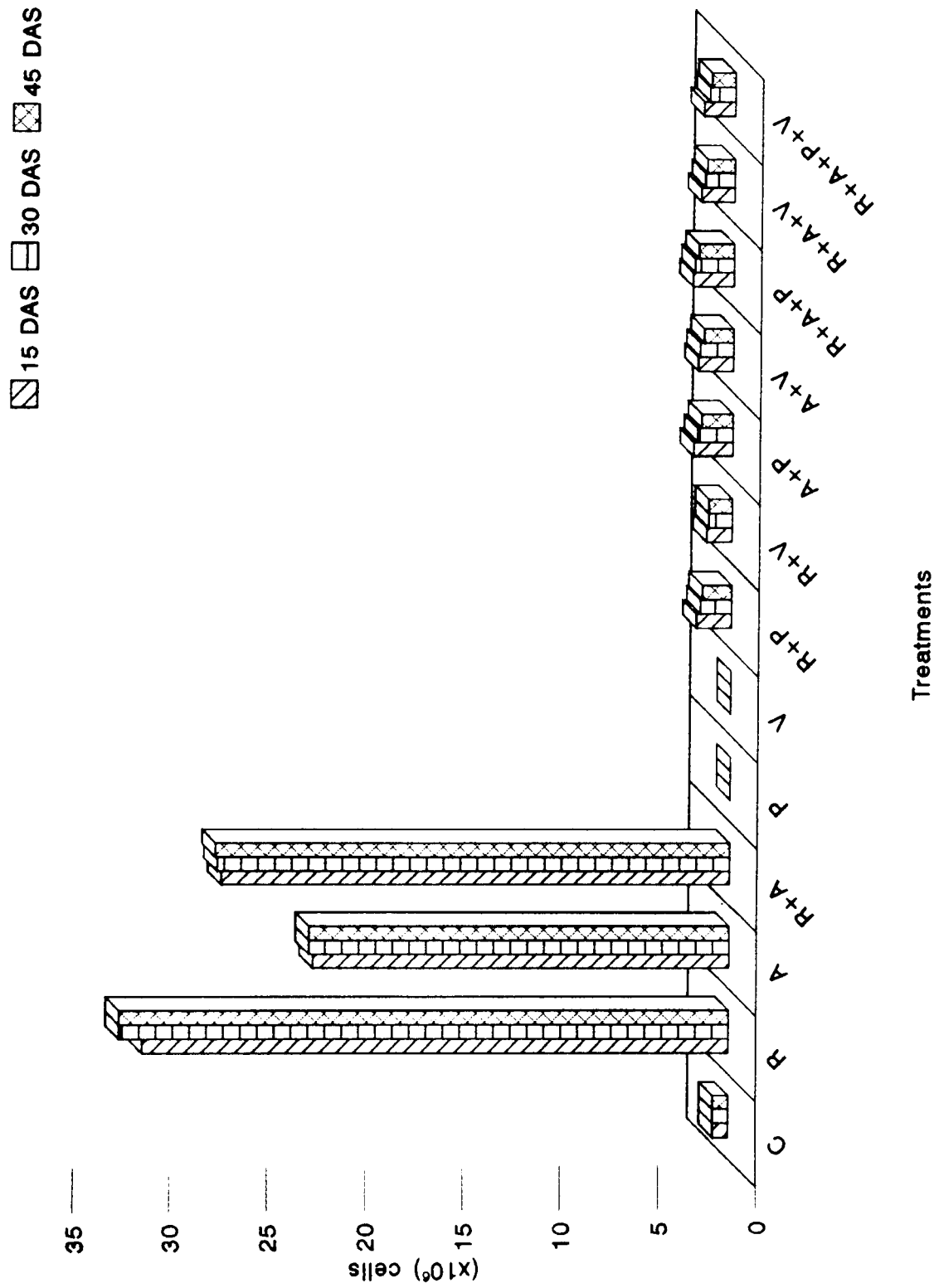
Treatments	15 DAS		30 DAS		45 DAS	
	B ($\times 10^6$)	P ($\times 10^2$)	B ($\times 10^6$)	P ($\times 10^3$)	B ($\times 10^6$)	P ($\times 10^4$)
Uninoculated (control)	0.8	0.2	0.8	0.02	0.8	0.002
<u>Rhizobium</u> (R)	30.0	-	31.2 (+4.0)	-	31.2 (+4.0)	-
<u>Azotobacter</u> (A)	21.3	-	21.5 (+0.9)	-	21.5 (+0.9)	-
R + A	30, 21.1	-	30.2, 21.2 (+0.66, +0.47)	-	31.2, 21.2 (+4.0, +0.47)	-
<u>Paramecium</u> (P)	-	8.0	-	0.82 (+2.5)	-	0.083 (+3.75)
<u>Vorticella</u> (V)	-	6.0	-	0.63 (+5.0)	-	0.065 (+8.33)
R + P	1.8 (-94.0)	8.8 (+10.0)	1.6 (-94.66)	9.0 (+1025)	1.5 (-95.0)	11.20 (+13900)
R + V	1.3 (95.66)	6.5 (+8.33)	1.2 (-96.0)	6.9 (+1050)	1.2 (-96.0)	11.50 (+19066)
A + P	2.0 (-90.61)	8.0 (0.0)	1.8 (-91.54)	8.3 (+937.5)	1.6 (-92.48)	10.92 (+13550)
A + V	1.8 (91.54)	6.2 (+3.33)	1.7 (-91.94)	6.5 (+983.3)	1.5 (-92.95)	10.85 (+17983)
R + A + P	2.2, 2 (-92.66, -90.52)	8.2 (+2.5)	2, 1.9 (-93.33, -90.99)	8.5 (+962.5)	1.8, 1.8 (94.0, -91.46)	10.88 (+13500)
R + A + V	2.0, 1.5 (92.33, -92.89)	6.2 (+3.33)	1.8, 1.2 (-94.0, -94.31)	6.7 (1016.6)	1.6, 1.11 (-94.66, -94.78)	10.92 (+18100)
R + A + P + V	1.8, 1.4 (24.0, -93.36)	9.6.8 (+12.5, -95.0, -94.31) (+13.33)	1.5, 1.2 (-95.0, -94.31)	9.2, 7.2 (+1050, +1100)	1.4, 1.0 (-95.33, -95.26)	11.2, 11.2 (+13900, 18566)

Note : Each value is a mean of four replications

Values in the parentheses indicate per cent increase or decrease

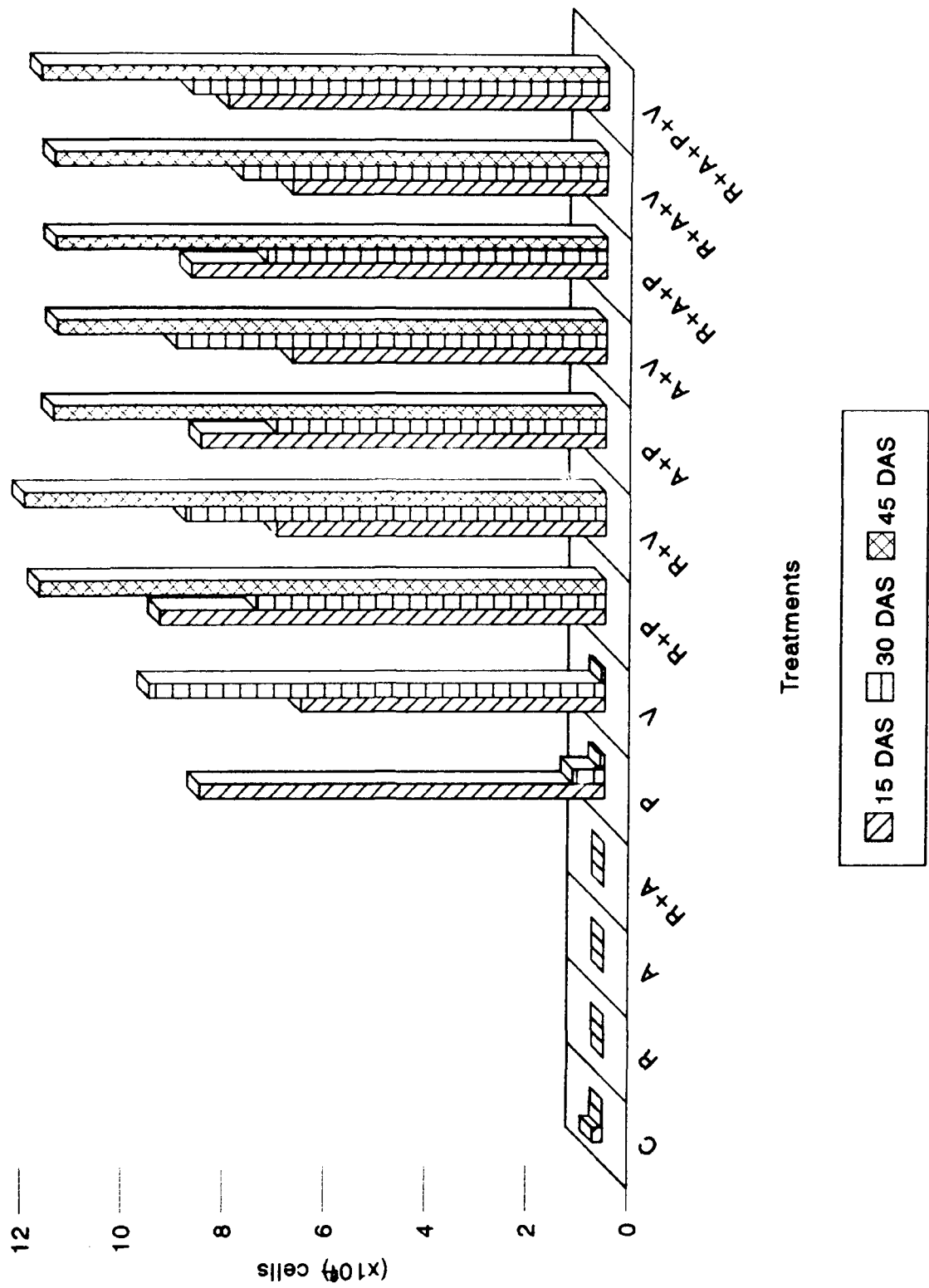
B = Bacteria : Rhizobium &/or Azotobacter

P = Protozoa : Paramecium &/or Vorticella



(N.B.: C-Control; R-Rhizobium; A-Azotobacter; P-Paramecium; V-Vorticella)

Va. Effect of protozoa on population of bacteria in cowpea at 15, 30 and 45 DAS



(N.B.: C-Control; R-Rhizobium; A-Azotobacter; P-Paramecium; V-Vorticella)

Vb. Increase in population of protozoa as influenced by bacteria

Vorticella compared to 31.2×10^6 in Rhizobium alone. Azotobacter decreased to about 1.6×10^6 with Paramecium and to 1.5×10^6 with Vorticella compared to 21.5×10^6 with Azotobacter alone. Rhizobium + Azotobacter decreased to about $(1.8 + 1.8) \times 10^6$ with Paramecium, $(1.6 + 1.1) \times 10^6$ with Vorticella and $(1.4 + 1.0) \times 10^6$ with Paramecium + Vorticella. Paramecium increased to about 11.2×10^4 with Rhizobium, 10.92×10^4 with Azotobacter and to 10.88×10^4 with Rhizobium + Azotobacter compared to 0.083×10^4 in Paramecium alone. Vorticella increased to about 11.5×10^4 with Rhizobium to 10.85×10^4 with Azotobacter and to 10.92×10^4 with Rhizobium + Azotobacter compared to 0.065×10^4 in Vorticella alone. In general, Vorticella reduced the bacteria more compared to Paramecium (Fig. 5a, 5b).

Results of Experiment - 2

The experiment with cowpea was repeated with limited number of treatments to test the validity of the results obtained in Experiment-I.

Effect of protozoa on the shoot characters of cowpea

The results of these parameters are presented in Table 11.

Plant height : It ranged from 23.50 to 36.25 cm. The maximum of 36.25 cm was found in the plants inoculated with

Table 11. Effect of protozoa on the shoot characters of cowpea.

Treatments	Plant height (cm)	Stem girth (cm)
Uninoculated (control)	30.00	0.425
<u>Rhizobium</u> (R)	32.50 (8.33)	0.462 (3.7)
<u>Rhizobium</u> + <u>Azotobacter</u> (A)	36.25 (20.83)	0.487 (6.2)
R + <u>Vorticella</u>	25.50 (-15.00)	0.412 (-1.3)
R + A + <u>Paramecium</u> + <u>Vorticella</u>	23.50 (-21.66)	0.400 (-5.88)

Note : Each value is a mean of four replications

Values in the parentheses indicate per cent increase or decrease (-) over uninoculated control

Rhizobium + Azotobacter and minimum of 23.50 cm in plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella followed by 25.50 cm in Rhizobium + Vorticella inoculated plants.

Stem girth : It ranged from 0.4 to 0.487 cm. The same trend was found here also.

Effect of protozoa on the plant biomass

Shoot dry weight : The results are presented in Table 12. The shoot dry weight ranged from 0.750 g in Rhizobium + Azotobacter + Paramecium + Vorticella inoculated plants to 2.8 g in Rhizobium + Azotobacter inoculated plants.

Root dry weight : It ranged from a maximum of 2.5 g in plants inoculated with Rhizobium + Azotobacter to a minimum of 0.45 g in Rhizobium + Azotobacter + Paramecium + Vorticella inoculated plants. Results are presented in Table 13.

Total biomass : A similar trend was observed in the total biomass also. It ranged from 1.2 g in Rhizobium + Azotobacter + Paramecium + Vorticella to 5.3 g in Rhizobium + Azotobacter inoculated plants.

Effect on nodulation : The results are presented in Table 14. The nodule number ranged from 44.5 to 94.00. The maximum was found in Rhizobium + Azotobacter inoculated

Table 12. Effect of protozoa on the plant biomass of cowpea.

Treatments	Shoot dry weight (g/pot)	Root dry weight (g/pot)	Total biomass (g/pot)
Uninoculated (control)	2.025	1.625	3.65
<u>Rhizobium</u> (R)	2.275 (12.34)	1.875 (15.38)	4.15 (13.69)
<u>Rhizobium</u> + <u>Azotobacter</u> (A)	2.800 (38.27)	2.500 (53.84)	5.30 (45.20)
R + <u>Vorticella</u>	1.125 (-44.44)	0.800 (-50.76)	1.925(-47.26)
R + A + <u>Paramecium</u> + <u>Vorticella</u>	0.750 (-62.96)	0.450 (-72.30)	1.200(-67.12)

F test	*	*	*
C.D. at 5%	0.66	0.62	1.28

Note : Each value is a mean of four replications

Values in the parentheses indicate per cent increase or decrease (-) over uninoculated control

Table 13. Effect of protozoa on nodulation in cowpea.

Treatments	Nodule number	Nodule weight (g/pot)
Uninoculated (control)	71.75	0.925
<u>Rhizobium</u> (R)	92.00 (28.22)	1.025 (10.81)
<u>Rhizobium</u> + <u>Azotobacter</u> (A)	94.00 (31.01)	1.062 (14.81)
R + <u>Vorticella</u>	49.75 (-30.66)	0.500 (-45.94)
R + A + <u>Paramecium</u> + <u>Vorticella</u>	44.50 (-37.97)	0.312 (-66.27)

F test	*	NS
C.D. at 5%	11.45	2.68

Note : Each value is a mean of four replications

Values in the parentheses indicate per cent increase or decrease (-) over uninoculated control

plants and minimum in Rhizobium + Azotobacter + Paramecium + Vorticella inoculated plants. The nodule weight ranged from 0.312 g to 1.062 g. The maximum was found in Rhizobium + Azotobacter inoculated plants and minimum in Rhizobium + Azotobacter + Paramecium + Vorticella inoculated plants.

Effect of protozoa on the population of bacteria in cowpea crop

A similar trend as explained in the results of experiment - 1 was found here also. The results are presented in Table 14. Vorticella was found to decrease the Rhizobium population more (from 3×10^6 to 1.2×10^6) and Paramecium and Vorticella when inoculated together had more effect it reduced the population of Rhizobium + Azotobacter from $(31 + 21.4) \times 10^6$ to $(1.0 + 0.96) \times 10^6$ by the end of 45 days.

Effect of protozoa on rice crop

Effect of protozoa on the plant height

The plant height of rice crop ranged from 44 cm to 56 cm. The maximum height of 56 cm was seen in the plants inoculated with Azotobacter + Azospirillum and minimum of 44 cm was seen in the plants inoculated with Vorticella at harvest. The results are presented in Table 15. A similar trend was observed in all the observations of plant height recorded at every 15 days interval. The first two

Table 14. Effect of protozoa on the population of bacteria in cowpea crop - II.

Treatments	15 DAS		30 DAS		45 DAS	
	B ($\times 10^6$)	P ($\times 10^2$)	B ($\times 10^6$)	P ($\times 10^3$)	B ($\times 10^6$)	P ($\times 10^4$)
Uninoculated control	0.8	0.2	0.8	0.02	0.8	0.002
<u>Rhizobium</u>	31.1	-	31.2 (+0.3)	-	31.2 (+0.3)	-
<u>Rhizobium</u> + <u>Azotobacter</u>	31 + 21.4	-	31.1 + 21.4 (+0.32, 0.0)	-	31.1 + 21.5 (+0.32, 0.46)	-
<u>Rhizobium</u> + <u>Vorticella</u>	1.6 (-94.85)	6.3 (+5.00)	1.3 (-95.83)	6.9 (+105.0)	1.2 (96.15)	10.98 (+18200)
<u>Rhizobium</u> + <u>Azotobacter</u> + <u>Paramecium</u> + <u>Vorticella</u>	21.3+11.8 (-31.29, -44.85)	8.5+6.6 (+6.25, 10.00)	2.0+1.6 (-93.56, -92.52)	8.1+6.4 (+912.5, +966.6)	1.4+1.1 (-95.48, -94.85)	11.0+10.96 (+13650, +18166.6)

Note : Each value is a mean of four replications

B = Bacteria - Rhizobium &/or Azotobacter

P = Protozoa - Vorticella &/or Paramecium

Values in the parentheses indicate per cent increase or decrease (-).

observations (15 DAT and 30 DAT) showed non-significant differences between the treatments but after that i.e., from 45 DAT onwards significant difference was noticed between the treatments.

Effect of leaf number

The leaf number ranged from 24 to 30.25 at harvest. The results on leaf number are presented in Table 16. A maximum of 30.25 leaves was observed in the plants inoculated with Azotobacter + Azospirillum and a minimum of 24 leaves was observed in the plants inoculated with Vorticella. A similar trend was observed in other observations also. But the treatments statistically did not differ significantly between themselves.

Effect of tiller number : The number of tillers in the rice crop ranged from 5.25 to 7.75. The results on the effect of protozoa on the number of tillers is presented in Table 17. A maximum of 7.75 tillers was noticed in the plants inoculated with Azotobacter + Azospirillum and a minimum of 5.25 tillers was noticed in the plants inoculated with Vorticella. But the treatments statistically did not differ significantly between themselves.

Effect of protozoa on the yield components in rice crop

Effect on number of panicles : The panicle number was found to be in the range of 4.75 to 7.00. The results are

Table 15. Effect of protozoa on the plant height of rice crop.

Treatments	Plant height (cm)					
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	100 DAT
Uninoculated control	15.075	17.750	23.500	30.500	49.000	49.000
<u>Azotobacter</u> (Azt)	15.000 (-0.49)	18.750 (5.63)	25.250 (7.44)	32.750 (7.37)	51.125 (4.33)	51.150 (4.38)
<u>Azospirillum</u> (Azs)	15.625 (3.64)	19.000 (7.04)	25.500 (8.51)	33.250 (9.01)	53.500 (9.18)	53.500 (9.18)
Azt + Azs	16.875 (11.94)	19.500 (9.85)	25.750 (9.57)	35.000 (14.75)	56.000 (14.28)	56.000 (14.28)
<u>Paramecium</u> (P)	16.625 (10.28)	18.375 (3.52)	23.750 (1.06)	30.500 (0.00)	45.750 (-6.63)	45.750 (-6.63)
<u>Vorticella</u> (V)	14.625 (-2.98)	17.250 (-2.81)	23.250 (-1.06)	28.750 (-5.73)	43.500 (-11.22)	44.000 (-10.28)
Azt + P	15.625 (3.64)	17.000 (-4.22)	21.000 (-10.63)	31.250 (2.45)	49.500 (1.02)	49.500 (1.02)
Azt + V	15.375 (1.99)	18.250 (2.81)	24.750 (5.31)	30.750 (0.81)	48.000 (-2.04)	48.250 (1.53)
Azs + P	16.750 (11.11)	18.750 (5.63)	25.500 (8.51)	33.000 (8.19)	48.750 (-0.51)	48.750 (-0.51)
Azs + V	15.250 (1.16)	18.500 (4.22)	25.000 (6.38)	31.500 (3.27)	48.500 (-1.02)	48.500 (-1.02)
Azt + Azs + P	17.750 (17.74)	18.750 (5.63)	25.250 (7.44)	32.500 (6.55)	51.500 (5.10)	51.500 (5.10)
Azt + Azs + V	15.000 (-0.49)	17.750 (0.00)	23.750 (1.06)	31.750 (4.09)	48.500 (-1.02)	48.500 (-1.02)
Azt + Azs + P + V	15.750 (4.47)	18.500 (4.22)	25.250 (7.44)	30.750 (0.81)	49.000 (0.00)	49.125 (0.25)
F test	NS	NS	*	*	*	*
C.D. at 5%	2.419	1.718	2.230	2.290	5.370	4.850

Note : Each value is a mean of four replications
 Values in the parantheses indicate per cent increase or decrease (-) over uninoculated control

Table 16. Effect of protozoa on the number of leaves in rice crop.

Treatments	Number of leaves					
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	100 DAT
Uninoculated control	16.00	19.50	27.00	31.25	37.75	28.50
<u>Azotobacter</u> (Azt)	15.75 (-1.56)	24.25 (24.35)	30.25 (12.03)	33.50 (7.20)	41.00 (18.60)	28.75 (0.87)
<u>Azospirillum</u> (Azs)	15.75 (-1.56)	27.00 (38.46)	30.75 (12.50)	34.00 (8.80)	44.25 (17.21)	29.50 (3.50)
Azt + Azs	21.00 (31.25)	27.25 (39.74)	31.25 (15.74)	34.50 (10.40)	44.50 (17.88)	30.25 (6.14)
<u>Paramecium</u> (P)	17.25 (7.81)	19.50 (0.00)	28.25 (3.70)	30.50 (-2.40)	38.25 (1.32)	25.00 (-12.28)
<u>Vorticella</u> (V)	17.50 (9.37)	24.00 (23.07)	27.50 (11.85)	30.00 (-4.00)	37.25 (-1.32)	24.00 (-15.78)
Azt + P	15.50 (-3.12)	23.00 (17.94)	29.00 (7.40)	29.25 (-6.40)	32.75 (-13.24)	26.00 (-8.77)
Azt + V	15.50 (-3.12)	20.75 (6.41)	30.00 (11.11)	30.75 (-1.60)	36.75 (-2.64)	27.00 (-5.26)
Azs + P	15.25 (-4.68)	22.50 (15.38)	30.25 (12.03)	34.00 (8.80)	42.00 (11.25)	27.00 (-5.26)
Azs + V	19.25 (20.31)	23.00 (17.94)	29.00 (7.40)	32.25 (3.20)	39.50 (4.63)	29.50 (3.50)
Azt + Azs + P	16.25 (1.56)	25.50 (30.76)	31.00 (14.81)	32.75 (4.80)	37.50 (-0.66)	26.75 (-6.14)
Azt + Azs + V	17.00 (6.25)	24.50 (25.64)	29.25 (8.33)	32.50 (4.00)	40.00 (5.96)	28.50 (0.00)
Azt + Azs + P + V	16.25 (1.56)	25.25 (29.48)	30.75 (12.50)	32.00 (2.40)	35.75 (-5.29)	26.75 (-6.14)
F test	NS	*	NS	NS	NS	NS
C.D.at 5%	4.23	4.51	5.39	5.26	8.26	6.13

Note : Each value is a mean of four replications
 Values in the parantheses indicate per cent increase or decrease (-) over uninoculated control

Table 17. Effect of protozoa on the number of tillers in rice crop.

Treatments	Number of tillers
Uninoculated control	5.75
<u>Azotobacter</u> (Azt)	7.00 (21.73)
<u>Azospirillum</u> (Azs)	7.00 (21.73)
Azt + Azs	7.75 (34.78)
<u>Paramecium</u> (P)	6.00 (4.34)
<u>Vorticella</u> (V)	5.25 (-9.52)
Azt + P	6.75 (17.39)
Azt + V	6.50 (13.04)
Azs + P	6.75 (17.39)
Azs + V	6.75 (17.39)
Azt + Azs + P	6.75 (17.39)
Azt + Azs + V	7.00 (21.73)
Azt + Azs + P + V	6.75 (17.39)

F test	NS
C.D.at 5%	1.70

Note : Each value is a mean of four replications

Values in the parantheses indicate per cent increase or decrease (-) over uninoculated control

presented in Table 18. The maximum number of panicles (7.00) was found in the plants inoculated with Azotobacter + Azospirillum and minimum of 4.75 panicles was found in the plants inoculated with Paramecium.

Effect on number of spikes per panicle : The average number of spikes per panicle ranged from 4.50 to 6.25. The results are presented in Table 18. The maximum number of spikes per panicle (6.25) was seen in the plants inoculated with Azotobacter + Azospirillum and the minimum number of spikes (4.50) was found in uninoculated control, Paramecium inoculated and also Vorticella inoculated plants.

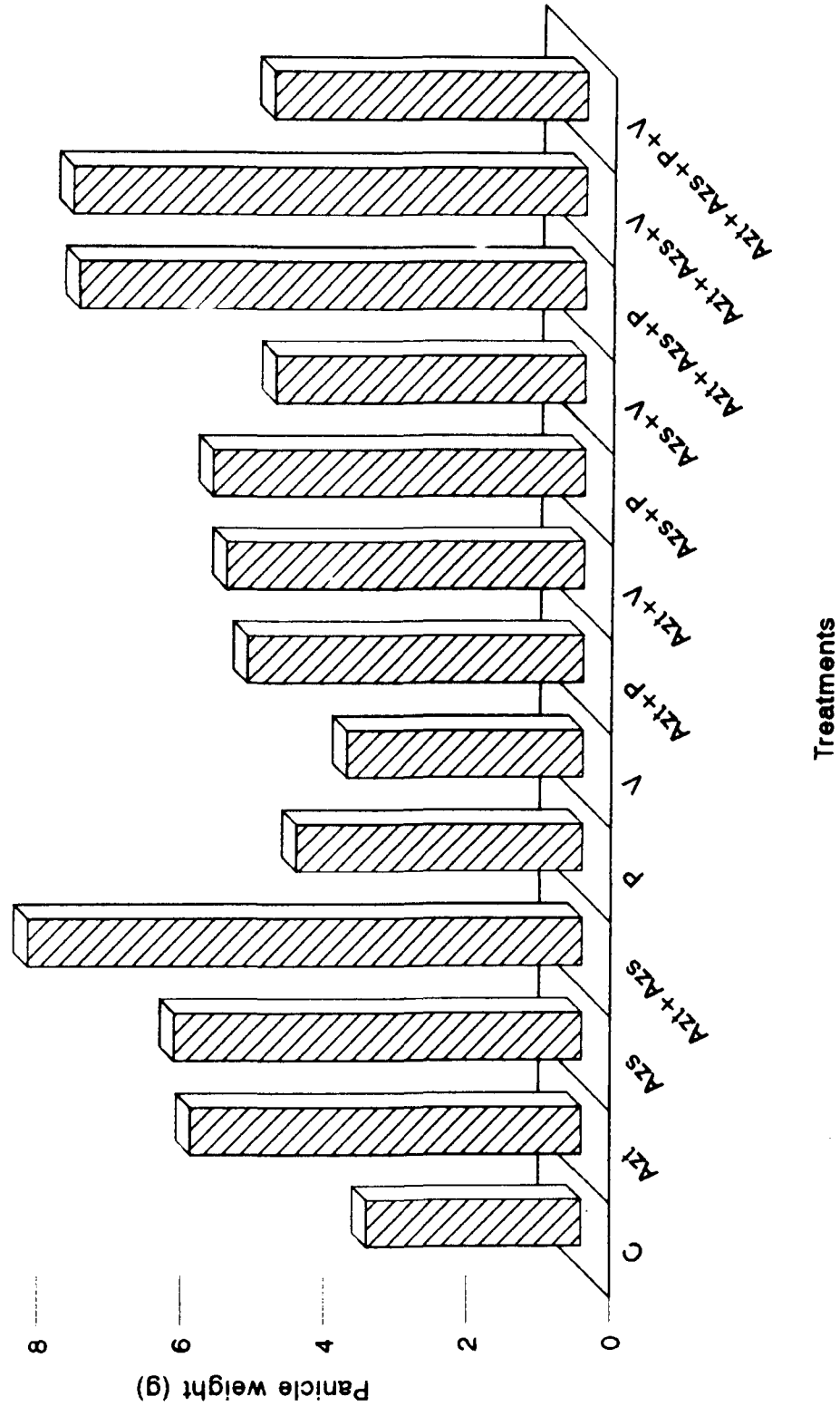
Effect on panicle length : The panicle length varied from a maximum of 13.75 cm to a minimum of 11.50 cm. The details of the results are presented in Table 18. The panicle length was found to be maximum (13.75 cm) in the plants inoculated with Azospirillum and also Azotobacter + Azospirillum. The minimum panicle length (11.50 cm) was seen in uninoculated control and plants inoculated with Azotobacter + Azospirillum + Paramecium + Vorticella.

Effect on dry weight of panicle : The dry weight of the panicles per pot varied from a maximum of 7.75 g to a minimum of 3.3 g. The results are presented in Table 18. The maximum weight of 7.75 g was found in the plants inoculated with Azotobacter + Azospirillum and the minimum weight of

Table 18. Effect of protozoa on the yield components in rice crop.

Treatments	Number of panicles	Number of spikes/panicle	Panicle length (cm)	Dry weight of panicle (g)/pot
Uninoculated control	5.50	4.50	11.50	3.300
<u>Azotobacter</u> (Azt)	6.25 (13.63)	5.00 (11.11)	12.25 (16.52)	5.475 (65.90)
<u>Azospirillum</u> (Azs)	6.50 (18.18)	5.25 (16.66)	13.75 (19.56)	5.700 (71.72)
Azt + Azs	7.00 (27.27)	6.25 (38.88)	13.75 (19.56)	7.750 (134.84)
<u>Paramecium</u> (P)	4.75 (13.63)	4.50 (0.00)	11.75 (2.17)	4.000 (21.21)
<u>Vorticella</u> (V)	5.75 (4.54)	4.50 (0.00)	11.75 (2.17)	3.300 (0.00)
Azt + P	5.25 (-4.54)	5.25 (16.66)	12.50 (8.69)	4.700 (42.42)
Azt + V	5.00 (-9.09)	4.75 (5.55)	12.50 (8.69)	5.000 (51.51)
Azs + P	6.50 (18.18)	5.25 (16.66)	12.50 (8.69)	5.250 (59.09)
Azs + V	5.50 (0.00)	4.75 (5.55)	12.25 (6.52)	4.320 (30.90)
Azt + Azs + P	5.75 (4.54)	5.00 (11.11)	11.75 (2.17)	7.075 (114.39)
Azt + Azs + V	6.50 (18.18)	6.00 (33.33)	13.50 (17.39)	7.175 (117.42)
Azt + Azs + P + V	5.75 (4.54)	5.25 (16.66)	11.50 (0.00)	4.375 (-32.57)
F test	*	*	*	*
C.D.at 5%	0.81	0.77	1.07	2.37

Note : Each value is a mean of four replications
 Values in the parantheses indicate per cent increase or decrease (-) over uninoculated control



(N.B.: C-Control; Azt-Azotobacter; Azs-Azospirillum; P-Paramecium; V-Vorticella)

Fig.6: Effect of protozoa on the panicle weight in rice crop

3.3 g was found in the uninoculated control plants and also in the plants inoculated with Vorticella (Fig. 6).

Influence of protozoa on the biomass of rice crop

Effect on shoot dry weight : The shoot dry weight of the rice crop ranged from 8.375 g to 11.675 g. The results on shoot dry weight are presented in Table 19. The maximum shoot dry weight of 11.655 g was found in the plants inoculated with Azotobacter + Azospirillum and the minimum shoot dry weight of 8.375 g was recorded in the plants inoculated with Azotobacter + Vorticella (Fig. 7).

Effect on root dry weight : The root dry weight of rice plants ranged from 7.275 g to 9.475 g. The details of the results are presented in Table 19. The maximum root dry weight 9.475 g was found in the plants inoculated with Azotobacter + Azospirillum and the minimum root dry weight of 7.275 g in the plants inoculated with Azotobacter + Vorticella.

Effect on total biomass : The total biomass of rice plants ranged from 15.650 g to 21.150 g. The results of total biomass are presented in Table 19. A similar trend as mentioned above was noticed in total biomass also. The maximum total biomass of 21.15 g was seen in Azotobacter + Azospirillum inoculated plants and minimum of 15.65 g in Azotobacter + Vorticella inoculated plants.

Plate 5. Effect of protozoa on Azotobacter in rice.

- 1 = Control
- 2 = Azotobacter
- 3 = Azotobacter + Azospirillum
- 4 = Azotobacter + Paramecium
- 5 = Azotobacter + Vorticella

Plate 6. Effect of protozoa on Azospirillum in rice.

- 1 = Control
- 2 = Azospirillum
- 3 = Azotobacter + Azospirillum
- 4 = Azospirillum + Paramecium
- 5 = Azospirillum + Vorticella



PLATE - 5



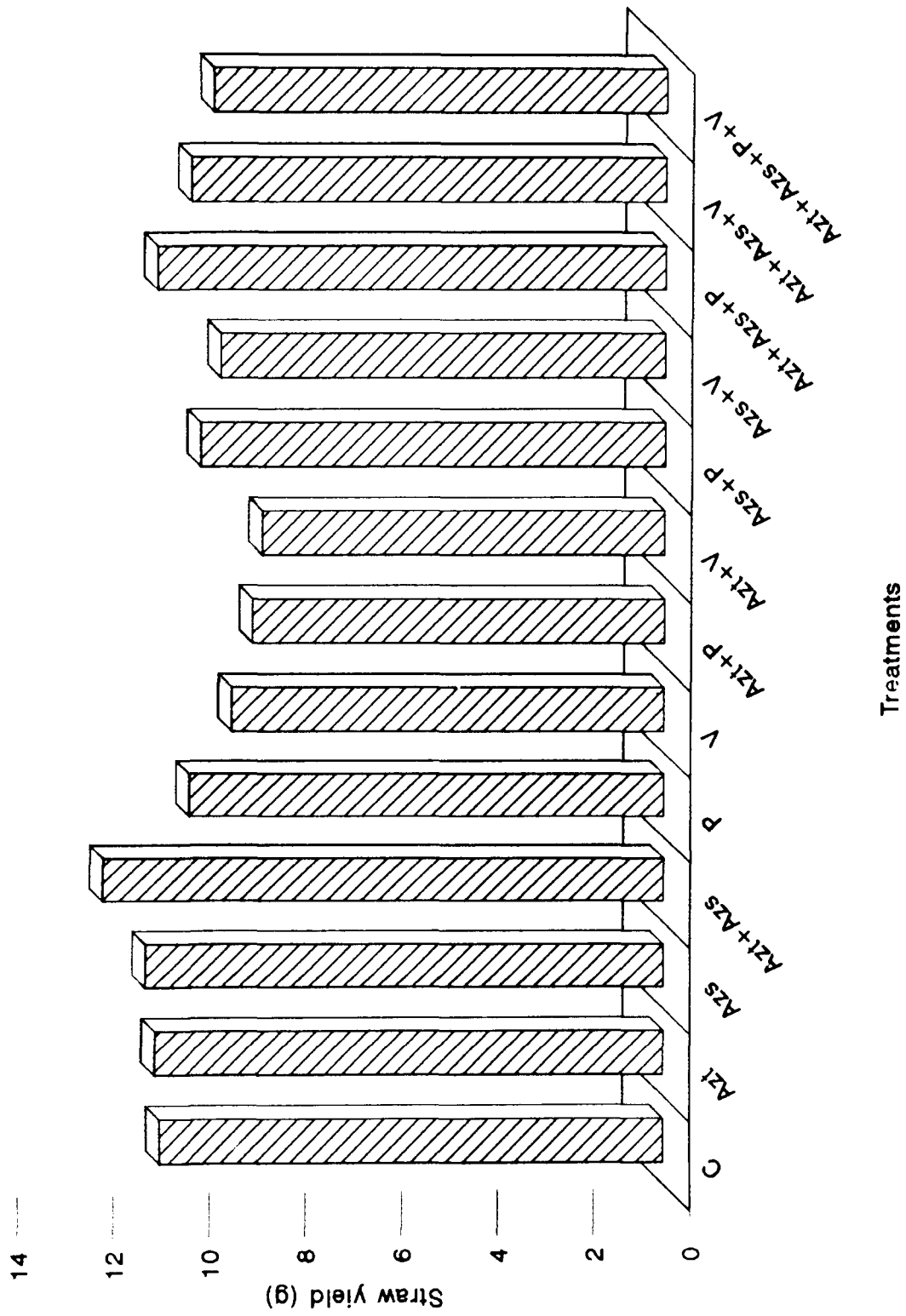
PLATE - 6

Table 19. Influence of protozoa on the shoot dry weight, root dry weight and total biomass of rice crop.

Treatments	Shoot dry weight (g)	Root dry weight (g)	Total biomass (g)
Uninoculated control	10.475	7.525	18.000
<u>Azotobacter</u> (Azt)	10.550 (0.95)	8.175 (8.63)	18.750 (4.16)
<u>Azospirillum</u> (Azs)	10.775 (2.86)	8.750 (16.27)	19.525 (8.47)
Azt + Azs	11.675 (11.45)	9.475 (25.91)	21.150 (17.50)
<u>Paramecium</u> (P)	9.875 (-5.72)	8.000 (6.31)	17.815 (-0.69)
<u>Vorticella</u> (V)	9.000 (-14.08)	7.400 (-1.66)	16.400 (-8.88)
Azt + P	8.575 (-18.13)	7.775 (3.32)	16.350 (-8.61)
Azt + V	8.375 (-20.04)	7.275 (-3.32)	15.650 (-13.05)
Azs + P	9.675 (-7.63)	8.300 (10.29)	17.975 (-0.13)
Azs + V	9.250 (-11.69)	8.100 (7.64)	17.350 (-3.61)
Azt + Azs + P	10.575 (0.95)	9.125 (21.26)	19.700 (9.44)
Azt + Azs + V	9.875 (-5.72)	9.100 (20.93)	18.975 (5.41)
Azt + Azs + P + V	9.425 (-10.02)	8.125 (7.97)	17.550 (-2.50)

F test	*	*	*
C.D. at 5%	1.10	0.47	1.57

Note : Each value is a mean of four replications
 Values in the parantheses indicate per cent increase or decrease (-) over uninoculated control



(N.B.: C-Control; Azi-Azotobacter; Azs-Azospirillum; P-Paramecium; V-Vorticella)

Fig.7: Influence of protozoa on the straw yield of rice crop

Effect of protozoa on the shoot N content of rice crop

Effect on per cent N : The per cent N in the rice plants ranged from 0.477 per cent to 0.757 per cent. The results are presented in Table 20. Maximum per cent N of 0.757 per cent was found in Azotobacter + Azospirillum inoculated plants and minimum of 0.477 per cent in Vorticella inoculated plants.

Effect on total shoot N : The total shoot N ranged from 42.93 mg to 88.37 mg. The results are presented in Table 20. The maximum shoot N of 88.37 mg was found in the plants inoculated with Azotobacter + Azospirillum. The minimum shoot N of 42.93 mg was found in the plants inoculated with Vorticella (Fig. 8).

Effect of protozoa on the population of bacteria in rice crop

The effect of protozoa on the growth and multiplication of Azotobacter and Azospirillum was studied in the rhizosphere soil of rice. The results are presented in Table 21. The bacterial number was found to be gradually decreasing with the increase in protozoan number. About 10^8 cells ml^{-1} of Azotobacter and Azospirillum with or without 800 ml^{-1} and 600 ml^{-1} of Paramecium and Vorticella respectively was inoculated at the time of transplanting. By the end of 100 DAT, Azotobacter decreased to about 8.1×10^4 with paramecium and to 5.7×10^4 with Vorticella.

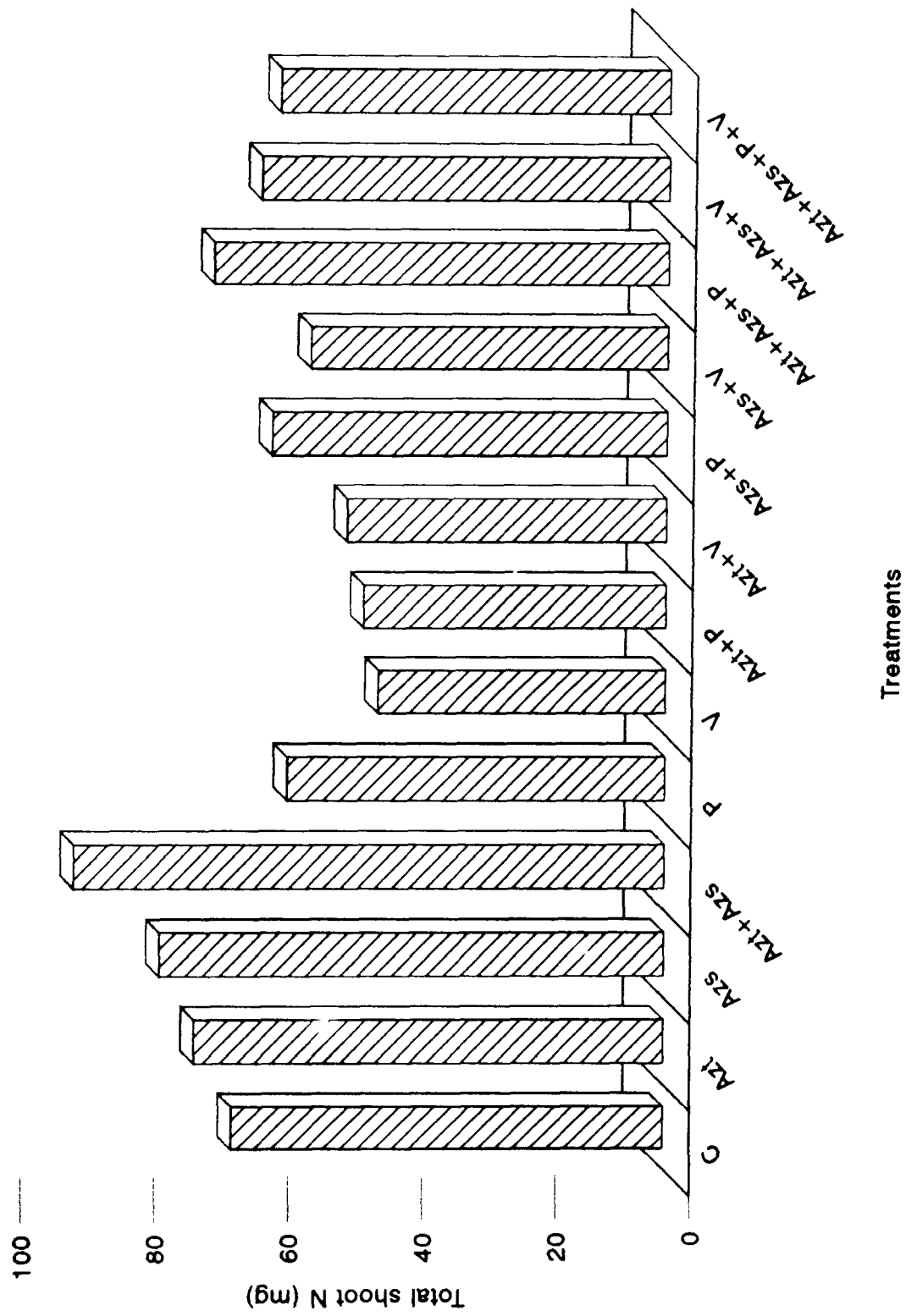
Table 20. Influence of protozoa on the shoot 'N' content of rice crop.

Treatments	Per cent 'N'	Total shoot 'N' (mg)
Uninoculated control	0.610	64.50
<u>Azotobacter</u> (Azt)	0.670 (9.83)	70.18 (8.80)
<u>Azospirillum</u> (Azs)	0.700 (14.75)	75.42 (16.93)
Azt + Azs	0.757 (24.09)	88.37 (37.00)
<u>Paramecium</u> (P)	0.572 (-6.22)	56.48 (-12.43)
<u>Vorticella</u> (V)	0.477 (-21.80)	42.93 (-33.44)
Azt + P	0.527 (-13.60)	45.19 (-29.93)
Azt + V	0.570 (-6.55)	47.73 (-26.00)
Azs + P	0.610 (0.00)	59.01 (-8.51)
Azs + V	0.577 (-5.4)	53.37 (-17.25)
Azt + Azs + P	0.642 (5.24)	67.89 (5.25)
Azt + Azs + V	0.617 (1.14)	60.92 (-5.55)
Azt + Azs + P + V	0.617 (1.14)	58.15 (-9.84)

F test	*	*
C.D.at 5%	0.054	0.594

Note : Each value is a mean of four replications

Values in the parantheses indicate per cent increase or decrease (-) over uninoculated control



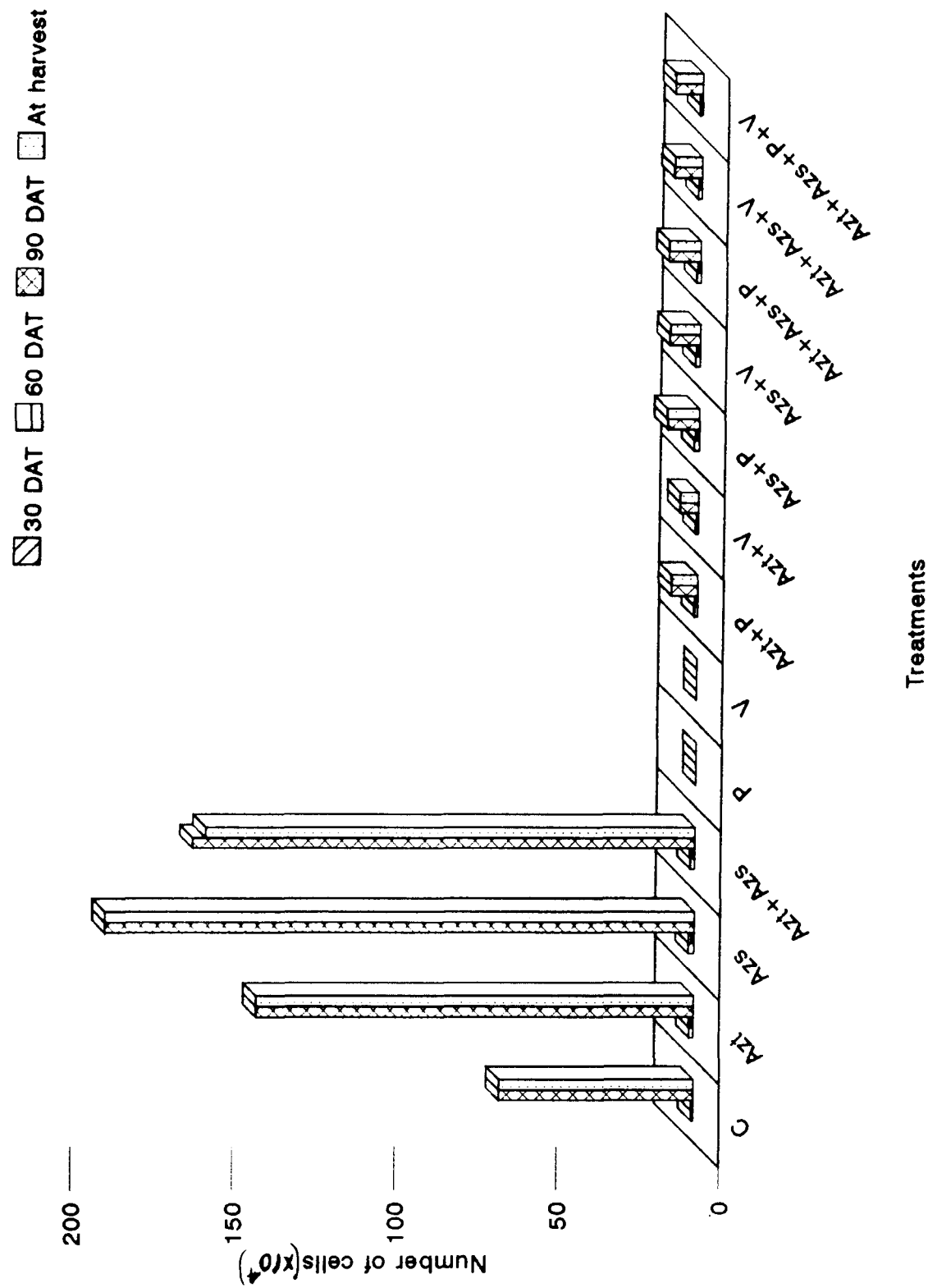
(N.B.: C-Control; Azl-Azotobacter; Azs-Azospirillum; P-Paramecium; V-Vorticella)

Fig.9: Influence of protozoa on the shoot N content of rice crop

Table 21. Effect of protozoa on the population of bacteria in rice crop.

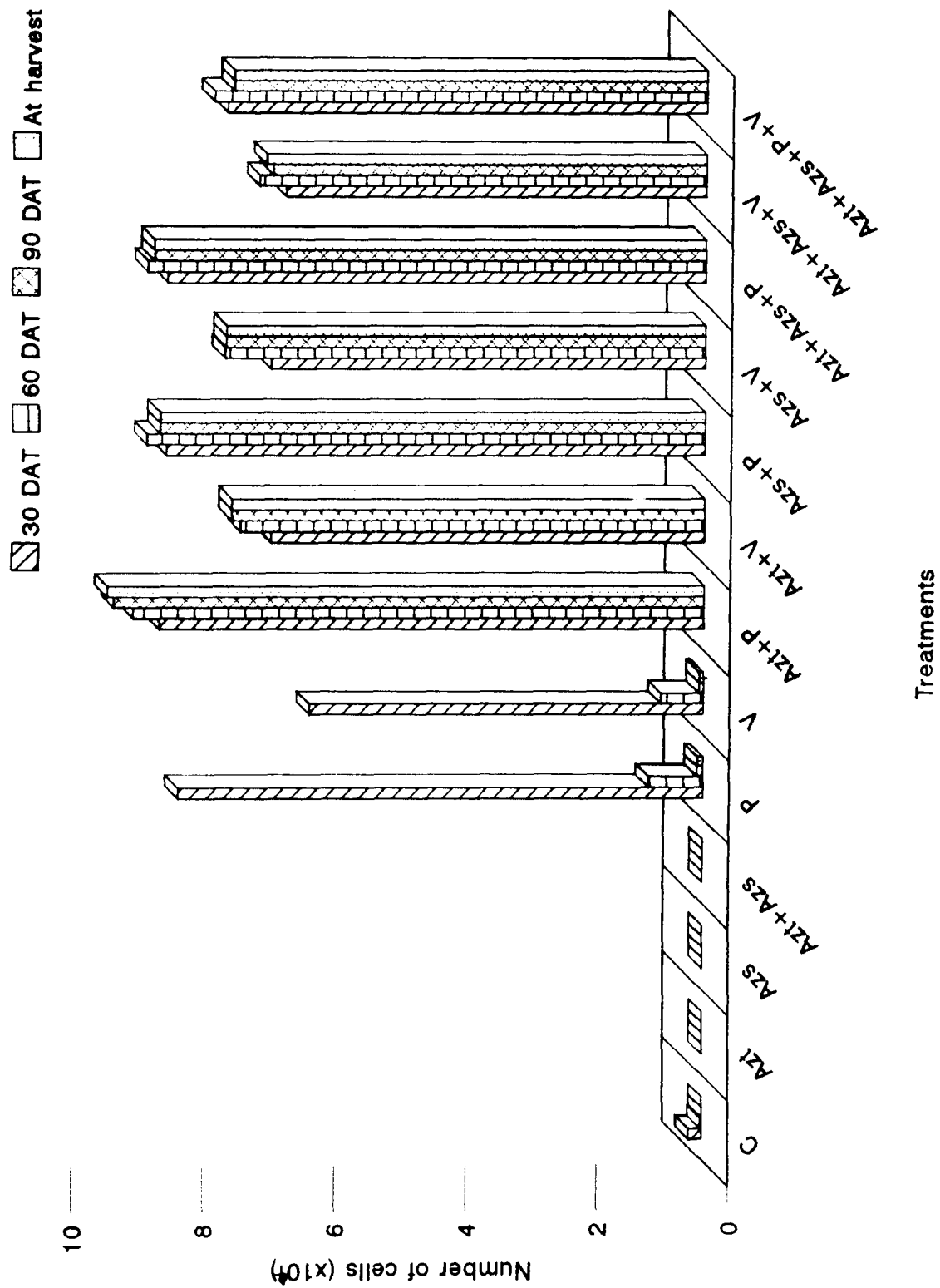
Treatments	30 DAS		60 DAS		90 DAS		At harvest	
	B ($\times 10^6$)	P ($\times 10^2$)	B ($\times 10^6$)	P ($\times 10^3$)	B ($\times 10^4$)	P ($\times 10^4$)	B ($\times 10^4$)	P ($\times 10^4$)
Uninoculated control	0.6	0.2	0.6	0.02	60	0.002	60	0.002
<u>Azotobacter</u> (Azt)	1.3	-	1.35 (+3.84)	-	135 (+3.84)	-	135 (+3.84)	-
<u>Azospirillum</u> (Axs)	1.8	-	1.82 (+1.11)	-	182 (+1.11)	-	182 (+1.11)	-
Azt + Axs	1.2+1.6	-	1.3+1.65 (+8.33, +3.12)	-	130 + 165 (+8.33, +3.12)	-	132 + 165 (+10.0, +3.12)	-
<u>Paramecium</u> (P)	-	8.0	-	0.83 (+0.37)	-	0.088 (+1.00)	-	0.086 (+0.75)
<u>Vorticella</u> (V)	-	6.0	-	0.65 (+0.83)	-	0.066 (+1.00)	-	0.062 (+0.33)
Azt + P	1.07 (-7.69)	8.3 (+3.75)	0.90 (-30.76)	8.7 (+987.5)	8.2 (-93.67)	9.0 (+11150)	8.1 (-93.76)	9.1 (+11275)
Azt + V	0.88 (-32.30)	6.6 (+10.0)	0.67 (-62.77)	7.0 (+1066.6)	5.7 (-95.61)	7.2 (+11900)	5.7 (-95.61)	7.2 (+11900)
Axs + P	1.58 (-12.22)	8.2 (+2.5)	1.20 (-33.33)	8.5 (+962.5)	9.9 (-94.5)	8.3 (+10275)	9.9 (-94.5)	8.3 (+10275)
Axs + V	1.47 (-37.22)	6.62 (+10.33)	1.13 (-37.77)	7.2 (+1100.0)	9.5 (-94.72)	7.3 (+12066.6)	9.3 (-94.80)	7.3 (+12066.6)
Azt + Axs + P	1.12+1.53 (-6.66, -4.37)	8.2 (+2.5)	1.0+1.32 (-16.66, -17.5)	8.5 (+962.5)	9.7+9.9 (-91.91, -93.8)	8.4 (+10400)	9.6+9.9 (-92.0, -93.8)	8.4 (+10400)
Azt + Axs + V	0.98 + 1.16 (-18.33, -27.5)	6.4 (+6.66)	0.8+1.0 (-33.33, -37.5)	6.8 (+1033.3)	7.8+9.2 (-93.5, -94.2)	6.6 (+10900)	7.7+9.2 (-93.58, -94.20)	6.7 (+11066.6)
Azt + Axs + P + V	0.98 + 0.85 (-18.33, -46.87)	8.1+6.4 (+1.25, +6.66)	0.8+0.7 (-33.33, -56.25)	8.4+6.6 (+975, +1000)	7.8+9.0 (-93.5, -94.37)	8.0+6.3 (+9900, +10400)	7.8+9.0 (-93.5, -94.37)	8.0+6.3 (+9900, +10400)

Note : Each value is a mean of four replications ; B = Bacteria - Azotobacter &/or Azospirillum
P = Protozoa - Paramecium &/or Vorticella ; Values in the parentheses indicate per cent increase or decrease(-)



(N.B.: C-Control; Azi-Azotobacter; Azs-Azospirillum; P-Paramecium; V-Vorticella)

Fig.9a: Effect of protozoa on the population of bacteria in rice crop



(N.B.: C-Control; Azi-Azotobacter; Azs-Azospirillum; P-Paramecium; V-Vorticella)

Fig.9b: Increase in the population of protozoa as influenced by bacteria

Azotobacter + Azospirillum to about $(9.6 + 9.9) \times 10^4$ with Paramecium and to $(7.7 + 9.2) \times 10^4$ with Verticella compared to $(132 + 165) \times 10^4$ with Azotobacter + Azospirillum without protozoa. Paramecium increased to about 9.1×10^4 with Azotobacter to 8.3×10^4 with Azospirillum and to 8.4×10^4 with Azotobacter + Azospirillum compared to 0.086×10^4 with Paramecium alone. Vorticella was found to increase to about 7.2×10^4 with Azotobacter to 7.3×10^4 with Azospirillum and to 6.7×10^4 with Azotobacter + Azospirillum compared to 0.062×10^4 with Vorticella alone. In general, Vorticella reduced the bacterial number more compared to Paramecium and after the bacterial number reached to about 10^4 cells the number almost remained constant (Fig. 9a, 9b).

DISCUSSION

V DISCUSSION

Greater understanding of the influence of protozoa on the growth and beneficial activities of bacteria is needed to effectively exploit the source of biofertilizers for N nutrition in cereals and legumes.

The effect of protozoa on bacteria has been investigated by several scientists and it has been well documented that several bacteria serve as food for various protozoa (Danso and Alexander, 1975). With this in view, the present investigation was carried out to know the effect of protozoa like Paramecium and Vorticella which are common ciliates found in wet soils on the growth and beneficial activities of the common soil bacteria like Rhizobium, Azotobacter and Azospirillum in both in vitro and pot culture studies using rice and cowpea crops.

Identifiatioin and enumeration of protozoa

The three predominant protozoa isolated from the rice growing soils of Karnataka were identified as Paramecium spp., Vorticella spp and Colpoda spp. Colpoda was found in larger quantities compared to Paramecium and Vorticella. Earlier Sardeshpande et al. (1977) studied the protozoa in Bangalore soils and reported that Colpoda spp. and Uroleptus spp. were found in the soil. However, Uroleptus spp could not be found in this study. This may be due to variations in

the soil samples, time of collection, soil pH, organic matter content etc.

Effect of protozoa on the population level of bacteria in broth culture studies

The protozoa viz., Paramecium, Vorticella and Colpoda when mixed with equal volume of the broth cultures of Rhizobium, Azotobacter and Azospirillum, were found to considerably decrease the population of the bacteria. Turbid cultures of bacteria were found to be cleared considerably in four days after addition of protozoa. The protozoa were found to increase with the decrease of bacteria (Table 3) (Fig. 1) compared to Paramecium and Vorticella, Colpoda did not reduce the bacterial numbers much. The increase in Colpoda number was also not high by the end of four days. Rhizobium decreased more compared to Azotobacter and Azospirillum.

These results are in accordance with the earlier findings by Sardeshpande et al. (1977) who reported that the numbers of Rhizobium decreased 5645 folds while the population of Azotobacter chroococcum decreased 2543 folds in 5 days after addition of a mixed culture of Colpoda spp. and Uroleptus spp in the broth culture studies. The present study supports the finding of Danso et al. (1975) and Sardeshpande et al. (1977) and also brings out that another ciliate

protozoa Vorticella spp. can also feed not only on Rhizobium but also Azotobacter and Azospirillum.

Colpoda was found to not decrease the bacterial population to a larger extent which is in accordance with the findings of Habte and Alexander (1978) who reported that Colpoda sp. inoculated with Rhizobium sp. only reduced the prey abundance from 10^9 to 10^8 /g soil. They concluded that this may be due to the ability of the bacteria to reproduce and replace the cells consumed by predation.

Effect of protozoa on the plant height

The maximum plant heights were recorded in the treatments without protozoa and minimum in treatments with protozoa both in cowpea and rice. This may be mainly due to reduction in the bacterial number by protozoa.

In cowpea, maximum plant height of 37 cm was recorded in Rhizobium + Azotobacter inoculated plants compared to 34.38 cm in Rhizobium alone inoculated plants and 31.88 cm in Azotobacter alone inoculated plants (Table 4). This shows that dual inoculation improves plant growth in cowpea. This is in accordance with the findings of El Mokadem et al. (1989) who reported that growth of chickpea was improved by adding Azospirillum and/or Azotobacter with Rhizobium.

Brown and Burlingham (1968) have reported that plant growth substances like IAA and gibberellins are produced by

Azotobacter chroococcum which altered the growth of stems leaves and flowers of pea and tomatoes. The role of such hormones in the interaction effect cannot be ruled out. This might have been a possible reason for the enhancement of plant growth in plants inoculated with Rhizobium + Azotobacter.

Protozoa inoculated plants showed minimum plant heights. Minimum plant height of 20.75 cm was recorded in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella which was followed by 25.5 cm in Vorticella alone inoculated plants. This shows that mixed culture of protozoa was more harmful compared to single cultures and Vorticella was more effective compared to Paramecium. However, not much work has been done on this aspect to support these results.

In rice also maximum plant heights were recorded in the plants inoculated with Azotobacter + Azospirillum (56 cm) (Table 15) followed by Azospirillum (53.5 cm). Such findings are also be reported by Hegazi et al. (1981) who observed that when wheat was inoculated with Azospirillum brasilense, the plant height was increased. Purushothaman et al. (1987) reported that inoculation of rice seeds with Azospirillum brasilense increased the shoot and root lengths in rice.

Rai and Gaur (1988) reported that there was significant increases in the crop growth and yield of wheat when the

seeds were inoculated with Azotobacter, Azospirillum or the combination of both the inoculants.

The minimum plant heights in rice were recorded in the plants inoculated with Vorticella (44 cm). Vorticella was found to reduce the plant height more compared to Paramecium (45.75 cm). No work has been done earlier on the effect of protozoa on rice crop.

Effect of protozoa on leaf number

The leaf number in rice also followed the same trend as explained in plant height. The maximum leaf number was seen in the plants inoculated with bacteria alone and minimum in the plants inoculated with protozoa.

In rice, the maximum number of leaves were seen in plants inoculated with Azotobacter + Azospirillum (30.25) (Table 16) followed by 29.5 in Azospirillum inoculated plants. This may be due to the fact that combined inoculation increases crop growth as reported by Rai and Gaur (1988). Minimum number of leaves (24) were seen in the plants inoculated with Vorticella followed by 25 in plants inoculated with Paramecium. This may be due to reduction in bacterial number by protozoa.

Effect of protozoa on the stem girth in cowpea

The stem girth in cowpea did not show any statistically significant difference between the treatments. But the stem

girth was lesser (0.4 cm) (Table 5) in the plants inoculated with Rhizobium + Azotobacter + Vorticella and Rhizobium + Azotobacter + Paramecium + Vorticella and it was highest (0.5 cm) in Rhizobium + Paramecium inoculated plants. This shows that stem girth cannot be considered as an effective parameter to evaluate the effect of protozoa on bacteria and growth of cowpea.

Effect of protozoa on the leaf colour in cowpea

The colour of the leaves in cowpea ranged from slightly yellow to dark green. Leaf/plant colour is one of the trait indicative of nitrogen nutrition status of the plant. Nitrogen imparts green colour to plant and colour of the plant growing under N-free condition can be attributed to the level of nitrogen fixation. Score for colour was given based on visual observations of the intensity of colour at the time of harvest. The score may vary from person to person but in general one can judge the large colour differences between the plants. In the present study also plant colour was closely related with total nitrogen content. Rhizobium + Azotobacter inoculated plants showed the maximum colour score of 2.75 and the plants inoculated with Vorticella, Rhizobium + Azotobacter + Paramecium showed a minimum colour score of 1.5 (Table 6) which shows their low total nitrogen content.

Effect of protozoa on the biomass of cowpea

Many workers have reported increase in dry matter yield due to inoculation of crop seeds with appropriate strains of nitrogen fixing organisms.

The total biomass or dry matter yield was maximum (6.21 g/pot) in the plants inoculated with Rhizobium + Azotobacter (Table 7) (Fig. 2) compared to the plants inoculated with Rhizobium alone (5.06 g/pot) and Azotobacter alone (4.59 g/pot). These results are in accordance to the findings of Gunawardena and Vlassak (1986) who reported that cowpea inoculated with a combination of Rhizobium and Azospirillum gave the highest value for shoot dry weight compared to single inoculations and control.

El Mokadem *et al.* (1989) reported that growth of chickpea was improved by adding Azospirillum and/or Azotobacter with Rhizobium.

Shashidhara (1992) reported that the cumulative dry matter yield of maize and cowpea was highest when inoculated with Rhizobium + Azospirillum.

The minimum plant biomass of 1.36 g was seen in the plants inoculated with Rhizobium + Azotobacter + Paramecium + Vorticella which without protozoa was highest followed by 2.29 g per pot in Vorticella alone inoculated plants. These

results suggests that mixed culture of protozoa with mixed culture of bacteria reduce the dry matter yield more compared to single cultures and also Vorticella is more harmful compared to Paramecium.

Effect of protozoa on nodulation in cowpea

The nodule number was found to be highest (97.5/pot) in the plants inoculated with Rhizobium + Azotobacter compared to 93 per pot in Rhizobium alone inoculated plants and 89.5 per pot in plants inoculated with Azotobacter alone. These results are in accordance with the findings of Burns et al. (1981) who reported that nodulation in leguminous plants is enhanced by inoculation with mixed cultures of Azotobacter vinelandii and Rhizobium.

Gunawardena and Vlassak (1986), Kundu (1988) and Yahalom et al. (1988) have reported that combined inoculation of Rhizobium and Azospirillum increased nodulation in cowpea, pigeonpea and siratro, respectively.

El Mokadem et al. (1989) reported that combination of Rhizobium with the diazotrophs Azotobacter/Azospirillum inoculation increased nodule branching in chickpea.

The lowest number of nodules (47/pot) were recorded in Rhizobium + Azotobacter + Paramecium + Vorticella inoculated plants (Table 8) followed by 49.75 per pot in Rhizobium +

Vorticella inoculated plants. The nodule number was reduced to lesser than half in the former and to about half in the latter. This shows that Vorticella affect the nodulation more compared to Paramecium (Fig. 3).

These results are in accordance with the findings of Sardeshpande et al. (1977) who reported that the presence of predatory protozoa in the soil do affect nodulation by the inoculated rhizobia to certain extent. But they do not completely eliminate nodulation. In the absence of protozoa, the rhizobia nodulate better.

The nodule weight was found to be maximum (1.3 g/pot) in the plants inoculated with Rhizobium eventhough the nodule number was lesser compared to Rhizobium + Azotobacter inoculated plants. This is because the nodules in Rhizobium alone inoculated plants were bigger than the nodules in plants inoculated with Rhizobium + Azotobacter (1.05 g/pot). The minimum nodule weight was found in Rhizobium + Azotobacter + Paramecium + Vorticella (0.41 g/pot) followed by Vorticella (0.47 g/pot). The nodule weight was reduced in the plants inoculated with protozoa compared to bacteria alone and uninoculated control.

It is well known that about 10^7 - 10^8 cells per g of Rhizobium is required for effective nodulation. As the Rhizobial number was reduced to about 10^6 cells per g soil,

it might have caused reduction in nodulation in cowpea plants.

Effect of protozoa on the shoot N content of cowpea

The per cent N and total shoot N were found to be highest (2.68% and 101.3 mg/pot) in Rhizobium + Azotobacter inoculated plants compared to Rhizobium alone (2.67% and 82.23 mg/pot) and Azotobacter alone inoculated plants (2.30% and 65.55 mg/pot). These results are in accordance to the findings of Gunawardena and Vlassak (1986) and Kundu (1988) who reported that combined inoculation of Rhizobium and Azospirillum increased nodulation and nitrogen fixation in cowpea and pigeonpea.

The nitrogen content of the plants inoculated with protozoa were decreased compared to the plants inoculated with bacteria alone. The per cent N was found to be lowest (1.73%) in Vorticella inoculated plants (Table 9) followed by 1.8 per cent in Paramecium, 1.83 per cent in Rhizobium + Vorticella and 1.86 per cent in Azotobacter + Vorticella inoculated plants. This shows that Vorticella had reduced the nitrogen fixing ability of the bacteria to a larger extent compared to Paramecium. The total shoot N was found to be lowest (13.65 mg/pot) in Rhizobium + Azotobacter + Paramecium + Vorticella inoculated plants followed by 21.28 mg in case of Vorticella inoculated and 25.25 mg in

Rhizobium + Vorticella inoculated plants depending on the shoot dry weight (Fig. 4). These results are almost same as the results of leaf colour score. This may be due to reduction in bacterial number.

Effect of protozoa on the tiller number in rice

The number of tillers were found to be highest in the plants inoculated with Azotobacter + Azospirillum (7.75) followed by Azotobacter inoculated plants and Azospirillum inoculated plants (7.00) (Table 17). This shows that dual inoculation increases tiller number in rice. Hegazi *et al.* (1981) found that Azospirillum brasilense inoculation of wheat in the field in Egypt increased the plant height and tiller number. Similarly Kapulnik *et al.* (1983) obtained significant increases in the number of fertile tillers per unit area with two wheat cultivars. Watanabe and Lin (1984) reported that inoculation of rice with Pseudomonas and Azospirillum promoted tillering.

The number of tillers in rice were found to decrease with the addition of protozoa lowest being 5.25 in Vorticella inoculated plants followed by 5.75 in uninoculated control and 6.00 in Paramecium inoculated plants. But the treatments did not differ significantly between themselves. So it can be said that protozoa did not have much effect on the number of tillers in rice.

Effect of protozoa on the yield components of rice

The number of panicles were more in the treatments with bacteria alone and were found to decrease in the treatments where protozoa was added. The highest number of panicles (7.00) was found in Azotobacter + Azospirillum inoculation followed by 6.5 in Azospirillum and 6.25 in Azotobacter inoculated plants (Table 8). It was reduced to 6.50, 5.50 and 5.00 respectively by addition of Vorticella and to 5.75, 6.50 and 5.25 respectively by addition of Paramecium. The lowest number of panicles (4.75) was seen in plants inoculated with Paramecium alone.

Number of spikes per panicle was highest (6.25) in Azotobacter + Azospirillum inoculated plants compared to 5.00 in Azotobacter and 5.25 in Azospirillum alone inoculated plants (Table 18). These were reduced to about 6.00, 4.75 and 4.75 respectively by addition of Vorticella and to 5.00, 5.25 and 5.25 respectively by addition of Paramecium. The lowest number of spikes (4.50) was found in Paramecium inoculated plants, Vorticella inoculated plants and uninoculated control plants. This shows that Paramecium equally reduced the number of spikes per panicle in rice as Vorticella.

The panicle length was highest (13.75 cm) in plants inoculated with Azospirillum and Azotobacter + Azospirillum.

In Azospirillum, it was reduced to about 12.5 cm by addition of Paramecium and to 12.25 cm by addition of Vorticella (Table 18). In Azotobacter + Azospirillum inoculated plants, it was reduced to about 11.75 by addition of paramecium, 13.5 by addition of Vorticella and to 11.5 cm by Paramecium + Vorticella inoculation. This shows that Paramecium reduced the panicle length to a greater extent than Vorticella.

The dry weight of the panicle which is the most important yield component was found to be highest (7.75 g) in Azotobacter + Azospirillum inoculated plants compared to 5.475 in Azotobacter alone inoculated plants and 5.70 g in Azospirillum alone inoculated plants. This was reduced to 7.075 g by addition of Paramecium, 7.175 g by addition of Vorticella and 4.375 g by Paramecium + Vorticella in Azotobacter + Azospirillum inoculated plants (Table 18). The lowest panicle weight of 3.3 g was recorded in Vorticella inoculated plants and uninoculated control plants in which grains were chaffy (Fig. 6).

From all these results of yield components, it can be known that combined inoculation of Azotobacter + Azospirillum increases the yield in rice and the protozoa affects the grain filling and yield in rice.

Rao et al. (1983) reported that root inoculation with Azospirillum in rice resulted in a significant increase in

the mean yields of both grain and straw. Kundu and Gaur (1984) reported that inoculation of rice seedlings with Azotobacter chroococcum, Pseudomonas striata and Aspergillus awamori increased the grain and straw yields of rice. Watanabe and Lin (1984) reported that inoculation of wetland rice seedlings with nitrogen fixing Pseudomonas sp. (Hg) and Azospirillum lipoferum (34H) promoted tillering and increased the rate of grain filling and grain yield per plant. Omar et al. (1987) reported that inoculation of seeds and seedlings of rice with live inoculum of Azospirillum brasilense gave 16-22 per cent increase in rice grain yield.

Effect of protozoa on the biomass of rice

The shoot dry weight or straw yield of rice was highest (11.675 g/pot) in the plants inoculated with Azotobacter + Azospirillum (Table 19) followed by 10.775 g in Azospirillum and 10.475 g in Azotobacter inoculated plants. It was reduced to about 10.575 g by addition of Paramecium, 9.875 g by addition of Vorticella and to 9.425 g by Paramecium + Vorticella addition in Azotobacter + Azospirillum inoculated plants. In Azospirillum inoculated plants, it was reduced to about 9.675 g by addition of Paramecium and to 9.250 g by addition of Vorticella. In plants inoculated with Azotobacter straw yield was reduced to about 8.575 g by addition of Paramecium and to 8.375 g by addition of Vorticella. The lowest straw yield of 8.375 g was recorded

in Azotobacter + Vorticella inoculated plants. In general, Vorticella affected the straw yield to a greater extent compared to Paramecium (Fig. 7).

The same trend was seen even in the root dry weight and total biomass of rice. The root dry weight was highest (9.475 g) in Azotobacter + Azospirillum inoculated plants and lowest (7.275 g) in Azotobacter + Vorticella inoculated plants.

The total biomass was highest (21.15 g) in the dual inoculated plants compared to 19.525 g in plants inoculated with Azospirillum alone and 18.650 g in Azotobacter alone inoculated plants. The lower biomass was recorded in protozoa inoculated plants, the lowest (15.65 g/pot) being in Azotobacter + Vorticella inoculated plants.

In general, inoculation with the diazotrophs increased the straw yield and total biomass in rice compared to uninoculated control. These results were in accordance with the early findings of Hegazi et al. (1981) who reported that when wheat was inoculated with Azospirillum brasilense, the yield of both grain and straw were increased. Rao et al. (1983) reported that root inoculation of rice with Azospirillum increased the grain and straw yield.

Kundu and Gaur (1984) reported that mixed inoculum of Azotobacter, Pseudomonas striata and Aspergillus awamori increased the grain and straw yields of rice.

Nandi and Sen (1985) reported that suspension spray of Klebsiella and Azotobacter chroococcum on phyllosphere of rice and wheat plants increased their dry weight. Gopalaswamy and Vidhyasekaran (1987) reported that inoculation of rice with Azospirillum brasilense increased the grain yield by 0-22 per cent and straw yield by 8-4 per cent.

Rai and Gaur (1988) reported that there was a significant increase in the yield of wheat when the seeds were inoculated with Azospirillum sp. or Azotobacter sp. or the combination of both. Protozoa decreased the straw yield also in rice which emphasises the need to control protozoa number in rice fields.

Effect of protozoa on the shoot N content of rice

The per cent N and total shoot N were found to be higher in the treatments with only bacteria or diazotrophs and lower in the treatments inoculated with protozoa (Table 20). The per cent N and total shoot N were highest (0.757% and 88.37 mg/pot) in the plants inoculated with Azotobacter + Azospirillum followed by Azospirillum (0.7% and 75.42 mg/pot) and then Azotobacter inoculated plants (0.67% and 70.18 mg/pot). In dual inoculated plants it was reduced to 0.642 per cent and 67.89 mg by addition of Paramecium (23% decrease) to 0.617 per cent and 60.92 mg by addition of

Vorticella (31% decrease) and to 0.617 per cent and 58.15 mg by Paramecium + Vorticella (34% decrease) compared to those without protozoa. In Azospirillum, inoculated plants it was reduced to 0.61 per cent and 59.01 mg and to 0.577 per cent and 53.37 mg by addition of Paramecium (22% decrease) and by addition of Vorticella (29% decrease), respectively. In Azotobacter inoculated plants it was reduced to about 0.527 per cent and 45.19 mg by addition of Paramecium (35% decrease) and to 0.57 per cent and 47.73 mg by addition of Vorticella (32% decrease in total shoot N) compared to those without protozoa. The lowest per cent N and shoot N (0.477% and 42.93 mg/pot) was found in Vorticella inoculated plants (Fig. 8).

In general inoculation with Azotobacter, Azospirillum and both in combination increased the shoot N content compared to uninoculated control. These were in accordance to the early findings by Nur *et al.* (1980) who reported that Azospirillum inoculation increased the total nitrogen content in Setaria italica and Zea mays. Dunigan *et al.* (1981) reported that nitrogen fixation was significantly increased by inoculation of rice with Azospirillum brasilense.

Kundu and Gaur (1984) reported that inoculation of rice seedlings with Azotobacter chroococcum, Pseudomonas striata and Aspergillus awamori increased the uptake of N and P with or without application of fertilizers. Rai and Gaur (1988)

reported that when seeds were inoculated with either Azospirillum or Azotobacter or combination of both, the uptake of nitrogen by the crop was increased over the control.

Influence of protozoa on the population of beneficial soil bacteria in cowpea and paddy soils

The numbers of Rhizobium, Azotobacter and Azospirillum were found to be considerably reduced by Paramecium and Vorticella in both cowpea and rice rhizosphere soils, with the increase in the protozoan numbers (Table 10).

In cowpea soil, there was a gradual decrease in the Rhizobium and Azotobacter number and by the end of 45 days Rhizobium was reduced to 1.5×10^6 by Paramecium, 12×10^6 by Vorticella compared to 31.2×10^6 in Rhizobium alone inoculation (95% decrease by Paramecium and 96% decrease by Vorticella compared to initial number). Azotobacter was reduced by 92.4 per cent by Paramecium and by 92.9 per cent by Vorticella. Rhizobium + Azotobacter was reduced by 55 folds (91%) by Paramecium, by 74 folds (94%) by Vorticella and by 83 per cent folds (95%) by Paramecium + Vorticella together (Fig. 5a). These results are in par with the findings of Sardeshpande *et al.* (1977) who reported that the reduction in the population of Rhizobium and Azotobacter was nearly 127 and 126 folds respectively in 60 days period by mixed culture of Colpoda and Uroleptus in soybean rhizosphere soil.

Paramecium increased to about 11.2×10^4 (1390 folds) with Rhizobium to 10.92×10^4 (1350 folds) with Azotobacter and to 10.88×10^4 (13500 folds) with Rhizobium + Azotobacter. Vorticella increased to about 11.5×10^4 (19066 folds) with Rhizobium to 10.85×10^4 (17983 folds) and to 10.92×10^4 (18100 folds) with Rhizobium + Azotobacter (Fig. 5b). These results are in accordance with the findings of Mallory (1983) who reported that when an alternate prey is present, the predation is increased in protozoa.

These results are also in accordance with the findings of Danso et al. (1975) who reported that as the abundance of Rhizobium meliloti and a cowpea Rhizobium strain in soil decreased, the protozoan density increased. Habte and Alexander (1977) gave further evidence for the regulation of bacterial populations in soil by protozoa. They found that after addition of a large number of cowpea Rhizobium to soil, the population decreased steadily until the numbers reached about 10^7 per g and protozoa rose to about 10^4 per g.

The same results were obtained in rice rhizosphere soil also. Azotobacter decreased to about half the original number with paramecium and to lesser than half with Vorticella. Azospirillum and Azotobacter + Azospirillum also showed the same results. Paramecium increased by 11275 folds with Azotobacter, by 10275 folds with Azospirillum and by 10400 folds with Azotobacter + Azospirillum. Vorticella

increased by 11900 folds with Azotobacter, by 12066 folds with Azospirillum and by 11066 folds with Azotobacter + Azospirillum. In rice, the bacterial population decreased steadily until they reached about 10^4 per g and later remained constant (Fig. 9a, 9b). These results are in accordance to the earlier findings of Danso and Alexander (1975) and Danso et al. (1975) who reported that the size of the prey population diminishes only until a density is attained at which the energy used by the predator in hunting for survivors equals to that obtained from feeding. The same is also reported by Sardeshpande et al. (1977) that bacteria are devoured by protozoa till their population density fall to a particular level and not beyond that.

From this study, the data clearly reveals that dual inoculation of nitrogen fixing bacteria increases the plant growth, yield and total N content when compared to single inoculations. Protozoa decreases the plant growth, yield and total N content both in cowpea and rice and controlling the protozoa number is essential for better growth and yield.

But this study was conducted under green house conditions and large number of protozoa were inoculated to the soil. Under natural conditions such large populations of protozoa are not usually found to effect the crop growth to a larger extent. Protozoan counting in the soils may be

necessary to increase the efficiency of biofertilizers in cereals and legumes.

The study was conducted in only one season. However, the study may be conducted in different agroclimatic seasons to confirm the results for the practical utility.

SUMMARY

VI SUMMARY

Influence of soil protozoa on the growth and activities of nitrogen fixing bacteria, Rhizobium, Azotobacter and Azospirillum was studied. The interaction effects of protozoa and bacteria on the growth and nitrogen fixation in cowpea and rice over a period of 45 and 120 days respectively was investigated.

The population of Rhizobium, Azotobacter, Azospirillum, Paramecium and Vorticella estimated from the rhizosphere soil of cowpea and rice at various time intervals showed that the population of the bacteria gradually decreased with the increase in protozoan number. The decrease of bacterial number was more by Vorticella than Paramecium. In rice, the bacterial number decreased only until it reached a threshold level and then remained constant. In broth culture studies the population of bacteria decreased with the increase in protozoan number. The rate of reduction was greater in broth culture compared to the soil culture. In general, the rate of reduction of bacterial number was greater in Rhizobium compared to Azotobacter and Azospirillum.

The plant growth, biomass production, nodulation and nitrogen fixation in cowpea inoculated with both Rhizobium and Azotobacter were higher over inoculation of protozoa alone. Dual inoculation of Rhizobium and Azotobacter had

more beneficial effects over single inoculations. All the growth parameters were reduced with the addition of protozoa. Nodulation in cowpea was reduced by about 50 per cent by the addition of protozoa. The reduction was higher by Vorticella compared to Paramecium and was highest by the mixed culture of Paramecium and Vorticella.

In rice, the plant growth, tiller number, straw yield, grain yield and nitrogen content were higher in the absence of protozoa compared to the plants inoculated with protozoa.

Dual inoculation of Azotobacter and Azospirillum was found to be more beneficial compared to single inoculations in terms of plant growth, grain yield, straw yield and nitrogen content in rice. There was an adverse effect on all the above parameters by protozoa. Straw yield and nitrogen content were reduced by about 30 per cent by protozoa and grain yield by about 35 to 40 per cent. Vorticella caused more harm than Paramecium.

The studies revealed that (i) dual inoculation of Rhizobium and Azotobacter to cowpea and Azotobacter and Azospirillum to rice increased the growth and yield. (ii) Soil protozoa in general, Vorticella in particular reduced the number of beneficial soil bacteria to the extent that would have detrimental effect on the nitrogen fixation, growth and yield of cowpea and rice.

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* = Original not seen

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APPENDICES

APPENDIX - I

Physico-chemical properties of the soil,
used for the experiment

Property	GKVK Soil
(a) Property of constituent	
Coarse (%)	30.29
Fine sand (%)	25.98
Silt (%)	16.36
Clay (%)	27.35
Texture	Clay loam
(b) Chemical property	
pH	6.50
CEC (m.mhos.cm)	7.10
Organic carbon (%)	0.77
Total N (kg/ha)	176.00
Available P (kg/ha)	13.50
Total K (kg/ha)	375.98

APPENDIX - II

Composition of media used :

(a) Yeast extract mannitol agar (YEMA) for Rhizobium

Mannitol	10.00 g
K ₂ HPO ₄	0.50 g
MgSO ₄ .7H ₂ O	0.20 g
NaCl	0.10 g
Yeast extract	0.50 g
Distilled water	1000 ml
pH	7.00
Agar	15.00 g

Congored 2.5 ml of one per cent solution per litre of YEMA

(b) Waksman-77 (W₇₇) media for Azotobacter

Mannitol	10.00 g
K ₂ HPO ₄	0.50 g
MgSO ₄ .7H ₂ O	0.20 g
NaCl	0.20 g
MnSO ₄	Trace
FeCl ₃	Trace
CaCO ₃	10.00 g
Distilled water	1000 ml
pH	7-7.2
Agar	15.00 g

APPENDIX II contd..)

(c) Malate medium for Azospirillum

K ₂ HPO ₄	0.50 g
MgSO ₄ .7H ₂ O	0.20 g
NaCl	0.10 g
CaCl ₂	0.02 g
FeCl ₃	0.01 g
Malic acid	5.00 g
KOH	4.00 g
Na.MoO ₄ .2H ₂ O	0.02 g
Bromo thymol blue (0.5% in Ethanol)	2.00 ml
Distilled water	1000 ml
pH	6.6-7.0
Agar	15.00 g

Broth solutions of all these media are prepared without adding agar.
